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Welcome

Dear friends and colleagues,

It is a great pleasure for me to welcome you to Istanbul, Turkey for the 7th European Congress of Pharmacology in 26-30 June 2016. The Congress is hosted by the Turkish Pharmacological Society. The members of the society, the Local Organising Committee and the Scientific Committee took great care to design a programme that combines hot topics in pharmacology. All the announcements are available through the congress web page www.ephar2016.org. After the congress the website will be accessed from www.tfd.org.tr/ephar/2016 in order to make it available for long term.

Turkish Pharmacological Society, established in 1966, is a rapidly expanding community looking forward to having international scientific and social collaborations. Our 23rd national meeting, held in Ankara between 7-10 September 2015 welcomed more than 300 delegates. 2016 will be 50th anniversary of our society, and it is going to be a great pleasure for us to celebrate both events together in Istanbul.

The 7th European Congress of Pharmacology is taking place in Military Museum and Cultural Centre, located in the heart of the city. This modern congress centre with easy transportation offers convenient facilities such as meeting rooms for ongoing parallel sessions allowing you to select your favourite topics, exhibition hall for poster sessions and companies, and networking places to meet with your colleagues.

Istanbul is the biggest city in Turkey, located in the north-western part of the country, with a pleasant climate in June. Istanbul is a real must see destination with many unique features, easily accessible by hundreds of direct flights from many countries. It is the only city in the world to connect two continents-Europe and Asia. Istanbul, which has been a capital for centuries, embraces many historical and cultural beauties of Turkey including ancient and modern attractions.

On behalf of the Organising Committee, I welcome you to Istanbul for a great meeting to exchange knowledge between basic and clinical fields of pharmacology throughout the World and to share good times.

We hope EPHAR 2016 Istanbul will always be in your best memories!

Öner SÜZER
Congress Chair EPHAR 2016

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Scientific programme

The programme includes the following sessions:
A **plenary session** is a session with no contemporary parallel, with invited lecturers and no oral communications. These sessions last for 60 minutes.
A **main session** is organized by a national pharmacological society or a sponsor in consultation with the Scientific Committee. They run in parallel and with invited lecturers and elevated talks chosen by the organizers of the session among relevant free submissions. A main session lasts for 150 minutes (plus a 30 minute coffee break inside the session).
An **oral presentation session** is composed of a variable number of selected oral communications from the same field. This session lasts 90 minutes (maximum).
A **workshop** is a session organized by an external organization.

Oral presentations
C001- C0120 refer oral presentations.
C001-C036 are elevated talks which are included in main sessions.
C037-C064 will be presented on oral presentation sessions at Monday, 27th June 2016 between 13:30-15:00 (oral sessions 1-5, halls A-E).
C064-C092 will be presented on oral presentation sessions at Tuesday, 28th June 2016 between 15:00-16:30 (oral sessions 6-10, halls A-E).
C093-C120 will be presented on oral presentation sessions at Wednesday, 29th June 2016 between 15:00-16:30 (oral sessions 11-15, halls A-E).

Poster presentations
P001-P276 refer poster presentations.
EPHAR Poster Award Winner poster will be presented at all poster session days.
P001-P092 will be presented at poster session day 1, Monday, 27th June 2016 between 08:30-17:30 (Poster Hall)
P093-P183 will be presented at poster session day 2, Tuesday, 28th June 2016 between 08:30-17:30 (Poster Hall)
P184-P276 will be presented at poster session day 3, Wednesday, 29th June 2016 between 08:30-17:30 (Poster Hall)

Meeting at a glance

Sunday, 26th June 2016

Opening Session, Hall A (18:00-20:00)

Monday, 27th June 2016

Main Sessions (09:00-12:00)

Hall A: Small molecules inhibitors of ion channels in chronic pain states
Hall B: Different immunopharmacological approaches to treat allergic diseases
Hall C: EPA/DHA: Cardiovascular health and omega-3 long-chain PUFAs
Hall D: Targeting coding and non-coding RNAs in pharmacological research

Lunch & Poster Viewing (12:00-13:30)

Oral Presentations (13:30-15:00)

Session 1, Hall A: Neuropharmacology / Psychopharmacology; Obesity and Metabolic Diseases (C037-C042)
Session 2, Hall B: Cardiovascular Pharmacology (C043-C048)
Session 3, Hall C: Clinical Pharmacology (C049-C053)
Session 4, Hall D: Drug Safety and Toxicology (C055-C060)
Session 5, Hall E: Neuropharmacology / Psychopharmacology (C061-C064)

Plenary Lecture Hall A (15:30-16:30): miRNAs as potential therapeutic targets in cardiovascular disorders

Tuesday, 28th June 2016

Main Sessions (09:00-12:00)

Hall A: How to improve translation from animals to humans
Hall B: Emerging therapeutic targets in chronic pain and inflammation
Hall C: Calcium signalling induced by sphingosine-1-phosphate and other agonists in urogenital tract
Hall D: Signalling pathways in vascular function and dysfunction

Lunch & Poster Viewing (12:00-13:30)

Plenary Lecture, Hall A (13:30-14:30): Purinergic signaling in adult neural progenitor cell functions: Experimental basis and possible therapeutic significance

Oral Presentations (13:30-15:00)

Session 6, Hall A: Neuropharmacology / Psychopharmacology (C065-C069)
Session 7, Hall B: Cardiovascular Pharmacology (C070-C075)
Session 8, Hall C: Pharmacokinetics and Drug Metabolism (C076-C081)
Session 9, Hall D: Receptors and Cell Signalling (C082-C087)
Session 10, Hall E: Drug Discovery, Development and Evaluation (C088-C092)

Wednesday, 29th June 2016

Main Sessions (09:00-12:00)

Hall A: Dopamine signaling in health and disease
Hall B: Basic and translational pharmacology of hydrogen sulfide: molecular targets and targeted diseases
Hall C: Trace amine associated receptors (TAARs): a promising target for pharmacotherapies?
Hall D: Molecular clocks and chronopharmacology
Hall E: ORPHEUS platform: "Best Practices for High Research Standards and PhD Students training in Responsible Research"

Lunch & Poster Viewing (12:00-13:30)

Plenary Lecture, Hall A (13:30-14:30): Poly(ADP-ribose) polymerase: pathomechanisms and therapeutic opportunities

Oral Presentations (13:30-15:00)

Session 11, Hall A: Pain and Inflammation; Gasotransmitters (C093-C098)
Session 12, Hall B: Cancer Chemotherapy; Respiratory Pharmacology; Genitourinary and Reproductive Pharmacology (C099-C104)
Session 13, Hall C: Gastrointestinal Pharmacology; Rational Drug Use (C105-C110)
Session 14, Hall D: Drug Discovery, Development and Evaluation; Drugs for Infectious Diseases (C111-C116)
Session 15, Hall E: Miscellaneous (C054-C120)

Thursday, 30th June 2016

Main Sessions

Hall A: Targeting inflammation in disease (09:00-12:00)
Hall B: European Registered Pharmacologist Project (09:00-10:30)
Hall B: EJP Educational Seminar (11:00-12:00)
Hall C: New insights in neuropsychiatric disorders: models & molecules with therapeutic potential (09:00-12:00)
Hall D: Biased signaling - far beyond arrestin (09:00-10:30)
Local workshop Hall E: Rational Drug Use (11:00-12:00)
End of congress: 12:00

Sunday, 26th June 2016

Opening Session, Hall A

18:00-20:00

18:00 Opening Ceremony

18:30 Welcome Reception; Turkish Pharmacological Society 50th anniversary

20:00 End of opening session

Monday, 27th June 2016

Main Sessions (09:00-12:00)

Hall A:
Small molecules inhibitors of ion channels in chronic pain states

(Organized by British Pharmacological Society)
Organizers and chairs:
Edward Stevens (Pfizer Neuroscience and Pain Research Unit, Cambridge, UK)
Gary Stephens (University of Reading, UK)
09:00-12:00

09:00 Introduction to the session
Gary Stephens
University of Reading, UK

09:15 Small molecule inhibitors of NaV1.7 voltage-gated sodium channels
Edward Stevens
Pfizer Neuroscience and Pain Research Unit, UK

09:45 Elevated talk (C001): Gene expression profile of sodium channel subunits in the anterior cingulate cortex during experimental paclitaxel-induced neuropathic pain
Willias Masocha
Kuwait University, KUWAIT
(Authors: Willias Masocha)

10:00 Role of M-type K+ channels in acute and chronic pain
Nikita Gamper
University of Leeds, UK

10:30 Automated Patch Clamp ion channel technology in chronic pain drug discovery - panacea or paucity?
Damian C Bell
Charles River Laboratories, UK

11:00 TRP channels in pain
Lucy Donaldson
University of Nottingham, UK

11:30 Elevated talk (C002): Targeting changes in inhibitory signalling in chronic pain
Wendy L. Imlach
The University of Sydney, AUSTRALIA
(Authors: Wendy L Imlach, Macdonald J Christie)

12:00 End of symposium

Introduction to the session

Gary J Stephens
University of Reading, Reading, UK

Chronic pain is reported to affect 1.5 billion people globally, in particular, neuropathic pain is believed to affect up to 4.5% of the world population, an amount increasing with the ageing worldwide population. The pharmaceutical industry estimates that the global market in pain therapeutics will reach US $600 billion this year. Moreover, chronic pain is associated with a large unmet clinical need, fuelling the development of small molecular entities (SMEs), often via biological drug sources, to target specific key proteins in dorsal root ganglia and dorsal horn nociceptive pathways. Thus, voltage-gated sodium channel subunits such as $Na_V1.7$ (and also $Na_V1.8$) represent important novel targets for development of SMEs and peptides derived from natural venoms. Voltage-gated potassium channels represent a diverse family that set basal properties and control neuronal firing frequency in pain sensory neurons; the focus here includes agents acting at 'M-type' potassium channels. In addition to being primary downstream targets for gold-standard opioid analgesics, voltage-gated calcium channels are directly blocked by ziconotide, derived from *Conus* sea snail; new therapeutic agents are targeting both $Ca_V2.2$ (N-type) and Ca_V3 (T-type) calcium channels. TRP channels are fast becoming major new therapeutic targets, in particular, TRPV1 and TRPA1 which are highly expressed in nociceptive pathways; potential targeting mechanisms include use of stimulatory agents that desensitize and block TRP channels (a mechanism characterised by the archetypal TRPV1 agonist capsaicin), but also channel blockers. There is also the clear possibility to target ligand-gated ion channel receptors. The symposium will discuss pre-clinical testing of novel therapeutic agents targeting ion channels in nociceptive pathways, their advance to *in vivo* models of chronic pain and their potential future routes to the clinic.

Small molecule inhibitors of NaV1.7 voltage-gated sodium channels

Edward Stevens
Pfizer Neuroscience and Pain Research Unit, UK

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Elevated talk (C001): Gene expression profile of sodium channel subunits in the anterior cingulate cortex during experimental paclitaxel-induced neuropathic pain

Willias Masocha
Department of Pharmacology and Therapeutics, Faculty of Pharmacy, Kuwait University, Kuwait.
Email address of corresponding author: masocha@hsc.edu.kw

The use of paclitaxel in the treatment of solid tumors is limited by development of painful neuropathy. There is a dearth of drugs for the prevention or treatment of this neuropathic pain because its pathophysiology is not well understood. There is considerable evidence indicating that dysregulation of sodium channel expression, mainly in the periphery and spinal cord level, contribute to the pathogenesis of neuropathic pain in animal models. This study was conducted to investigate the supraspinal expression of sodium channel subunits in the anterior cingulate cortex (ACC), an area in the brain involved in pain perception and modulation, during paclitaxel-induced neuropathic pain.

BALB/c mice were treated for 5 consecutive days with paclitaxel (2 mg/kg, i.p.) or its vehicle. The reaction latency times to thermal stimuli (hot-plate test) were recorded before (baseline) and at 7 days after treatment. Gene transcripts of sodium channel alpha and beta subunits were quantified by real time PCR in the ACC of brains dissected out from mice sacrificed at 7 days post first dose administration of paclitaxel or vehicle.

Paclitaxel treatment reduced reaction latency time to thermal stimuli at 7 days post-first drug administration compared to baseline. Amongst the 10 sodium channel alpha subunits analysed treatment with paclitaxel did not significantly alter the mRNA expression of Nav1.3 and Nav1.9, but significantly increased the expression of Nav1.1, Nav1.2, Nav1.4, Nav1.5, Nav1.6, Nav1.7, Nav1.8 and Nax. Amongst the 4 sodium channel beta subunits analysed treatment with paclitaxel significantly increased the expression of Navβ1 and Navβ3, but not Navβ2 and Navβ4.

Our results indicate that during paclitaxel-induced neuropathic pain there is differential up-regulation of sodium channels in the ACC, which might contribute to the increased neuronal activity observed in the area during neuropathic pain.

This study is supported by Kuwait University, Grant numbers: PT01/09 and SRUL02/13.

Keywords: paclitaxel, neuropathic pain, sodium channels, anterior cingulate cortex, gene expression

Role of M-type K+ channels in acute and chronic pain

Nikita Gamper
Faculty of Biological Sciences, University of Leeds, Leeds, UK
Department of Pharmacology, Hebei Medical University, Shijiazhuang, CHINA

Kv7 (KCNQ or M-type) K^+ channels are increasingly recognised among the most important regulators of nociceptive sensory neuron excitability. M channels conduct slow, non-inactivating K^+ currents with a threshold for activation well below the resting membrane potential of nociceptors (~ -60 mV). These properties, in combination with outwardly-rectifying voltage-dependence, allow M channels to function as an 'intrinsic voltage-clamp' mechanism that controls resting membrane potential, firing thresholds and accommodation within the action potential trains. Functional M channels are expressed in cell bodies, peripheral axons, nerve endings as well as in dorsal roots and central terminals of nociceptive fibres. M channel activity strongly contributes to afferent fibre excitability *in vivo*. For instance, intraplantar hind paw injections of the M channel inhibitors trigger nocifensive behaviour in rats while peripheral injections of M channel enhancers ('openers') produce analgesia. Growing evidence suggests that functional deficiencies of M channels contribute to multiple painful conditions. Thus, acute, receptor-mediated inhibition of M channels in nociceptive nerve endings contributes to inflammatory pain while downregulation of M channel expression has been reported in several models of neuropathic and cancer pain. Multiple studies report analgesic activity of pharmacological M channel enhancers in a variety of animal pain models. One M channel opener, flupirtine, is a clinically used analgesic while a closely related compound, retigabine, is clinically approved as anticonvulsant. Moreover, growing evidence suggests that M-channel enhancement may contribute to analgesic activity of some NSAIDs such as celecoxib. Since first recognition of analgesic activity of M channel enhancing drugs, thousands of new M channel openers were described and some of them are moving through analgesic drug discovery pipelines. In this report I will discuss current developments in M channel and pain research as well as the perspectives of therapeutic targeting of M channels in nociceptors.

Automated Patch Clamp ion channel technology in chronic pain drug discovery - panacea or paucity?

Damian C Bell
Charles River Labs, Chesterford Research Park, Saffron Walden, UK.

Automated Patch Clamp (APC) technology was first developed at the start of the millennium. The increased throughput it afforded promised a new dawn in ion channel recordings: it offered the potential to overcome the time-consuming, low-throughput bottleneck arising at manual patch clamp that had long stymied ion channel drug discovery.

Through selected examples, ion channel targets in chronic pain drug discovery using different APC technologies will be discussed. The first generation of APC included: PatchLiner (Nanion), PatchXpress (Molecular Devices Corp., MDC), QPatch (Sophion), SyncroPatch96 (Nanion) and IonWorks (MDC). With the field maturing, midway through its second decade, second generation platforms (APC 2.0) are providing higher throughput, improved fluidics (e.g. including exchange of intracellular solution) and additional recording capabilities (current-clamp, temperature control). The impact of the second generation of APC (e.g. Qube, Sophion; SyncroPatch 384PE, Nanion) and the prospects for chronic pain therapeutics will be evaluated.

TRP channels in pain

Lucy F Donaldson
School of Life Sciences, University of Nottingham, Nottingham UK.

TRP channels are fundamental to transduction in many sensory neurons, being involved in the transduction of noxious heat, cold, and chemical stimuli. As such TRP channels have been the target of many analgesic drug discovery programmes, but with limited success. Many pro-nociceptive molecules exert their effects on nociceptive afferents through stimulation of intra-cellular signalling that modulates ion channel function, including TRP channels particularly TRPV1 and TRPA1, leading to changes in nociceptor properties such as lowered activation threshold and increased stimulus-evoked responses.

Both TRPV1 and TRPA1 have been implicated in neuropathic pain and neuronal damage, particularly in diabetic neuropathy. We have identified novel mechanisms of TRP channel sensitisation in sensory neurons, through the actions of pro-nociceptive splice variants of vascular endothelial growth factor-A, and agonists of Receptor for Advanced Glycosylation End-product (RAGE). TRP channel sensitisation and altered nociception can be blocked *in vitro* and *in vivo* by anti-nociceptive VEGF-A alternative splice variants. We are investigating the potential of small molecule inhibitors for control of alternative pre-mRNA splicing of VEGF, and hence modulation of TRP channel function, as a novel analgesic strategy.

Elevated talk (C002): Targeting changes in inhibitory signalling in chronic pain

Wendy L Imlach, Macdonald J Christie
Discipline of Pharmacology, Sydney Medical School, The University of Sydney, Sydney, Australia.
Email address of corresponding author: wendy.imlach@sydney.edu.au (W. L.Imlach)

Chronic pain can be difficult to manage with current therapeutics. A large body of evidence in animal models and humans suggests that chronic pain involves persistent pathological adaptations, some of which are potential therapeutic targets. Much of the fast inhibitory neurotransmission in the spinal cord is mediated by glycine, which when removed, results in the pathological symptoms of neuropathic pain.

This study investigates glycinergic signalling and the effect of GlyT2 inhibitors on spinal cord nociceptive signalling in a rat model of neuropathic pain.

In this study we used a partial sciatic nerve ligation (PNL) model of chronic pain in adult rats. Inhibitory synaptic currents were measured in whole-cell voltage-clamp from laminae II cells. Tungsten electrodes placed in the inner laminae were used to elicit eIPSCs.

We have found that glycinergic neurotransmission is reduced, or occasionally abolished, in a subset of neurons in the superficial laminae of the dorsal horn of animals with neuropathic pain. Here, we investigated the effect of GlyT2 inhibitors on these neurons to determine whether the loss of glycinergic neurotransmission in the neuropathic pain model could be restored. These inhibitors increase glycinergic neurotransmission by prolonging the synaptic current.

Our results suggest that inhibition of GlyT2 may be another potential target for neuropathic pain.

Keywords: neuropathic pain, glycine, GlyT2, spinal cord, dorsal horn

Hall B:
Different immunopharmacological approaches to treat allergic diseases

(Organized by Danone-Nutricia Research, Utrecht, the Netherlands and IUPHAR/Immunopharmacology)
Organizers and chairs:
Francesca Levi-Schaffer (The Hebrew University of Jerusalem, ISRAEL)
Aletta Kraneveld (Utrecht University, The NETHERLANDS)
09:00-12:00

09:00 Pro-resolving mediators: potential new treatment for the allergy and asthma
Oliver Haworth
Barts and the London School of Medicine & Dentistry, UK

09:30 Microbiome manipulation for the management of allergic disorders
Johan Garssen
Utrecht University, The NETHERLANDS

10:00 Activate inhibition and inhibit activation of mast cells and eosinophils with antibodies to treat allergic diseases
Francesca Levi-Schaffer
The Hebrew University of Jerusalem, ISRAEL

10:30 Coffee break

11:00 Elevated talk (C003): Novel mechanism of action with therapeutic potential: the chemokine neutraligands for asthma
Nelly Frossard
Université de Strasbourg, FRANCE
(Authors: Nelly Frossard, François Daubeuf, Dominique Bonnet, Dayana Abboud, Virgile Beckaert, Ali Ouadi, Patrice Marchand, David Brasse, Marcel Hibert, Jean Luc Galzi)

11:15 Elevated talk (C004): Problems of standardizing specific activity of allergen products
Viacheslav Ivanov
Ministry of Health of the Russian Federation, RUSSIA
(Authors: Viacheslav Ivanov, Viacheslav Mosyagin, Vladimir Bondarev, Yury Olefir)

11:30 Elevated talk (C005): Involvement of CXCR6/CXCL16 axis in platelet-leukocyte adhesion to the dysfunctional arterial endothelium in COPD patients
Patrice Marques
University of Valencia, SPAIN
(Authors: Patrice Marques, Aida Collado, Cristina Rius, Paula Escudero, Cruz González, Emilio Servera, Laura Piqueras, Maria Jesus Sanz)

11:45 Elevated talk (C006): Galectin-9 promotes ALDH activity in dendritic cells to support the differentiation of Treg cells in vitro
Aletta Kraneveld
Utrecht University, The NETHERLANDS
(Authors: Sander De Kivit, Atanaska I Kostadinova, Joann Kerperien, Mary E Morgan, Leon M Knippels, Aletta D Kraneveld, Johan Garssen, Linette Willemsen)

12:00 End of symposium

Pro-resolving mediators: potential new treatment for the allergy and asthma

Oliver Haworth
Barts and the London School of Medicine & Dentistry, UK

Asthma is a chronic inflammatory disease of the airways that is often un-responsive to current treatments indicating the need to develop new approaches to treatment. Omega-3 fatty acids have long known to be beneficial to health, but the precise mechanism by which they dampen inflammation is not been clear. Pro-resolving lipid mediators (PRLMs) derived from omega-3 fatty acids include resolvins, lipoxins and maresins offer the potential to treat asthma by dampening inflammation and promoting the resolution of allergic airway inflammation.
PRLMs accelerate the resolution of allergic airway inflammation in part by regulating innate lymphocytes such as Natural Killer (NK) cells and innate lymphoid cells (ILCs). Resolvin E1 (RvE1) acted in part through NK cells as depletion of NK cells blunted the ability of RvE1 to mediate resolution of inflammation. The percentage of NK cells in the peripheral blood of patients with severe asthma was diminished compared to healthy individuals. ILC2 are potent producers of pro-inflammatory cytokine IL-13 and the PRLM Lipoxin A4 (LXA4) inhibited the ability of ILC2 cells to make IL-13. Maresin-1 (MaR1) decreased ILC2 production of IL-5 and IL-13 and increased the percentage of regulatory T cells within the lung during allergic airway inflammation. Together these results demonstrate that pro-resolving lipid mediators have many powerful actions to promote resolution of allergic airway inflammation.

Microbiome manipulation for the management of allergic disorders

Johan Garssen
Utrecht Institute for Pharmaceutical Sciences, division Pharmacology, Beta faculty, Utrecht University, the NETHERLANDS and Nutricia-research Utrecht / SINGAPORE

Prebiotic oligosaccharides, probiotic microbes and combinations thereof (synbiotics) are more and more accepted as immunomodulatory ingredients for the prevention and or treatment of immune related disorders such as infections, obesity, certain brain/behavior disorders and last but not least allergy related disorders such as atopic eczema, food allergy, asthma, and rhinitis.

The human body has an effective defense system against foreign non-self organisms or substances. This defense consists of a non-specific first line defense, a non-specific innate immunity and a specific adaptive immunity. Altogether this is called the immune system which has the ability to recognize, to remember, to destroy (non-self) cells and/or to inactivate damaging substances. The intriguing feature of immune development is that it is a continuous process which never ends. A complex highly flexible organ-system able to react and change very fast depending on the danger signal at all ages. During pregnancy the immune system of the unborn baby is educated to inhibit the rejection between mother and child even if they are so-different. After birth the immune system should develops as fast as possible in order to recognize self from non-self and react to any other danger signal. Although there is no consensus at what age the immune system is fully developed compared to adult immune responsiveness it is generally accepted that the majority of immune functions reach adult levels during early puberty although scientists agree that the immune system is never ready and under development during our whole life. Especially during the first few months of life the immune system changes enormously. For this reason during this period the immune system is highly susceptible to both positive as well as negative triggers affecting a healthy immune development. More and more research indicates that early events on immune development might have serious consequences on immune related diseases later at adult ages such as allergies, asthma and even autoimmunity. Several factors are playing a crucial role in immune development and as a consequence immune related disorders. Genes, epigenetic stimuli, environmental triggers, pollution, infections, and diet are recognized as example factors playing a pivotal role in immune development especially during early life. A divers microbiome seems to play a pivotal role in developing and the maintenance of a healthy balanced immune system. With respect to diet breast milk is the best early “immune” nutrition with well described immune benefits early and later in life which might play a role in lowering the incidence of immune related disorders and of metabolic diseases such as obesity. Unique molecules from breastmilk receives lots of attention for the development of new concepts for immune regulation involving microbiome manupilation. Breastmilk prebiotic oligosaccharides or oligosaccharides mimicking breastmilk oligosaccharides, probiotics and combinations of both pro and prebiotics (called synbiotics) are extensively studied by many immunologists in order to find out whether these nutritional ingredients can decrease the incidence and/or severity of immune related disorders and especially allergy related disorders. Published and peer reviewed data indicate that these unique ingredients might be a useful tool to manage e.g. allergic disorders in a preventive as well as therapeutic way. Underlying mechanism indicate a pivotal role for a divers microbiome. However, not all prebiotics and/or probiotics are similar and

translational research is a must to select the optimal combination for the best and safest effect regarding allergy management. The current presentation will focus on some unique examples containing pro-, pre- or synbiotics with a validated effect on allergy related features and some other immune related disorders such as HIV and certain cancers.

Activate inhibition and inhibit activation of mast cells and eosinophils with antibodies to treat allergic diseases

Francesca Levi-Schaffer
Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, POB 12065, Jerusalem 91120, ISRAEL

Mast cells (MCs) and eosinophils are the key effector cells of allergic inflammatory diseases such as asthma, allergic rhinitis, atopic dermatitis (AD), etc. Drugs are available to downregulate the symptoms of allergy such as anti-histamines or limit the ongoing inflammation and tissue/organ damage such as corticosteroids. While mild/moderate forms of allergy are well controlled by a combination of these approaches, severe asthma and AD remain unmet clinical needs.

In the last decades monoclonal antibodies (mAbs) therapies especially for autoimmune diseases and cancer have made tremendous progress. In allergy anti-IgE antibodies are already on the market and useful for some forms of asthma. However a global approach suitable for most patients and with minimal side effects is warranted.

We have taken a different approach following the discovery of the novel activating receptor (AR) CD48 and of the inhibitory receptors (IRs) CD300a and Siglec-7 on both MCs and eosinophils. We reasoned that blocking ARs and stimulating IRs with specific mAbs will inhibit these cell functions and hence allergy.

We have fully characterized these receptors on mouse and human MCs (bone narrow and cord blood derived) and eosinophils (bone marrow derived and peripheral blood isolated) by assessing their expression and signal transduction. For down or upregulating CD48 or Siglec-7 commercially available blocking or activating mAbs were used, while for CD300a we synthesized bi-specific Abs to target specifically MCs and eosinophils. We have tested the anti-inflammatory/anti-allergic properties of the Abs in mouse models of asthma, PCA, allergic peritonitis and AD.

In vitro we demonstrated that our approach significantly inhibited MC and eosinophil functions such as degranulation, cytokine production, chemotaxis and in vivo it downregulated the allergic responses. Therefore mAbs for ARs or IRs specifically expressed on MCs and eosinophils can be a better pharmacological tool than existing drugs for the treatment of allergy.

Elevated talk (C003): Novel mechanism of action with therapeutic potential: the chemokine neutraligands for asthma

Nelly Frossard[1], François Daubeuf[1], Dominique Bonnet[1], Dayana Abboud[3], Virgile Beckaert[2], Ali Ouadi[2], Patrice Marchand[2], David Brasse[2], Marcel Hibert[1], Jean Luc Galzi[3]
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The CXCL12 chemokine and its receptors CXCR4-CXCR7 are involved in normal tissue patterning, as well as in the physiopathology of inflammatory diseases, including allergic asthma. We recently reported in a murine model of airway hypereosinophilia, the anti-inflammatory action of a non-peptidic CXCL12 neutraligand, chalcone 4. We synthesized an 123I-chalcone 4, exhibiting similar activities to chalcone 4, to visualize its bioavailability when administered intranasally. We show rapid elimination of 123I-chalcone 4 from the lung in less than 30 min (97±2%), and elimination in the bile, feces and urine. PK analysis confirms the elimination half-life (T½<5 min) in the lung. This is concomitant to a decrease in CXCL12 in lung (-20±3%), and its increase in blood (x1000), suggesting draining of the neutraligand and CXCL12 from the lung. Although chalcone 4 is eliminated, we report the inhibition of all features of asthma: airway hyperresponsiveness (-45±7%), inflammatory cell recruitment, in particular eosinophils (-54±2%) and M1 macrophages (-65±4%), and remodeling with decreased mucus hypersecretion (-84±2%) and collagen deposition (-78±8%). In addition, we show an inhibition of the alveolar macrophage M1-M2 polarization accompanied by inhibition of cytokine release ex vivo in response to ovalbumin or to CXCL12 itself, suggesting a role of the macrophage in modulating the recruitment of eosinophils. In conclusion, the CXCL12 neutraligand binds endogenous CXCL12 to prevent binding to its receptor and glycosaminoglycans, thereby collapsing the extracellular density gradient of CXCL12 to escape the immune response, and inhibits macrophage activation and differentiation in response to allergen.

Keywords: respiratory system, asthma, inflammation, anti-inflammatory strategy

Elevated talk (C004): Problems of standardizing specific activity of allergen products

Viacheslav Ivanov, Viacheslav Mosyagin, Vladimir Bondarev, Yury Olefir
Federal State Budgetary Institution "Scientific Centre for Expert Evaluation of Medicinal Products" of the Ministry of Health of the Russian Federation, Moscow, Russia.
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At present there is not a uniform system of allergen products standardization. Currently manufactured allergen products are standardized in units reflecting the extent of their total allergenic activity. Different approaches to standardization of allergen products complicate comparative assessment of allergenic activity of products that are produced by different manufacturers. For instance, American allergen products are standardized in BAU (Bioequivalent Allergy Units), European products – in BU (Biological Units), IU (International Units), IR (Index of reactivity) and other units. Most producers use in vitro methods (e.g. enzyme-linked immunosorbent assay, radioallergosorbent test inhibition) and a well-characterised internal reference product to measure allergenic activity.

Allergen products manufactured in Russia are standardized in PNU (Protein nitrogen Units). However this parameter which describes the content of protein in allergenic material does not reflect true allergenic activity of product, does not guarantee the consistency of activity across different batches and complicates dosing during allergen-specific immunotherapy. Therefore it is necessary to standardize therapeutic allergen products in units of allergenic activity, develop standards of allergenic activity and common requirements for allergen-specific sera used in allergen standardization studies.

The standardization of therapeutic allergen products includes, among other things, the demonstration of the presence of main allergens in a product. Therefore, it is necessary to consider the use of competitive binding of allergen-specific IgE antibodies for assessing activity of allergen products produced in Russia and for developing an assay for determination of main allergenic components.

Therapeutic allergen products manufactured with regard to individual indications for a particular patient are becoming increasingly popular in many countries. Development of requirements for quality control of allergen products tailored to a particular patient is also a pressing problem in Russia.

The use by Russian manufacturers of modern approaches to allergen products quality assessment will increase safety and efficacy of allergen-specific immunotherapy of allergic diseases.

Keywords: allergen products, allergenic activity, standardization

Elevated talk (C005): Involvement of CXCR6/CXCL16 axis in platelet-leukocyte adhesion to the dysfunctional arterial endothelium in COPD patients

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Cardiovascular disease (CVD) is a major co-morbidity in chronic obstructive pulmonary disease (COPD), yet the pathways involved in its development remain unknown. Therefore, the potential link between CXCR6/CXCL16 axis and COPD-induced endothelial dysfunction were investigated. Whole blood from 21 COPD patients and 15 age-matched controls was analysed by flow cytometry. Platelet activation (P-selectin expression and circulating PAC-1+ platelets) and CXCR6 and CXCL16 expression was determined. CXCR6 expression on different leukocyte subsets was also evaluated. Parallel-plate flow chamber assay was employed to evaluate platelet-leukocyte and leukocyte adhesion to cigarette smoke extract (CSE)-stimulated arterial endothelium. Flow cytometry analysis revealed that COPD patients presented greater numbers of activated circulating platelets (PAC-1+) with increased expression of P-selectin, CXCL16 and CXCR6 compared with age-matched controls. Additionally, COPD patients presented augmented numbers of platelet-neutrophil, platelet-monocyte and platelet-lymphocyte aggregates than age-matched control subjects. This correlated with enhanced platelet-leukocyte and leukocyte adhesiveness to CS extract (CSE)-stimulated arterial endothelial cells and was partly dependent on endothelial CXCL16 up-regulation and increased CXCR6 expression on platelets and leukocytes. We provide the first evidence that increased CXCR6 expression on circulating platelets and leukocytes from COPD patients may constitute a prognostic marker for adverse cardiovascular events.

Keywords: cigarette smoke, chemokines, arterial dysfunction, platelets-leukocytes, endothelium

Elevated talk (C006): Galectin-9 promotes ALDH activity in dendritic cells to support the differentiation of Treg cells in vitro

Sander De Kivit[1], Atanaska I Kostadinova[1], Joann Kerperien[1], Mary E Morgan[1], Leon M Knippels[2], Aletta D Kraneveld[1], Johan Garssen[2], Linette Willemsen[1]
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Intestinal epithelial cells (IEC) are known to drive the development of Treg responses by promoting the development of aldehydedehydrogenase (ALDH) expressing CD103+ tolerogenic dendritic cells (DC). IEC express and secrete galectin-9 which is promoted by short-chain galacto-oligosacharides and long-chain fructo-oligosaccharides (GF). While galectin-9 may support a Treg response, it is not known whether IEC-derived galectin-9 induces Treg cell differentiation via DC.

HT-29 and T84 IEC transwell cultures were basolaterally exposed to human TNF-alpha and IFN-γ. After overnight culture, IEC were washed and apically exposed to 5 µM TLR9 ligand (CpG) in presence or absence of 0.5%w/v of a 9:1 mixture of GF for 24h. Basolateral conditioned medium was collected and stored at -20oC.

Human monocyte-derived DC (moDC) and bone marrow-derived DC (BMDC) from Balb/c mice were differentiated in the presence of recombinant galectin-9 or conditioned medium from IEC. Tim3Fc fusion protein was used to neutralize galectin-9. DC expression of CD103 and ALDH activity was determined by flow cytometry (FACS) and the capacity of DC to induce functional Treg cells (allogenic DC-T cell cultures and suppression assay) was assessed. MAPK and PI3K inhibitors were used to study signaling cascades in galectin-9 exposed BMDC.

ALDH activity was increased in moDC and BMDC differentiated in the presence of galectin-9, while the expression of CD103 remained unaltered. In addition, moDC exposed to galectin-9 or IEC-conditioned medium showed increased capacity to induce Treg cell differentiation with suppressor activity in vitro. The latter could be block by galectin-9 neutralization. The induction of ALDH activity in BMDC by galectin-9 was dependent on MAPK p38 and PI3K signaling pathways.

Treg cell induction by galectin-9 is dependent on ALDH activity in DC. These data contribute to gain insight in the molecular mechanisms by which IEC-derived galectin-9 induced by TLR9 supports tolerance induction in the intestinal mucosa.

Keywords: dendritic cell, intestinal epithelial cell, T^{reg} cells, galectin-9

Hall C:
EPA/DHA: Cardiovascular health and omega-3 long-chain PUFAs

(Organized by GOED, Global Organization for EPA and DHA omega3s)
Organizers and chairs:
Gerard Bannenberg (GOED (Global Organization for EPA and DHA omega3s), USA)
Ali Moderrisoğlu (Sifar Pharmaceuticals, TURKEY)
09:00-12:00

09:00 A current view of the relation of lipid intake to cardiovascular health, including the bioavailability of various lipid forms
Rob Winwood
DSM Nutritional Products, SWITZERLAND

09:30 Overview of the evidence for omega-3 LCPUFA and cardiovascular health
Philip Calder
University of Southampton, UK

10:00 Elevated talk (C008): Niacin and olive oil promote the skewing to M2 phenotype in bone marrow-derived macrophages of mice with metabolic syndrome
Maria C Naranjo
Instituto de la Grasa, CSIC, SPAIN
(Authors: Maria C Naranjo, Sergio Montserrat De La Paz, Sergio Lopez, Rocio Abia, Francisco J.g. Muriana, Beatriz Bermudez)

10:30 Coffee break

11:00 CYP450 epoxygenases as sources of protective omega-3 lipid mediators in vascular inflammation and resolution
David Bishop-Bailey
University of London, UK

11:30 Omega-3 Index - why the omega-3 status matters
Clemens Von Schacky
University of Munich, GERMANY

12:00 End of symposium

A current view of the relation of lipid intake to cardiovascular health, including the bioavailability of various lipid forms

Rob Winwood
DSM Nutritional Products, Kaiseraugst, SWITZERLAND and chair of the GOED science committee.

The overall efficiency of fat absorption in human adults is about 95%, more or less independent of the amount of fat consumed. However, the qualitative nature of the dietary fat influences overall efficiency. In general, efficiency increases with the degree of fatty acid unsaturation and reduces with overall chain length (1). The entire process of digestion and absorption of fats will typically take 16-24 hours. Ingested fats are predominately triglycerides (TAG). These are insoluble and need to be hydrolysed and emulsified before they can be transferred to the cells lining the intestinal wall (enterocytes). The TAG's are converted into monoacylglycerols (MAG's) and free fatty acids (FFA's) to facilitate absorption. They are then usually packed into chylomicrons with other lipids and proteins and transported via the lymphatic system to the blood. The physical nature of fats in foods and supplements can affect their rate of digestion.

The marine omega-3 polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have a range of benefits for cardiovascular health, including reducing blood pressure and blood triglyceride levels. There is considerable controversy as to whether the commercially available forms of DHA and EPA oils vary in their bioavailability. It seems plausible that phospholipid (PL) fat forms will be metabolized faster than TAG forms, but this is probably not relevant to long-term tissue uptake. A comparative study (2) in 2015, failed to find significant differences of uptake into red blood cells of PL, TAG and the ethyl ester form (the latter being form of choice in Rx Blood Triglyceride/blood pressure lowering pharmaceutical preparations).

In 2016, the GOED (Global Organisation of EPA and DHA omega-3s) has reviewed the current scientific evidence as regards intake and risk of cardiovascular disease and made the following recommendations:For the healthy adults, a daily intake of 500mg per day of EPA and DHA will lower the risk of coronary heart disease (CHD). For secondary prevention of CHD, a daily intake of a minimum of 1,000mg per day of EPA and DHA is required (3). http://www.goedomega3.com/healthcare

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Overview of the evidence for omega-3 LCPUFA and cardiovascular health

Philip C. Calder
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Fatty fish, other seafood, fish oils and their concentrates, and some algal oils are sources of long chain, highly unsaturated omega-3 fatty acids (O3LCPUFA). Functionally the most important O3LCPU

FA appear to be eicosapentaenoic acid (EPA) and docosahexaenoioc acid (DHA), although roles for docosapentaenoic acid (DPAn-3) are emerging. Intakes of EPA and DHA are typically low and much below what is recommended. Increased intakes are reflected in greater incorporation into blood lipid, blood cell and tissue (including heart) pools. Increased content of EPA and DHA can modify the structure of cell membranes and also the function of membrane proteins. EPA and DHA also modify the production of lipid mediators and through effects on cell signaling can alter patterns of gene expression. Through these actions EPA and DHA act to beneficially modify a number of risk factors for cardiovascular disease (CVD) including blood pressure, platelet reactivity and thrombosis, plasma triglyceride concentrations, vascular function, heart rate and heart rate variability, and inflammation. Consistent with these effects, epidemiological studies show an inverse association between EPA and DHA intake or status and risk of cardiovascular morbidity and mortality. Thus, there is a key role for O3FA in prevention and slowing progression of cardiovascular disease. Furthermore, some, but not all, supplementation studies with O3FA have demonstrated reduced mortality in at risk patients, such as post-myocardial infarction, indicating a therapeutic role. Meta-analyses based upon the inconsistent evidence base produce mixed findings. This will be further explored.

Elevated talk (C008): Niacin and olive oil promote the skewing to M2 phenotype in bone marrow-derived macrophages of mice with metabolic syndrome

Maria C Naranjo[1], Sergio Montserrat De La Paz[1], Sergio Lopez[1], Rocio Abia[1], Francisco J.g. Muriana[1], Beatriz Bermudez[2]
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Metabolic syndrome (MetS) is associated with obesity, dyslipemia, type 2 diabetes and chronic low-grade inflammation. The aim of this study was to determine the role of high-fat low-cholesterol diets (HFLCDs) rich in SFAs (HFLCD-SFAs), MUFAs (HFLCD-MUFAs) or MUFAs plus omega-3 long-chain PUFAs (HFLCD-PUFAs) on polarisation and inflammatory potential in bone marrow-derived macrophages (BMDMs) from niacin (NA)-treated Lepob/obLDLR-/- mice.

Animals fed with HFLCD-SFAs had increased weight and serum triglycerides, and their BMDMs accumulated triglycerides over the animals fed with HFLCD-MUFAs or -PUFAs. Furthermore, BMDMs from animals fed with HFLCD-SFAs were polarised towards M1 phenotype with functional competence to produce pro-inflammatory cytokines, whereas BMDMs from animals fed with HFLCD-MUFAs or -PUFAs were skewed to anti-inflammatory M2 phenotype.

These findings open opportunities for developing novel nutritional strategies with olive oil as the most important dietary source of MUFAs (notably oleic acid) to prevent development and progression of metabolic complications in the NA-treated MetS.

Keywords: olive oil, niacin, bone marrow-derived macrophages, metabolic syndrome, inflammation

CYP450 epoxygenases as sources of protective omega-3 lipid mediators in vascular inflammation and resolution

David Bishop-Bailey
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A number of lipid mediators are known to contribute to cardiovascular health, inflammation and inflammatory resolution. Fatty acid metabolites produced by CYP450 enzymes are of considerable current interest. Altering diet with omega-3 lipid supplementation (docosahexaenoic acid, and eicosapentaenoic acid) strongly elevates circulating omega-3 derived CYP450 products in humans and animal models. Elevating the levels of endogenous CYP450 products by disrupting (knockout) or inhibiting the enzyme soluble epoxide hydrolase, reduces atherosclerosis development, abdominal aortic aneurysm formation, dyslipidaemia, hypertension and diabetes in different mouse models. Using targeted lipidomics we find CYP450-dervied epoxy-oxylipins from arachidonic acid, linoleic acid, eicosapentaenoic acid and docosahexaenoic acid are produced by vascular and inflammatory cells and are regulated by inflammatory stimuli. These CYP-derived oxylipins regulate vascular cell inflammation, and inflammatory resolution, in particular by targeting migration and activation of cells of the monocyte linage. The contribution of omega-6 and in particular omega-3 derived CYP450 products to these anti-inflammatory processes will be discussed.

Omega-3 Index - why the omega-3 status matters

Clemens von Schacky
Omegametrix, Martinsried, and Preventive Cardiology, University of Munich, both GERMANY

The Omega-3 Index has been defined as the percentage of eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) in erythrocytes, as determined with a specific and highly standardized analytical method. The availability of this method has sparked the interest of many scientific groups and large companies, which so far resulted some 200 publications, and some 50 ongoing research projects all over the world. Results demonstrated that erythrocytes represent all other human cells so far investigated in EPA plus DHA, and thus the status of an individual in EPA plus DHA. A low Omega-3 Index has been demonstrated to be a novel biomarker for cardiovascular risk according to the criteria of the American Heart Association. In the design of large intervention trials with cardiovascular endpoints with EPA plus DHA, the Omega-3 Index was not included, and therefore issues like baseline levels and bioavailability were ignored, which explains their overall neutral results. However, other cardiac diseases, like congestive heart failure, are characterized by a low Omega-3 Index, and the pertinent trial with EPA plus DHA was positive. This raises the hopes for new trials in the cardiovascular field, based on the Omega-3 Index.
Brain structure and function also depend on EPA and DHA. A low Omega-3 Index has been demonstrated in issues of brain development or cognition and in diseases like attenttion-deficit hyperkinetic disorder or major depression. Meta-analyses of pertinent intervention trials clearly demonstrate positive effects of EPA plus DHA in these issues of brain health. Interestingly, in some trials, it has been demonstrated that EPA plus DHA decelerate “physiologic” ageing of brain and muscle, pointing to a deficit in EPA and DHA as an underlying cause. Such a deficit can be found in many Western countries, and diagnosed only with the Omega-3 Index.

Hall D:
Targeting coding and non-coding RNAs in pharmacological research

(Organized by Turkish Pharmacological Society)
Organizers and chairs:
Bahar Tunçtan (Mersin University Faculty of Pharmacy Department of Pharmacology)
09:00-12:00

09:00 Targeting miRNAs in the treatment of cardiovascular and renal diseases
Bahar Tunçtan
Mersin University, TURKEY

09:30 Targeting the store-operated calcium entry
Metiner Tosun
İzmir University of Economics, TURKEY

10:00 miRNAs as biomarkers for early diagnosis of certain diseases
Mehmet Sami Serin
Mersin University, TURKEY

10:30 Coffee break

11:00 Elevated talk (C009): The effect of S-nitrosoglutathione (GSNO) in a rat model of isoproterenol induced myocardial infarction
Deniz Kaleli Durman
İstanbul University, TURKEY
(Authors: Deniz Kaleli Durman, Uğur Aksu, Duygu Terzioğlu, Işık Ikbal Barış, Dilek Yılmaz Bayhan, Birsel Sönmez Uydeş Doğan)

11:15 Elevated talk (C010): Protective and therapeutic effects of doxycycline against renal ischemia-reperfusion injury in rats
Mustafa Sağır
Gaziosmanpasa University, TURKEY
(Authors: Mustafa Sağır, Hakan Parlakpinar, Fatih Oguz, Alaaddin Polat, Gul Pelin Odabasi)

11:30 Elevated talk (C011): PYK2 as a therapeutic target for myocardial infarction
Sofia Iris Bibli
National and Kapodistrian University of Athens, GREECE
(Authors: Sofia Iris Bibli, Zongmin Zhou, Sven Zukunft, Beate Fisslthaler, Ioanna Andreadou, Csaba Szabo, Peter Brouckaert, Ingrid Fleming, Andreas Papapetropoulos)

11:45 Elevated talk (C012): The effect of beta 3-ARs on Na+/K+-ATPase in cardiac hypertrophy
Gizem Kayki Mutlu
Ankara University, TURKEY
(Authors: Gizem Kayki Mutlu, Ebru Arioglu Inan, Irem Karaomerlioglu, Vecdi Melih Altan)

12:00 End of symposium

Targeting miRNAs in the treatment of cardiovascular and renal diseases

Bahar Tunçtan
Department of Pharmacology, Faculty of Pharmacy, Mersin University, Mersin, TURKEY

MicroRNAs (miRNAs) are a family of small noncoding RNA species that post-transcriptionally regulate gene expression by binding to their target messenger RNAs (mRNAs), leading to mRNA degradation, suppression of translation, or gene activation. miRNAs have been implicated in a variety of processes such as cell proliferation, differentiation, migration, invasion, immune response, inflammation, apoptosis, angiogenesis, and metastasis. In recent years, the diagnostic and prognostic value of circulating miRNAs as biomarkers have been proved for several diseases, including cardiovascular and renal diseases as well as different types of malignancies, diabetes mellitus, stroke, infectious diseases, and pregnancy. Since miRNAs are up- or down-regulated in several disease states, the miRNA-based drug is required to reduce or enhance the miRNA expression for the particular therapeutic purpose. Antagomirs (also called anti-miRs or blockmirs), one of a novel class of chemically engineered oligonucleotides, have been used to silence miRNA expression by preventing other molecules from binding to a desired site on a mRNA molecule. Conversely, agomirs (also called miRNA mimics) are small, chemically modified double-stranded RNAs that mimic endogenous miRNAs and enable miRNA functional analysis by up-regulation of miRNA activity. The present lecture is intended to highlight the rationale for the use of antagomirs or agomirs for preventing the consequences of ischemic or inflammatory cardiovascular and renal diseases.

Targeting the store-operated calcium entry

Metiner Tosun

Dept. of Pharmacology, Faculty of Medicine, Izmir University of Economics, 35330 Izmir, TURKEY

Receptor- and store-operated Ca^{2+} (SOC) channels play important roles in the regulation of intracellular Ca^{2+} homeostasis in vasculature. Proteins coded by canonical subfamily of the transient receptor potential (TRPC) genes are suggested to participate in both channel types. Among the family, TRPC1 and TRPC6 are the most predominant members expressed in vascular smooth muscle cells (VSMCs). Altered expression pattern of TRPC genes appear to have significant functional consequences in blood vessels. Due to our early observation regarding a conditional coupling of SOC entry to contraction in rat aorta, we further monitored changes in TRPCs' expression in longitudinal aging model to find out a possible association with age related changes in vascular responses. Upon observing TRPC1 downregulation and TRPC6 upregulation with aging, we silenced TRPC1 in A7r5 embryonic rat VSMCs to see if there is a cross-talk. Data showed the existence of a reciprocal expression pattern between the two and unexpected elevation in SOC. Based on these observations in rats, we investigated whether this interaction is also operational in man. For this purpose, TRPC1 silencing and TRPC1 overexpression (OE) vector-transfected primary human aortic smooth muscle cells (HASMCs) cells were used in expressional and functional analyses. Transcriptome analysis of TRPC1-OE cells showed at least 1.5-fold-change in 155 transcripts involved in different signaling pathways. Furthermore, at least twenty candidate miRNAs were detected for HASMCs *via* next generation sequencing. In fura-2-loaded and TRPC1-knocked-down cells, SOC was also elevated two fold. Real time cellular analysis showed antiproliferative and proliferative effects of TRPC1-silencing and overexpression, respectively. In summary TRPC1 might be a regulatory channel subunit of SOC in VSMCs as we also observed in Huh7 hepatocellular carcinoma and A7r5 rat aortic cell lines. Supported by The Scientific and Technological Research Council of Turkey (TUBITAK, 103S176, 104S568, 108S072 and 110S096 to MT).

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miRNAs as biomarkers for early diagnosis of certain diseases

Mehmet Sami Serin
Mersin University Faculty of Pharmacy, Department of Pharmaceutical Microbiology, TURKEY

MicroRNAs (miRNAs) are a class of short non-coding RNA molecules. They have attracted significant attention from biomedical research communities over the past two decades. Facilitated by high-throughput genomics and bioinformatics in conjunction with traditional molecular biology techniques and animal models, miRNA research is now positioned to make the transition from laboratories to clinics to deliver profound benefits to public health.
MicroRNAs (miRNAs) are small, however important regulators of post-transcriptional gene expression that have been linked to various cellular processes. Alterations of miRNAs are associated with a number of disease pathologies. With over 5000 miRNAs discovered in humans to date, many of them have already been implicated in common human disorders such as cancer, viral diseases, immune-related diseases, Neurodegenerative diseases. miRNAs have important potential to becoming the next generation of diagnostics and therapeutics.

Biomarker potential of several miRNAs for the early diagnosis of hepatocellular carcinoma related with HBV and HCV infections will be focused in this presentation.

Elevated talk (C009): The effect of S-nitrosoglutathione (GSNO) in a rat model of isoproterenol induced myocardial infarction

Deniz Kaleli Durman[1], Uğur Aksu[2], Duygu Terzioğlu[3], Işık Ikbal Barış[4], Dilek Yılmazbayhan[4], Birsel Sönmez Uydeş Doğan[1]

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S-nitrosothiols (RSNOs) are thought to represent a circulating endogenous reservoir of NO, and may have potential as donors of NO. Although NO is clearly established as a mediator of cardioprotection, there are few studies that have examined the role of RSNOs in cardioprotection. In this study, we aimed to investigate the effect of S-nitrosoglutathione (GSNO) as an NO donor in a rat model of myocardial infarction.

Myocardial infarction in rats was induced by subcutaneous injection of isoproterenol (ISO, 85 mg/kg, s.c.) on 1st and 2nd days with an interval of 24h. Male wistar rats (300-350g) were divided into four groups (n=8); 1) Control, 2) ISO (85mg/kg, sc), 3) ISO (85mg/kg,sc) + GSNO (5mg/kg,i.p.), 4) GSNO (5mg/kg,i.p.). At the end of the administration period, electrocardiogram (ECG) changes were monitored in rats. Moreover, plasma levels of; Troponin-I for the assessment of myocardial infarction; IL-1beta for determining cytokine profiling and also the histopathological examination of the heart tissues was performed. Additionally, glycocalyx integrity was evaluated by measuring the plasma hyaluronan levels.

Plasma levels of Troponin-I, IL-1beta and hyaluronan levels were significantly increased by ISO administration. Whereas, GSNO-administrated group displayed lower plasma levels of these reagents similar to that of control values. However, with the administration of GSNO in ISO treated group; plasma troponin levels tended towards decrease although no significant difference was evident, IL-1beta levels were significantly decreased ($p<0.05$) while, hyaluronon levels were found unchanged compared to corresponding values in ISO group. GSNO administration did not produce any histological improvement in comparison to ISO group.

Although GSNO administration partly restored the cell compartment but not the glycocalyx, it seems to have a protective effect on myocardial infarction. A better understanding of the mechanism regulating the cardioprotective effect of NO and the possible role of GSNO in cardioprotection may provide new therapeutic opportunities and targets for cardiovascular diseases

Keywords: S-nitrosoglutathione, GSNO, myocardial infarction, isoproterenol

Elevated talk (C010): Protective and therapeutic effects of doxycycline against renal ischemia-reperfusion injury in rats

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Ischemia-reperfusion (I/R) injury to native kidneys results from pathological processes that cause organ hypo-perfusion such as trauma, dehydration and sepsis. I/R injury is a leading cause of acute kidney injury and describes a major clinical problem with high combined levels of morbidity and mortality. This study was designed to investigate the protective and therapeutic effects of doxycycline (DOX), a member of the tetracycline family of antibiotics with antioxidant properties, on renal damage induced by renal I/R in rats.

Thirty-two Wistar albino rats were randomly distributed into four groups: (1) sham group, in which the rats only underwent right nephrectomy (n=8); (2) right nephrectomy and left renal ischemia (1h) and reperfusion (24h) group (I/R) (n=8); (3) right nephrectomy and 10 mg/kg DOX i.p. before left renal ischemia (1h) and reperfusion (24h) group (DOX+I/R) (n=8); (4) after right nephrectomy, left renal ischemia (1h) and 10 mg/kg DOX i.p. before reperfusion (24h) group (I/R+DOX) (n=8). At the end of the study, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), total oxidative status (TOS), total antioxidant capacity (TAC), were assayed in the kidney tissue. Also, blood levels of urea nitrogen (BUN) and creatinine (Cr) were determined.

In the I/R group, tissue MDA and TOS levels were found to be significantly higher, whereas SOD, CAT and GPX levels were lower when compared to the sham group. Also in this group BUN and Cr levels were found as significantly elevated. DOX treatment significant decrease in the MDA production and TOS levels when compared to the I/R group (Table 1).

According to our results, it is possible to say that apart from its antibiotic effects, DOX provides protective and therapeutic effects on I/R-induced renal injury in rats by inhibiting oxidative stresss and lipid peroxidation.

Keywords: doxycycline, renal ischemia-reperfusion, oxidative stress, antioxidant, rat

Table 1. Oxidative and antioxidative status of the kidney and blood levels of urea nitrogen (BUN) and creatinine (Cr).

Groups	MDA nmol/g tissue	TOS mmol H2O2 equivalent /g protein	TAC mmol Trolox equivalent /L	SOD U/mg protein	CAT K/g protein	GPX U/mg protein	BUN (mg/dL) Mean ±SD	Cr (mg/dL) Mean ±SD
1. Sham	21.38± 6.01	7.11± 1.29	0.94± 0.09	0.83± 0.06	30.31± 4.38	106.35± 11.74	26.75± 3.69	0.62± 0.04
2. I/R	32.92± 6.80*	13.94± 5.28*	0.84± 0.11	0.70± 0.09*	19.52± 3.51*	76.60± 5.89*	152.50± 26.87*	2.36± 0.65*
3. DOX+I/R	21.21± 6.34**	9.24± 2.27**	0.89± 0.06	0.66± 0.04*	23.12± 3.46*	78.41± 5.91*	155.87± 24.92*	2.49± 0.56*
4. I/R+DOX	24.5± 7.42**	7.25± 1.51**	0.86± 0.068	0.68± 0.06*	24.52± 5.21*	76.55± 6.52*	154.25± 28.28*	2.55± 0.86*

**p<0.05 vs Sham; **p<0.05 vs I/R. Results are presented as mean±SD.*

DOX: Doxycycline; MDA: Malondialdehyde; TOS: Total oxidative stresss; TAC: Total antioxidative capacity; SOD: Superoxide dismutase; CAT: Catalase; GPX: Glutathione peroxidase; BUN: Blood urea nitrogen; Cr: Creatinine.

Elevated talk (C011): PYK2 as a therapeutic target for myocardial infarction

Sofia Iris Bibli[1], Zongmin Zhou[1], Sven Zukunft[2], Beate Fisslthaler[2], Ioanna Andreadou[1], Csaba Szabo[3], Peter Brouckaert[4], Ingrid Fleming[2], Andreas Papapetropoulos[1]
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Although endothelial nitric oxide (NO) synthase (eNOS) is known to play a protective role against reperfusion injury, the molecular mechanisms regulating eNOS activity during ischemia/reperfusion (I/R) injury are only partially understood. Herein, we investigated the effect of PYK2 kinase, a negative regulator of eNOS activity, in myocardial injury. Oxidative stress injury (H2O2) in differentiated H9c2 cardiomyocytes increased phosphorylation of PYK2 on its activator site and promoted eNOS phosphorylation on Y657 and S1177. Phosphorylation of eNOS on Y657 was the result of a direct PYK2 action, while for S1176 is was mediated by the PI3K/Akt cascade. Activity assays demonstrated that Y657 exerts a dominant effect on eNOS activity, limiting enzyme activity when both Y657 and S1177 were phosphorylated. Cell viability assays revealed increased cell survival under either oxidative stresss or oxygen-glucose deprivation/recovery injury upon PYK2 inhibition; this effect was reversed by inhibiting NO production. In vivo ischemia-reperfusion activated PYK2 during early reperfusion, leading to eNOS phosphorylation on Y656 and reduced NO output, as judged by the low tissue cGMP levels. Pharmacological blockade of PYK2 (PF-431396; 5μg/g iv prior to ischemia) alleviated eNOS inhibition and prevented cardiac damage following I/R in wild-type (17.9%±1.8% PYK2-inhibited group vs 45.4%±3.9% control group), but not in eNOS KO mice (59.3%±2.0% PYK2 inhibited group vs 56.2%±3.7% control group). Our studies identify PYK2 as a pivotal regulator of eNOS function in myocardial infarction and propose this kinase as a novel therapeutic target for cardioprotection.

Keywords: nitric oxide, PYK2, cardioprotection

Elevated talk (C012): The effect of beta 3-ARs on Na+/K+-ATPase in cardiac hypertrophy

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Beta 3 adrenoreceptors (β_3-ARs), which mediate negative inotropic effects, are known to be upregulated in cardiac pathologies associated with sympathetic overactivation and their effects become more prominent. β_3-ARs are shown to stimulate cardiac Na^+/K^+ pump in healthy heart. However, their effects on the pump in the presence of a cardiac pathology are still not known. For this purpose, we aimed to investigate the effects of β_3-ARs on Na^+-K+ pump in hypertrophy related to sympathetic overstimulation.

Rats were divided into three groups: control (sham), noradrenaline-treated, noradrenaline+BRL treated groups. Rats received noradrenaline (4 mg/kg/day) and BRL 37344 (10 mg/kg/day) for 14 days. Cardiac function of these rats was investigated by the method of left ventricle pressure-volume analysis. Basal hemodynamic parameters and load-independent functional indexes were calculated. After isolating heart plasma membrane exctracts, enzymatic Na^+/K^+-ATPase activity was assessed. Finally, protein expression levels were measured in whole heart lysates.

Systolic function parameters ESP and dPmax, diastolic function parameter EDP were found to be increased and dPmin to be decreased in noradrenaline-treated rats with higher heart weight/body weight ratio. Heart rate was also increased in these rats. Moreover, load-independent contractility indexes PRSW, Ees and stiffness were increased in hypertrophic rats. On the other hand, BRL 37344 improved these parameters. Na^+/K^+ pump activity was also increased in hypertrophic rats. Na^+/K^+ pump activity returned to control values with BRL 37344 treatment. Moreover, ANP, which is an hypertrophy marker, was increased with hypertrophy and decreased with β_3-AR stimulation. In hypertrophic rats, an increase was observed in β_3-AR expression which was decreased by BRL treatment.

Our study which shows spesific in vivo β_3-AR agonism has an antihypertrophic effect via the stimulation of Na^+/K^+ pump may be a promising therapeutic option for hypertrophy and provides better understanding of the pathophysiology of hypertrophy.

Keywords: hypertrophy, Na+/K+ ATPase, beta 3-AR, noradrenaline, pressure-volume analysis

Oral Presentation Sessions (13:30-15:00)

Oral Presentation Session 1, Hall A

Neuropharmacology / Psychopharmacology; Obesity and Metabolic Diseases(C037-C042)

Chairs: Charis Liapi (National and Kapodistrian University of Athens, Greece)
Ahmet Ulugöl (Trakya University, Turkey)

13:30 C037: Unilateral AAV-mediated alpha-synuclein overexpression model of Parkinson disease's to study motor and cognitive dysfunction
Banu Cahide Tel, Sevgi Uğur Mutluay, Elif Çınar, Gül Yalçın Çakmaklı, Esen Saka, Ayşe Ulusoy, Bülent Elibol

13:45 C038: Continuity of antipsychotic prescription in a homeless cohort: results of a randomized controlled trial
Stefanie Nadya Rezansoff, Julian Myles Somers, Akm Moniruzzaman

14:00 C039: The effect of acute stress and the role of mineralocorticoid and glucocorticoid receptors present in the prefrontal cortex in the memory extinction learning
Jessica Rosa, Daniela Lescano Martins Uliana, Leandro Antero Da Silva, Leonardo Barbosa Moraes Resstel

14:15 C040: Effect of a functional food on the PLIR (peroxidation of leukocytes index ratio) during post-prandial stress in healthy subjects
Husseen Manafikhi, Ilaria Peluso, Raffaella Reggi, Yaroslava Longhitano, Christian Zanza, Maura Palmery

14:30 C041: Cardiac remodeling alterations in choline-deprived rats: An overview
Charis Liapi, Athina Strilakou, Ahmed Al Humadi

14:45 C042: Expression of trpv1 receptors increased in hippocampus following pentylenetetrazole induced kindling in male rats
Ali Shamsizadeh, Farangis Fatehi, Iman Fatemi, Gholamhossein Hassanshahi, Mohammad Kazemi Arababadi

C037: Unilateral AAV-mediated alpha-synuclein overexpression model of Parkinson disease's to study motor and cognitive dysfunction

Banu Cahide Tel[1], Sevgi Uğur Mutluay[1], Elif Çınar[1], Gül Yalçın Çakmaklı[2], Esen Saka[3], Ayşe Ulusoy[4], Bülent Elibol[3]
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In this study, we aimed to establish loss of motor and cognitive functions with AAV-mediated alpha-synuclein (a-syn) overexpression in dentate gyrus (DG) and substantia nigra (SN) unilaterally in an animal model of Parkinson's disease (PD). We investigated behavioral and histopatalogical changes which occur due to excessive a-syn aggregation.

Female Sprague-Dawley rats (200-250g) were injected with AAV-carrying either a-syn (n=12 DG, n=12 SN, n=12 SN+DG), green fluorescent protein (GFP; n=12 DG, n=11 SN, n=12 SN+DG) or saline (n=4 DG, n=4 SN, n=6 SN+DG) stereotactically. Further 14 animals used as naïve controls. The animals were tested between 15-17th weeks with cylinder test for motor asymmetry, apomorphine-induced open field test for locomotor activity, novel object recognition test for memory; Morris's water maze for spatial learning; elevated plus maze for anxiety and sucrose preference for anhedonia. A-syn and GFP expression levels and synaptophysin for synaptic loss were evaluated by western blot analysis.

We showed a-syn and GFP protein expression in all injection sites by western blotting. In hippocampus, synaptophysin levels decreased in all a-syn groups compare to control but only in DG-a-syn group reached statistical significance ($p<0,001$). In cylinder test, SN+DG a-syn group used less contralateral forelimb compare to control ($p<0.05$). In apomorphine-induced open field test, SN-a-syn group had higher numbers of contralateral turns compare to control ($p<0.05$), and increased the locomotor activity, DG-a-syn group spent less time with novel object compare to control ($p<0,001$). SN-a-syn group spent less time in open arm ($p<0.05$) and consumed less sucrose ($p<0,001$) compare to SN-GFP.

A-syn overexpression in SN+DG group caused motor asymmetry and motor impairment, in SN groups caused anhedonia and anxiety, in DG groups caused memory impairment due to synaptic loss. This model may help to investigate cognitive and motor dysfunctions in PD.

This study is supported by Hacettepe University Scientific Research Projects Coordination Unit (ID:701).

Keywords: a-synuclein, adeno-asociated viral vector (AAV), hippocampus, substantia nigra, Parkinson's disease

C038: Continuity of antipsychotic prescription in a homeless cohort: Results of a randomized controlled trial

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Adherence to antipsychotic medication is very low among homeless mentally ill individuals, and is significantly related to duration of homelessness. This is the first experimental study to investigate the impact of supported housing on adherence to antipsychotics among homeless people with schizophrenia. We hypothesized that supported housing would lead to a significantly higher level of antipsychotic adherence compared with usual care.

Comprehensive prescription drug data were examined among participants diagnosed with schizophrenia. Participants were randomized to one of three conditions: Treatment As Usual (reference); Scattered Housing; and Congregate Housing. Adherence was operationalized using the medication possession ratio (MPR). One-way analysis of variance and post-hoc pairwise comparisons were used to estimate the effect of each intervention.

165 participants met inclusion criteria. MPR pre-randomization ranged from 0.44-0.48. Following randomization (2.6 years), overall MPR among study participants increased to 0.64, with a standard deviation (SD) of 0.32. Individuals randomized to Scattered Housing had an MPR of 0.78 (SD=0.21). Congregate and Treatment As Usual resulted in ratios of 0.61 (SD=0.32) and 0.55 (SD=0.37), respectively. ANOVA demonstrated that MPR was significantly different between study arms (P<0.001), and post-hoc comparisons showed that Scattered Housing resulted in a significant intervention effect (relative difference: 0.24; 95% Confidence Interval (CI): 0.10-0.37; adjusted P<0.001). MPR was higher in Congregate Housing compared to Treatment As Usual, but the difference was not statistically significant (relative difference: 0.06; 95% CI:-0.10-0.21; adjusted P=0.643).

This study is the first randomized controlled trial demonstrating the benefits of supported housing on antipsychotic medication adherence among people experiencing homelessness and schizophrenia. Results indicate that guideline-level adherence (defined as MPR ≥0.80) is achievable with this sub-population. Further implementation of supported housing is strongly indicated, particularly in the Scattered format.

Trial registration: ISRCTN57595077

Keywords: antipsychotic, medication adherence, medication possession ratio, homeless, housing

C039: The effect of acute stress and the role of mineralocorticoid and glucocorticoid receptors present in the prefrontal cortex in the memory extinction learning

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It is proposed that the exposure to stress before a trauma can be a risk factor for the development of psychopathologies, such as posttraumatic stress disorder (PTSD) also, the extinction of aversive memories appears to be impaired in this disorder. The ventral portion of medial prefrontal cortex (vmPFC), which comprise the prelimbic (PL) and infralimbic (IL) regions, is involved in the modulation of neuroendocrine and behavior responses in stressful situations. Additionally, there is a high expression of glucocorticoid receptors in this area. The aim of the current study is to investigate the effect of acute stress, along with the role of mineralocorticoid and glucocorticoid receptors present in the vmPFC in the memory extinction learning. Male Wistar rats will be submitted to stereotaxic surgery for the bilateral implantation of guide cannulae in the PL or IL regions of the vmPFC for drug administration. After surgery recovery, they will be divided in two groups: 1) Stressed group (restraint stress for 1 hour); 2) Control group (without restraint stress). After 7 days, both groups will be exposed to contextual fear conditioning protocol, followed by extinction and extinction retention sessions (each session is 24h apart from the previous). The mineralocorticoid receptor antagonist (RU 23318), or glucocorticoid receptor antagonist (RU 486) or vehicle will be microinjected into the PL or IL immediately after the extinction session. The animals which were submitted to restraint stress appears an increase of the expression of aversive memory, and have a deficit in the extinction of conditioned fear memory. Mineralocorticoid and glucocorticoid receptors modulate the extinction of aversive memories, also that acute stress produces deficit in the conditioned fear extinction learning due to alteration in the expression of these receptors into the vmPFC, and morphological modifications in this brain structure.

This study is supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

Keywords: extinction of aversive memory, acute stress, PTSD, prefrontal cortex, glucocorticoid and mineralocorticoid receptors

C040: Effect of a functional food on the PLIR (peroxidation of leukocytes index ratio) during post-prandial stress in healthy subjects

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The Peroxidation of Leukocytes Index Ratio (PLIR) measures both the resistance of leukocytes to an exogenous oxidative stresss and their functional capacity of the oxidative burst in response to activation. Postprandial dysmetabolism has been linked to atherosclerosis and inflammation; therefore, fatty meal consumption represents a model of acute inflammatory response and has been applied to study the effect of antioxidant beverages. A pilot study aimed to study the relationship between the PLIR and the Improvement of Postprandial Metabolic Stress by a Functional Food.

We investigated the relationship between postprandial dysmetabolism and the PLIR. Following a blind, placebo controlled, randomized, crossover design, ten healthy subjects ingested, in two different occasions, a high fat and high carbohydrates meal with Snello cookie (HFHCM-S) or with control cookies (HFHCMC). Snello cookie, a functional food covered by dark chocolate and containing glucomannan, inulin, fructooligosaccharides, and Bacillus coagulans strain GanedenBC30.

The functional food Snello cookie significantly improved postprandial metabolic stress (insulin, glucose, and triglycerides) and reduced the postprandial increase of uric acid. HFHCM-S improved PLIR of lymphocytes, but not of monocytes and granulocytes. Both meals reduced the lipoperoxidation (RATIO of fluorescence), on granulocytes, induced by both exogenous free radicals (AAPH) and reactive oxygen species (ROS) produced by oxidative burst (PMA).

The relationship between PLIR and postprandial dysmetabolism requires further investigations.

Keywords: functional food, postprandial metabolic stress, oxidative stresss, peroxidation of leukocytes index ratio

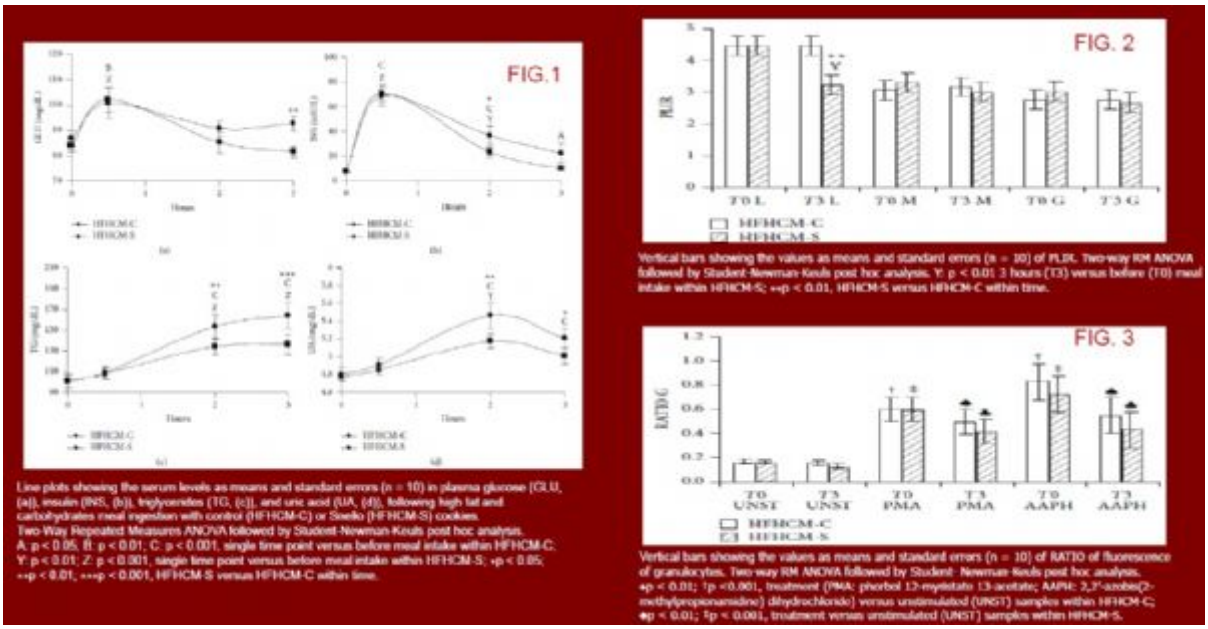

Figure 1. Line plots showing the serum levels as means and standard errors (n = 10) in plasma glucose (GLU, (a)), insulin (INS, (b)), triglycerides (TG, (c)), and uric acid (UA, (d)), following high fat and carbohydrates meal ingestion with control (HFHCM-C) or Snello (HFHCM-S) cookies. Two-Way Repeated Measures ANOVA followed by Student-Newman-Keuls post hoc analysis. A: $p<0.05$; B: $p<0.01$; C: $p<0.001$, single time point vs before meal intake within HFHCM-C; Y: $p<0.01$; Z: $p<0.001$, single time point vs before meal intake within HFHCM-S; $*p<0.05$; $**p<0.01$; $***p<0.001$, HFHCM-S vs HFHCM-C within time.

C041: Cardiac remodeling alterations in choline-deprived rats: An overview

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Choline is considered an essential nutrient and its deficiency resembles the metabolic syndrome phenotype that is usually associated with prediabetes and diabetes, obesity, dyslipidemia and fatty liver deposit. Choline dietary deprivation disrupts the normal function of vital body organs, among which cardiac impairment has gained increased interest. In the management of cardiac diseases carnitine, a structurally relevant to choline compound, has been used as adjunct. The aim of the study was to investigate the dietary choline deprivation impact on: a) myocardial performance and heart histology, b) the activity of myocardial AChE (cholinergic marker), Na^+/K^+-ATPase, and Mg^{2+}-ATPase, c) the role of specific metalloproteinases and d) define possible modifications after carnitine administration.

Adult Male Wistar Albino rats were fed with standard or choline deficient diet supplemented with or without carnitine in drinking water 0.15% w/v for four weeks. We assessed cardiac function, serum markers (homocysteine and BNP levels), performed histopathology analyses, echocardiography and determined the enzyme activities in the myocardium homogenate.

In the choline deficient group a compromised myocardium contractility was noted with an increase in serum BNP concentration, which were attenuated by carnitine. Homocysteine presented contradictory results. Heart histopathology revealed a lymphocytic infiltration along with inhibition of MMP-2 and increase of TIMP-2 immunohistochemical expression. The combination of dietary choline deprivation and carnitine supplementation increased the myocardial Na^+/K^+-ATPase activity with a concomitant decrease in the activities of Mg^{2+}-ATPase and AChE.

Choline deficiency impairs heart performance and carnitine exerts a cardio-protective role against these changes by modulating cholinergic myocardial neurotransmission and ATPase activity in favor of cardiac work efficiency. The observed pattern of TIMP and MMP modulation appears to promote fibrosis, but carnitine does not seem to act beneficially through this mechanism

Keywords: choline, heart, enzymes, matrix, carnitine

C042: Expression of trpv1 receptors increased in hippocampus following pentylenetetrazole induced kindling in male rats

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The exact pathogenesis of epilepsy is not clear well. Recent studies showed the possible involvement of TRPV1 receptors in this disease. In this study we measured the expression of TRPV1 receptors in hippocampus following pentylenetetrazole induced kindling in male rats. 14 male Wistar rats were allocated in two experimental groups. The kindling group received a subconvulsive dose of pentylenetetrazole (PTZ) (35 mg/kg, i.p.) every 48h for 15 sessions while the control rats were injected with an equal dose of saline. At the end of injections, rats were decapitated, and the brains were removed. TRPV1 receptor expression in hippocampus region was assessed by real-time PCR method. This study demonstrated that expressions of TRPV1 receptors were increased in hippocampus following PTZ induced kindling in male rats. As the expression of TRPV1 receptors increased in hippocampus of epileptic rats, the TRPV1 receptors may be a good candidate for epilepsy treatment.

Keywords: kindling, pentylenetetrazole, hippocampus, TRPV1 receptors

Oral Presentation Session 2, Hall B

Cardiovascular Pharmacology (C043-C048)

Chairs: María Jesus Sanz (University of Valencia, Valencia, Spain)
Buket Reel (Ege University, Turkey)

13:30 C043: Physiologically relevant concentrations of hydrogen sulfide negatively regulate platelet aggregation in vitro and in vivo
Michael Emerson, Francesca Rauzi, Erica Smyth, Milos Filipovic, Mark E Wood, Matthew Whiteman

13:45 C044: Abdominal aortic aneurysm (AAA) formation and mononuclear cell adhesion induced by angiotensin II are partly dependent on CXCR6/CXCL16 axis
Aida Collado, Patrice Marques, Cristina Rius, Elena Domingo, Paula Escudero, Laura Piqueras, María Jesus Sanz

14:00 C045: The nitric oxide mediated effects of nebivolol in cardiorenal syndrome
Guldem Mercanoglu, Caglar Macit, Semen Yesil, Burak Pamukcu, Nurhas Safran, Hafize Uzun, Ayfer Yalcin, Fehmi Mercanoglu

14:15 C046: Cardioprotective effect of cyclosporin A against doxorubicin cardiotoxicity
Meryem Şeyda Kaya, Meral Erdinç, Ilker Kelle, Hasan Akkoç, Emre Uyar, Zeynep Erdoğmuş Özgen, Levent Erdinç

14:30 C047: Doxycycline improves the impaired contractile responses induced by oxidative stresss in human saphenous vein grafts
Mazen Saeed, Buket Reel, Mehmet Zuhuri Arun, Mehmet Guzeloglu, Goksel Gokce, Bekir Ugur Ergur, Ceren Korkmaz

14:45 C048: Cardioprotective effects of pharmacologically decreased long-chain acylcarnitine contents in experimental models of myocardial infarction, atherosclerosis, and diabetes
Maija Dambrova, Kristine Volska, Marina Makrecka Kuka, Elina Makarova, Janis Kuka, Reinis Vilskersts, Edgars Liepinsh

C043: Physiologically relevant concentrations of hydrogen sulfide negatively regulate platelet aggregation in vitro and in vivo

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The generation of hydrogen sulfide (H_2S) by platelets is undefined. Studies with non-physiological mM concentrations of sulfide salts which release a short burst of H_2S suggest inhibitory roles. We aimed to determine whether and by which enzymes platelets generate H_2S. Secondly, we determined the impact of slow, consistent exposure to H_2S at physiologically relevant concentrations upon platelet function and investigated the role of endogenous H_2S in regulating platelets in vivo.

Expression and enzymatic activity of the two principle H_2S-generating enzymes cystathione-γ-lyase (CSE) and cystathionine-β-synthase (CBS) were assessed by western blotting, thialysine and Bindschelder's assays. H_2S generation was measured via the H2S-specific probe WSP-1 and endogenous S-sulfhydration measured by a tag-switch assay in human platelet lysates. In vitro and in vivo platelet aggregation were assessed by light transmission aggregometry of isolated human platelets and in a mouse model of platelet thromboembolism.

Robust expression of CBS but not CSE was detected in human platelets. Platelets also contained CBS but not CSE catalytic activity. H_2S generation by resting platelets could be detected and was significantly reduced upon pharmacological inhibition of CBS. Platelets contained S-sulfhydrated proteins. Exposure of platelets to the slow release donor GYY4137 at concentrations that release H_2S in the nM range led to significant inhibition of aggregation and AP67 which releases H_2S at a greater rate had proportionally higher potency. Pharmacological inhibition of endogenous H_2S generation in mice significantly increased collagen-induced aggregation in vivo.

Platelets generate H_2S catalytically from CBS which is associated with S-sulfhydration of as yet unidentified proteins suggesting novel H_2S-mediated signalling events. Slow consistent exposure to H_2S inhibits platelet aggregation in the nM range through mechanisms that remain to be identified. Finally, endogenous H_2S is a negative systemic regulator of platelet aggregation and may be hypothesised to exert anti-thrombotic activity in vivo.

Keywords: platelet, cardiovascular, thrombosis, pharmacology

C044: Abdominal aortic aneurysm (AAA) formation and mononuclear cell adhesion induced by angiotensin II are partly dependent on CXCR6/CXCL16 axis

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Abdominal aortic aneurysm (AAA) is a degenerative disease of the aorta that mainly affects elderly population over the age of 65. Nowadays the pathways involved in its onset and progression remain unknown and angiotensin-II (Ang-II) has been widely implicated. Therefore, the potential link between CXCR6/CXCL16 axis in AAA was investigated. Apolipoprotein E-deficient mice (apoE-/-) were subjected or not to a high-fat diet and infused with Ang-II (500 ng/kg/min) for 28 days. Some of the animals were daily treated with losartan at 10 or 30 mg/kg/day. Parallel-plate flow chamber assay was employed to evaluate leukocyte adhesion to Ang-II (1 µM)-stimulated human endothelium. Mice subjected to a high-fat diet and infused with Ang-II showed higher incidence of AAA, increased macrophage, CD3+ lymphocyte and CXCR6+ cell infiltration and enhanced neovascularization than unchallenged animals. These effects were accompanied by increased MCP-1/CCL2, CXCL16, CXCR6 and VEGF mRNA expression within the lesion. These events were reduced when losartan was administered at 30 but not at 10 mg/kg/day. When human umbilical vein or artery endothelial cells (HUVEC and HUAEC, respectively) were stimulated with 1 µM Ang-II (24h), a significant increase in CXCL16 expression was detected by flow cytometry and immunofluorescence. However, neutralization of CXCL16 activity only significantly inhibited Ang-II-induced mononuclear leukocyte-HUAEC interaction by 49% without affecting their interaction with HUVEC. These results suggest that the CXCR6/CXCL16 axis could constitute a new therapeutic strategy in the treatment of cardiovascular diseases associated with activation of the renin-angiotensin system (RAS).

Keywords: mononuclear cell, abdominal aortic aneurysm, chemokines, angiotensin II

C045: The nitric oxide mediated effects of nebivolol in cardiorenal syndrome

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Heart Failure (HF) is the clinical expression of impaired cardiac performance resulting from the complicated interaction between impaired cardiac performance and abnormalities in related physiologic systems particularly the kidneys. This complex heart-kidney relation was recently redefined as cardiorenal syndrome (CRS). The pathophysiology of CRS is complex and involves many alterations such as hemodynamic alterations, endothelial dysfunction and oxidative stresss among which endothelial dysfunction is the promoter. In this study we aimed to evaluate the renal protective effects of nebivolol after MI, based on the NO mediated mechanisms.

Male Sprague Dawley rats were divided into 4 groups of 12 each: Sham operated control (sham-control); MI induced control (MI-control); MI induced and immediate intravenous load followed by oral nebivolol treated group (MI-neb1) and MI induced and delayed nebivolol treated group (MI-neb2). MI was induced by 30 min ligation of the left anterior descending coronary artery (LAD). Hemodynamic parameters, LV and kidney functions, together with tissue oxidative/nitrosative damage and antioxidant capacity were assessed both in early (2nd day of MI) and late period (28th day of MI) of MI. NO mediated mechanisms were investigated immuno- histochemically by NOS labeling (both three isoform of NOS).

Compared to MI-control, structural, physiological and hemodynamic functions of LV (LVEDd, LVEDP, EF) were prevented in both MI-neb groups ($p<0.05$). Moreover, oxidative (characterized by decreased MDA and increased SOD levels) and nitrosative (characterized by decreased ONOO- levels) damage were limited in these groups ($p<0.05$). Compared to MI-control, MI-neb groups were characterized with decreased eNOS, iNOS and prevented nNOS labeling. No difference was observed in NOS labeling between MI-neb groups.

NOS mediated mechanisms of nebivolol can be summarized as: prevention of iNOS mediated inflammation and maintenance of physiological regulation of renal hemodynamics and sodium homeostasis by prevention of deterioration in nNOS.

Keywords: nebivolol, nitric oxide, heart failure, cardiorenal syndrome

Table 1. NOS labelling intensities

	Sham-control		MI-control		MI-neb1		MI-neb2	
	Day 2	Day 28	Day 2	Day 28	Day 2	Day 28	Day 2	Day 28
eNOS	2+	2+	2+	3+	±	2+	±	2+
iNOS	2+	2+	3+	3+	2+	1+	2+	1+
nNOS	3+	3+	2+	1+	3+	3+	3+	3+

3+: strong, 2+: moderate 1+: weak immunolabelling eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; nNOS, neuronal nitric oxide synthase

C046: Cardioprotective effect of cyclosporin A against doxorubicin cardiotoxicity

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Doxorubicin is a wide spectrum antibiotic used against various cancers. One of the most severe and significant side effects is cardiotoxicity that limits clinical usage of doxorubicin. The mechanism of Doxorubicin cardiotoxicity is not completely understood; however, it was shown that doxorubicin metabolites and reactive oxygen species (ROS) which activates necrotic signaling pathways have an important role in these cellular damage (1).Also p53 expression is declared to be a contributor to oxidative stresss related necrosis (2). In this study, investigation of cardioprotective effects of cyclosporine A against doxorubicin cardiotoxicity was aimed. Male Sprague-Dawley rats were grouped as; 1- control, 2-DOX group: Treated with a single dose doxorubicin (10 mg/kg i.p.), 3- DOX + CsA group-: Treated with a single dose doxorubicin (10 mg/kg i.p.) + cyclosporine A (3 mg/kg) for 7 days,4- CsA group:treated with cyclosporine A (3 mg/kg) for 7 days.In all groups, isolated hearts were perfused by Langendorff system. Heart coronary perfusion pressure (PP), left ventricular developed pressure (LVDP) and heart rate per minute (HR), LV(dP/dt)max and LV(dP/dt)min which shows systole and diastole rate were recorded.Moreover, isolated mRNA's from cardiac tissue samples were analyzed for p53 gene expression with Real Time PCR.

In Dox group versus to control group PP and LV (dP/dt) min values significantly increased ($p<0.05$); and, HR, LVDP and LV (dP/dt) max values significantly decreased ($p<0.001$). In Dox+CsA group versus to Dox group PP and LV (dP/dt) min values significantly decreased ($p<0.05$); and, HR, LVDP and LV (dP/dt) max values significantly increased ($p<0.001$). The p53 gene expression significantly reduced in Dox+CsA group versus to Dox group ($p<0.001$). It is concluded that CsA has protective effects against doxorubicin cardiotoxicity by conserving the cardiac functions.

1-Halestrap et al. (2009). Biochim. Biophys. Acta 1787, 1402–1415.
2-Vaseva et al. (2012). Cell 149(7). 1536–48.

Keywords: doxorubicin, cyclosporin A, cardiotoxicity, p53 expression

C047: Doxycycline improves the impaired contractile responses induced by oxidative stresss in human saphenous vein grafts

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Increased levels of reactive oxygen species (ROS) are a considerable clinical problem in cardiovascular surgery. Doxycycline at sub-antimicrobial dose acts as a potent matrix metalloproteinase inhibitor and also ROS scavenger. In this study, we aimed to investigate the hypothesis that impaired vascular reactivity caused by hydrogen peroxide (H_2O_2)-induced oxidative stresss in human saphenous vein (HSV) segments may be normalized by doxycycline.

HSV segments were collected from patients who undergoing coronary artery bypass grafting operation. After gently removing endothelial monolayer by mechanical scratching, the segments were divided into 4 groups and incubated with 10 µM H_2O_2 and/or 10 µM doxycycline for 16 hours. Untreated HSV segments served as control. In order to evalute the changes in vascular reactivity, concentration-response curves to noradrenaline, potassium chloride, serotonin and papaverine were performed in vein rings which are confirmed not having functional endothelium. In parallel experiments, ROS levels in the vein rings were assessed by using lucigenin- and luminol-enhanced chemiluminescence method.

Incubation of HSVs with H_2O_2 caused 5-fold and 4-fold increments in ROS and superoxide levels, respectively. Doxycycline significantly reduced the increased ROS and superoxide anion levels. Contractile responses to noradrenaline, potassium chloride and serotonin significantly decreased in HSV segments from H_2O_2 group. Treatment with doxycycline augmented the impaired contractile responses to noradrenaline and potassium chloride but not to serotonin. Sensitivity to these contractile agents remained unchanged. Maximum relaxation responses and sensitivity to papaverine were not affected neither by H_2O_2 nor doxycycline.

In our study, doxycycline attenuated H_2O_2-induced oxidative stresss and improved the impaired vascular contractile responses in HSV rings. These findings suggest that pre-treatment with doxycycline may normalize the hypocontractility of HSV grafts. Doxycycline may be useful in the prevention of ROS accumulation caused by ischemia during operation and maintenance of long term functional performance of HSV grafts.

Keywords: human saphenous vein, doxycycline, matrix metalloproteinase, oxidative stresss, hydrogen peroxide

C048: Cardioprotective effects of pharmacologically decreased long-chain acylcarnitine contents in experimental models of myocardial infarction, atherosclerosis, and diabetes

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Long-chain acylcarnitines are harmful fatty acid intermediates that accumulate in ischemic mitochondria and induce detrimental effects in energy metabolism pathways. We hypothesised that decreasing the acylcarnitine content via cardioprotective drugs may represent a novel metabolic treatment strategy. To lower long-chain acylcarnitine contents we used a novel compound, 4-

[ethyl(dimethyl)ammonio]butanoate (methyl-GBB), which effectively inhibits synthesis of L-carnitine by gamma-butyrobetaine dioxygenase (IC50 3 µM) and transport and uptake of L-carnitine by organic cation transporter 2 (IC50 3 µM).

The methyl-GBB treatment induced a substantial decrease in tissue and plasma acylcarnitine concentrations in both fed and fasted states of animals in all experimental models in mice and rats. As a result, in methyl-GBB-treated isolated rat hearts, the uptake and oxidation rates of labeled palmitate were significantly decreased by 40%, while glucose oxidation was significantly increased 2-fold. Methyl-GBB at doses of 5 mg/kg and 20 mg/kg decreased the infarct size by 45-48%. In vivo pretreatment with methyl-GBB attenuated the infarct size by 45% and significantly improved 24 h survival of rats. In apolipoprotein E knockout mice, methyl-GBB treatment at a dose of 10 mg/kg reduced the size of atherosclerotic plaques by 36%. Both in db/db and high fat diet fed C57BL/6 mice methyl-GBB administration (5mg/kg) improved insulin sensitivity and reduced blood glucose levels.

Methyl-GBB treatment decreases the acylcarnitine contents and leads to cardioprotective effects by limiting long-chain fatty acid oxidation and facilitating glucose metabolism. The pharmacologically reduced long-chain acylcarnitine content represents an effective strategy to improve insulin sensitivity and to protect the heart against ischemia–reperfusion-induced damage and development of atherosclerosis.

This study is supported by the Latvian State Research Program BIOMEDICINE. M. Dambrova received travel grant from the FP7 project InnovaBalt.

Keywords: long-chain acylcarnitine, methyl-GBB, atherosclerosis, infarct, diabetes

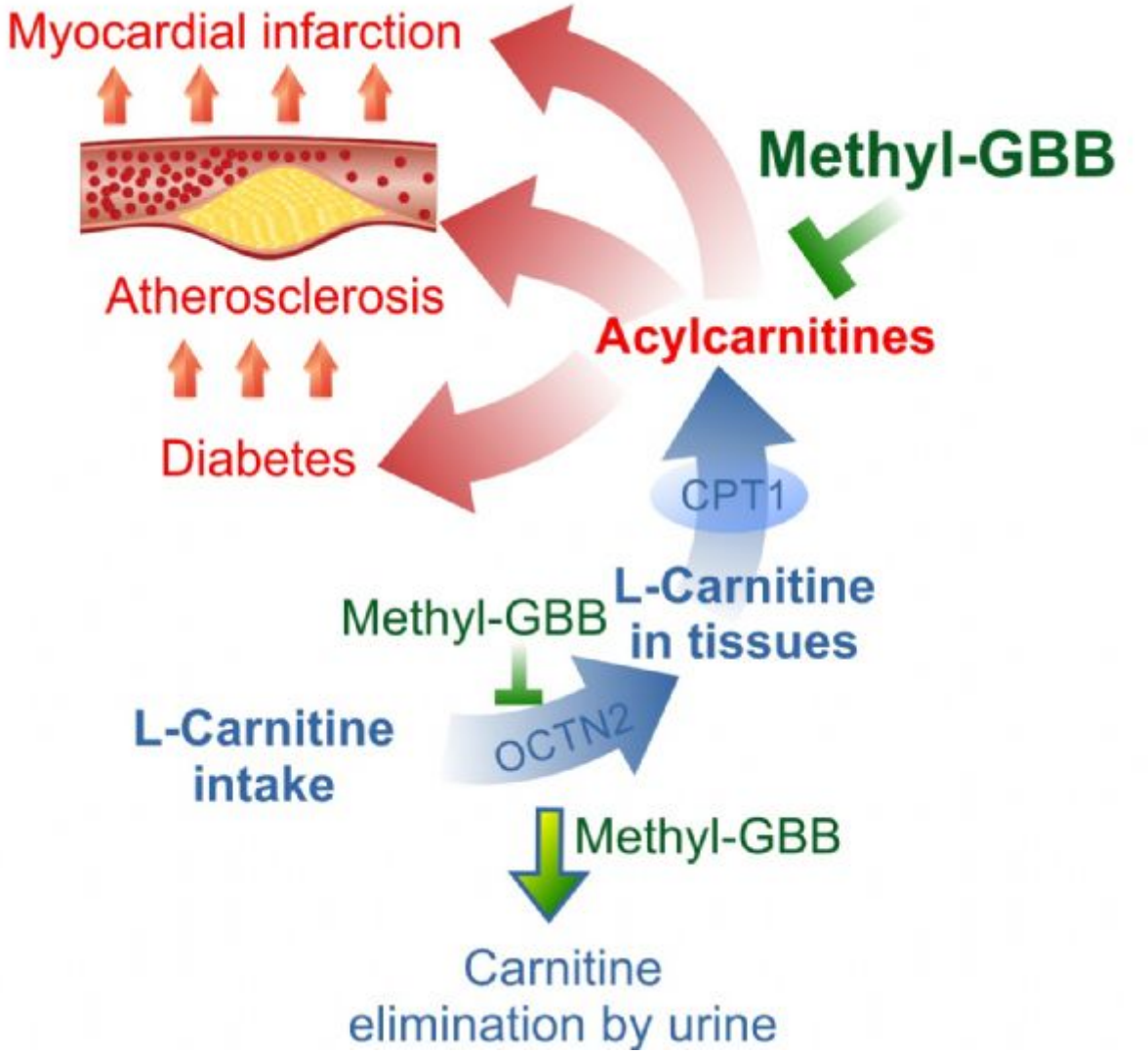

Figure 1. Cardioprotective effects of pharmacologically decreased long-chain acylcarnitine contents

Oral Presentation Session 3, Hall C

Clinical Pharmacology (C049-C054)

Chairs: Ondrej Slanar (Charles University, Czech Republic)
Bekir Faruk Erden (Kocaeli University, Turkey)

13:30 C049: Two phase 1 clinical studies evaluating the effects of the CYP3A4 inhibitor clarithromycin and the CYP3A4 inducer rifampin on systemic exposure to pacritinib in healthy volunteers
Suliman Al Fayoumi, Sherri Amberg, Huafeng Zhou, Lindsey Millard, Jack W. Singer, Mary Campbell

13:45 C050: Pattern of use of incretin-based medicines in a large sample of the Italian general population
Giuseppe Roberto, Francesco Barone Adesi, Francesco Giorgianni, Valeria Pizzimenti, Carmen Ferrajolo, Francesco Lapi, Paolo Francesconi, Gianluca Trifirò, Elisabetta Poluzzi, Fabio Baccetti, Rosa Gini

14:00 C051: ABCB1 and OPRM1 polymorphisms alter maternal efficacy and neonatal safety of remifentanil in women undergoing cesarean section
Hana Bakhouche, Pavlina Noskova, Svatopluk Svetlik, Jan Blaha, Ondrej Slanar

14:15 C052: Evaluation of positive reflections of activities encouraging awareness regarding rational use of medicines
Arzu Kiroglu, Volkan Aydin, Ahmet Akici

14:30 C053: Homozygous familial hypercholestrolemia patient with heterozygous genotype of MTTP gene (rs11944752) variation does not response to lomitapide therapy
Meral Kayıkcıoglu, Aslı Tetik Vardarli, Zuhal Eroglu

14:45 C054: Investigation of antibiotic utilization for dental infections
Cenker Zeki Koyuncuoglu, Mehtap Aydin, Ipek Neriman Kirmizi, Mesil Aksoy, Fatma Isli, Ahmet Akici

C049: Two phase 1 clinical studies evaluating the effects of the CYP3A4 inhibitor clarithromycin and the CYP3A4 inducer rifampin on systemic exposure to pacritinib in healthy volunteers

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Pacritinib is an oral kinase inhibitor with specificity for JAK2, FLT3, CSF1R, and IRAK1 currently being investigated in hematologic malignancies. Two phase 1 studies were conducted to evaluate effects of the CYP3A4 inhibitor clarithromycin and the CYP3A4 inducer rifampin on pacritinib exposure.

Subjects received 400 mg oral pacritinib on day 1 then oral clarithromycin (500 mg bid) or rifampin (600 mg qd) on days 8-12 or 8-17, respectively, with pacritinib co-administration on the final day. Each study evaluated effects of co-administration on pacritinib and primary metabolite M1 pharmacokinetics including maximum observed concentration (C_{max}), time to C_{max} (T_{max}), and area under the plasma concentration-time curve (AUC). Drug-drug interactions were assessed by examining 90% CIs for geometric mean ratios of pacritinib co-administered with clarithromycin or rifampin *vs* pacritinib alone.

Mean pacritinib plasma C_{max}, and AUC values were 1.3- and 1.8-fold higher, respectively, with similar mean $t_{1/2}$ when co-administered with clarithromycin *vs* pacritinib alone. With rifampin co-administration, mean pacritinib C_{max} and AUC values were 51% and 87% lower, respectively, with mean $t_{1/2}$ approximately 65% shorter for co-administration *vs* pacritinib alone. For M1 metabolite, mean C_{max} value was 65% lower, with longer median T_{max} for co-administration with clarithromycin vs. pacritinib alone (24 vs. 4 hours), while mean C_{max} value was 52% lower with similar T_{max} for co-administration with rifampin *vs* pacritinib alone. The most frequent adverse events observed with pacritinib when co-administered with clarithromycin or rifampin were diarrhea (35%; grade 3=10%) and nausea (5.9%; no grade >1), respectively. Only 1 grade >1 event was observed with pacritinib alone.

These results suggest dose adjustments may not be necessary for pacritinib when co-administered with CYP3A4 inhibitors due to limited increase in pacritinib exposure. Co-administration of pacritinib with strong CYP3A4 inducers should be avoided due to a marked decrease in exposure.

Keywords: myelofibrosis, pacritinib, phase 1, CYP3A4

C050: Pattern of use of incretin-based medicines in a large sample of the Italian general population

Giuseppe Roberto[1], Francesco Barone Adesi[2], Francesco Giorgianni[3], Valeria Pizzimenti[3], Carmen Ferrajolo[4], Francesco Lapi[5], Paolo Francesconi[1], Gianluca Trifirò[3], Elisabetta Poluzzi[6], Fabio Baccetti[7], Rosa Gini[1]

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The aim of this study was to describe the pattern of use of Incretin-based medicines (IBM) in a large sample of the Italian general population.

Administrative data from Tuscany region (Italy) were used. All prescriptions of antidiabetics (ATC A10*) dispensed for outpatient consumption and reimbursed by the National Health Service were analyzed. Active subjects aged ≥18 with at least one year of lookback were selected between 2008 and 2014. Annual trends of IBM incidence and prevalence of use were observed per pharmacological subgroups: Dipeptidyl peptidase-4 inhibitors (DPP4i) and glucagon-like peptide-1 analogues (GLP1a). Characteristics of new IBM users (age, sex, previous antidiabetic therapies) were also described per year of initiation.

On a total population of almost 3.3 millions adults, 31,750 patients received ≥1 prescription of IBM between 2008 and 2014. During the study period, incidence of use of IBM increased from 0.4‰ to 1.4‰, with a peak in 2011 (2.3‰). The highest incidence of use was observed in 2013 for DDP4i (2.1‰) and in 2011 for GLP1a (0.5‰). Prevalence of use increased from 0.2‰ to 1‰ for GLP1a and from 0.2‰ to 5.8‰ for DPP4i. Similar prevalence trends was observed among antidiabetic users GLP1a:0.4-1.6%; DPP4i:0.4-9.3%). Among IBM new users, the percentage of women decreased from 50.9% in 2008 to 43.5% in 2014 while the portion of those aged ≥65 years increased from 30% to 61.1%. Patients on hypoglycaemic monotherapy during the year preceding the first IBM prescription also increased from 23.4% to 32.9%.

IBM use in Tuscany rose steeply during the first half of the observation period and stabilized in the second. DPP4i have rapidly become the drugs of choice for the majority of new IBM users. Moreover, IBM therapy is increasingly started in elderly patients and as first add-on hypoglycaemic treatment.

Keywords: dipeptidyl peptidase-4 inhibitors, glucagon-like peptide-1 analogues, drug utilization

C051: ABCB1 and OPRM1 polymorphisms alter maternal efficacy and neonatal safety of remifentanil in women undergoing cesarean section

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The aim of our study was to evaluate possible effect of ABCB1, and OPRM1 polymorphisms on the efficacy and safety of remifentanil in women undergoing elective cesarean section under general anesthesia. Women received remifentanil (1 ug/kg i.v.) 30 s prior to the induction to standardized general anesthesia. The ABCB1 (rs2032582, rs1045642) and OPRM1 (rs1799971) polymorphisms were analyzed from maternal peripheral blood. The basal hemodynamic and demographic parameters in the study population (n=54) were similar in all the subgroups. The median +/- SD increase of systolic blood pressure at 5 min from the baseline was practically completely abolished in homozygous carriers of ABCB1 variants in comparison with wild-type subjects -2.67+/-25.0 vs. 16.57+/-15.7 mm Hg, $p<0.05$ for rs2032582, and 2.00+/-23.9 vs. 22.13+/-16.8 mm Hg, $p<0.05$, for rs1045642, respectively. While, no neonate belonging to ABCB1 wild-type homozygous or OPRM1 variant carrying mothers needed any resuscitative measure, 10.5 % of the neonates belonging to OPRM1 wild-type homozygous mothers received resuscitative support similarly as 11.1 %, and 12.5 % of neonates of mothers carrying variants of rs2032582, and rs1045642, respectively. Decreased stabilizing effects of remifentanil on maternal hemodynamics has been observed in ABCB1 wild type mothers, while the adaptation of the neonates was clinically worse in OPRM1 wild type, and ABCB1 variant allele carriers.

This study is supported by Charles University Project No: PRVOUK P25/LF1/2.

Keywords: pharmacogenetics, sympathetic stress response, opioids

C052: Evaluation of positive reflections of activities encouraging awareness regarding rational use of medicines

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Keeping unnecessary drugs at home is a situation showing both causes and consequences of irrational use of medicine (IUM). This study aimed at evaluation of drug utilization behaviors of employees of a call-center company.

An online survey was answered by 1121 employees of Global Information company, participating from eight provinces of Turkey in 2016. The survey consisted of questions assessing participants' drug handling and storage behaviors.

Participants who declared that "they did not keep drugs which they do not use/consider to use" were 31.0%, while those keeping such drugs and those failing to remember what they did were 41.0% and 28.0%, respectively. The survey item questioning handling of unused drugs showed a balanced distribution among participants such that 32.1% declared "they gave these drugs to medical unit of the company", 34.0% to "pharmacy, family health center, hospital, etc.", and 33.9% to dispose such drugs to "waste-bin, washbasin, closet, etc." Nearly half of participants (46.5%) stated a behavioral change in this manner in recent years. While 45.9% of participants declared that "they did not keep expired drugs", the rest either had such drugs or failed to remember whether they had or not. Participants who claimed a change in their "self-medication" behaviors recently constituted 39.6%, of which 79.1% stated that they gave up purchasing drugs without prescription.

Although our findings showed that substantial amount of participants still had unused drugs at home and disposed them inappropriately, it is understood that they started to exhibit more favorable changes in their behaviors in recent years, compared to the results of a previous survey performed by the same company. This may be attributed to the internal campaign within the company, encompassing rational management of drug handling, storage, and disposal. These type model activities should be encouraged to resolve IUM problems.

Keywords: rational use of medicine, drug handling, drug storage, drug disposal, self-medication

C053: Homozygous familial hypercholestrolemia patient with heterozygous genotype of MTTP gene (rs11944752) variation does not response to lomitapide therapy

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Homozygous Familial hypercholestrolemia (HoFH) is an autosomal dominant inherited metabolic disorder, characterized with abnormal LDL-cholesterol levels leading to premature atherosclerosis. Early diagnosis and effective treatments have a crucial role in controlling of FH development. Microsomal triglyceride transfer protein (MTTP) plays a central role in lipoprotein metabolism. MTTP is responsible for assembling and secretion of very low density lipoproteins. Lomitapide is an inhibitor of the MTTP. Use of Lomitapide approved by the FDA whereas In Turkey, approved by the ministry of health, has just been used for 'compassionate use' only 2 HoFH patient. Lomitapide therapy is one of the new treatment options in HoFH patients for minimizing morbidity and mortality by ensuring effective lipid reduction therefor side-effect/response to drug profiles and long-term clinical results of Lomitapide treatment have not known yet exactly. We aimed to investigate the effects of variations in MTTP gene region on drug response with HoFH patients during Lomitapide treatment.

Our study group includes 1 man and 1 woman homozygous HoFH cases treated with Lomitapide who does not respond to drug theraphy and response to drug theraphy, respectively. DNA isolation was performed from peripheral blood samples. Next generation Sequence analysis of the MTTP gene were carried out on Ion-torrent PGM instrument.

Our results showed that case with heterozygous genotype of the MTTP rs11944752 gene variation does not respond to the Lomitapide treatment.

This is the first study to show the candidate gene variation which effects the response to the Lomitapide treatment in HoFH patients. This result will guide possibility identification of HoFH patients responsive to MTTP inhibitors such as Lomitapide before initation of the therapy.

Keywords: pharmacogenetic, lomitapide, MTTP, next generation sequencing

C054: Investigation of antibiotic utilization for dental infections

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Antibiotics are prescribed by dentists for treatment or prevention to bacterial infections. However, these agents can be used irrationally in non-indicated clinical cases such as gingivitis, dry socket, and pulpitis. The knowledge of dental antibiotic prescribing pattern is limited in Turkey. The aim of this study was to investigate the antibiotic utilization prescribed by dentists for dental infections in Turkey.

The systemic antibacterial drugs contained data that prescribed by the dentists from Turkish Medicines and Medical Devices Agency's Prescription Information System (PIS) were investigated retrospectively from 1 January 2013 to 31 August 2015. Single diagnosed and antibiotic included prescriptions (n=10.442.962) were analyzed. Indications were classified according to the "International Statistical Classification of Diseases and Related Health Problems" (ICD)-10. Since this study involves extensive coverage of collected data, a part of study findings that related indication centered dental prescription data has been presented in this abstract.

Results: A total of 10.535.625 antibiotics were prescribed (the number of antibiotics per prescription was 1.01). The "dental caries" were the most frequently used indications for antibiotic prescribing (34.7%) and followed by "diseases of pulp and periapical tissues" (32.2%) and "dental examination" (Z01.2),(18.3%) within the most ten common reasons/indications in prescriptions. In all of these indications, amoxicillin-clavulanic acid (J01CR02) was the most prescribed antibiotic. This antibiotic (J01CR02) was written by dentists in 59.5% of dental caries" and in 59.3% of "periapical abscess".

In general, the antibiotic used indications in dentistry are expected as limited as possible. However, this study showed that dentists preferred broad-spectrum antibiotics and they used these drugs excessively in inappropriate indications and in some undiagnosed cases. These results reflect irrational prescribing performance of dentists that need urgently core activities.

Keywords: dental infections, antibiotic, irrational drug use

Oral Presentation Session 4, Hall D

Drug Safety and Toxicology (C055-C060)

Chairs: Michael Emerson (Imperial College London,UK)
Dimitrios Kouvelas (Aristotle University of Thessaloniki, Greece)

13:30 C060: The knowledge and attitudes of nursing students towards rational drug use
Yasemin Özatik, Ulken Tunga Babaoglu, Ayse Ozkaraman, Semra Yigitaslan, Kevser Erol

13:45 C056: Study enhancer drugs among future physicians in Maribor, Slovenia
Ziga Volgemut, Jan Schmidt, Katja Jerenec, Polonca Ferk

14:00 C057: Evaluation of renal function in patients using metformin
Neda Taner, Emine Karatas Kocberber, Cengizhan Ceylan, Barkın Berk

14:15 C058: Urinary N-acetylated cysteine-disulfides as indicators of kidney disease progression in HIV-infected patients
Clara Gonçalves Dias, Nuno Ramos Coelho, Lucília Neves Diogo, Ana Rita Lemos, Judit Morello, Emília Carreira Monteiro, Karina Soto, Sofia Azeredo Pereira

14:30 C059: How much signal do we get from spontaneous vs. solicited adverse drug reaction (ADR) reports? Defining an ADR-signal index
Gulnihal Ozcan, Emel Aykac, Nigar Demet Aydinkarahaliloglu

14:45 C055: Assessment of the cardiovascular risk profile of antiretroviral therapies independent of HIV infection
Erica Smyth, Brian Gazzard, Mark Nelson, Michael Emerson

C055: Assessment of the cardiovascular risk profile of antiretroviral therapies independent of HIV infection

Erica Smyth[1], Brian Gazzard[2], Mark Nelson[2], Michael Emerson[1]
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Abacavir sulphate (ABC) and tenofovir disoproxil fumarate (TDF) are alternative components of antiretroviral therapy (ART) that effectively treat HIV. Observational and clinical studies suggest that ABC may be associated with increased risk of myocardial infarction (MI). It is not clear whether increased cardiovascular risk is driven pharmacologically by ART or pathophysiologically by HIV. Since MI is platelet-driven, our hypothesis was that ABC increases cardiovascular risk via pharmacological modulation of platelet aggregation.

The direct effect of ART on platelets was determined by assessing aggregation of isolated platelets from HIV negative volunteers in the presence of approximate Cmax concentrations of metabolites of ABC or TDF. The ability of ABC and TDF to interrupt inhibition of platelet aggregation nitric oxide (NO) was compared. Platelet thromboembolism was assessed in the circulation of animals treated with ABC or TDF allowing for assessment of the effects of ART in the presence of endogenous NO.

Tenofovir (TFV, active metabolite of TDF) significantly inhibited platelet aggregation in vitro, however, no effect was detected with carbovir triphosphate (CTP, active metabolite of ABC). CTP blocked NO-mediated inhibition of platelet aggregation. In contrast, no significant effect was observed for TFV. Administration of ABC to mice significantly enhanced platelet thromboembolism 30 mins after treatment. The effect of ABC in mice dissipated within 4 hours indicating a reversible effect. No effect was observed following treatment of mice with TDF at any time point.

The increased cardiovascular risk associated with ABC in patients may be mediated by reversible pharmacological modulation of platelet and endothelial function rather than by HIV infection or differences in confounding factors between patient groups. In contrast, TDF exerts effects upon platelets that would not be expected to increase the incidence of platelet-driven events such as MI.

Keywords: platelet, cardiovascular, HIV, antiretroviral therapy

C056: Study enhancer drugs among future physicians in Maribor, Slovenia

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To improve study performance, various pharmaceutical products have been used and abused among future physicians worldwide. There is scarce information on study enhancing drug abuse among medical students in Slovenia. Our aim was to investigate the abuse of prescription drugs for improving academic results among students at the Faculty of Medicine, Maribor, Slovenia.

Online anonymous self-administered questionnaire was developed. Questions were related to age, gender, year of study, basic demographic information as well as to consumption of common prescription drugs, such as methylphenidate, benzodiazepines (diazepam, alprazolam, etizolam), amphetamines, modafinil, racetams, ephedrine, sertraline, bupropion. Students were invited to participate in completing the questionnaire through email and the Faculty website. Data was anonymously collected and stored on servers of an independent survey provider. Results were evaluated descriptively.

288 out of 645 (45%) students responded. A representative (similar to the actual gender ratio at the faculty) proportion of male and female students responded (31% vs. 69%). Mean age of participating students was (22.0±2.2) years. A higher proportion of male (18% vs. 8% female) students reported consumption of listed drugs. 7.5% of students used prescription drugs to increase study performance. Of the respondents (214) that answered the questions on drugs, 50% (16/32) of students consuming drugs reported taking them with the intention of increasing study performance. We also observed a higher proportion of abuse with students of final study years compared to students in their first years of study. Drugs most commonly used for this purpose were diazepam (2.8%), ephedrine (1.9%) and alprazolam (1.9%). Cognitive enhancers were less commonly used: piracetam (1%), modafinil (0.5%), methylphenidate (0.5%), amphetamines (0.5%).

Abuse of study performance enhancing drugs was confirmed among participating medical students. Further detailed investigations are needed to improve education of future physicians and public health awareness on academic doping.

Keywords: academic doping, study enhancers, drug abuse, medical students

C057: Evaluation of renal function in patients using metformin

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Metformin's the most important side effect is developing lactic acidosis. Metformin is contraindicated if serum creatinine levels are more than 1.5 mg/dL in men and 1.4 mg / dL in women. A retrospective randomized study was conducted in 66 adult patients who were hospitalized in a University hospital. Renal function of patients receiving metformin at the time they receive inpatient treatment will be assessed by serum creatinine levels. GFR calculated by the Cockcroft - Gault formula to calculate the patient were assessed according to the stage of renal failure.

The study uses a randomized 13 different departments as inpatient treatments and 65 patients using metformin. During the treatment, serum creatinine level of 15% patients couldn't be monitored. administration of drugs that were contraindicated for 3 patients who hospitalized at cardiology department with their high serum creatinine , was continued. Patients who were enrolled for the study and received treatment in infectious disease, serum creatinine level was not viewed. There is a significant correlation ($p \leq 0.01$) between the departments in monitoring of serum creatinine. Renal function of the patients who were taken for this study are 1, 2, 3, 4 stages and in patients with end-stage renal failure rates of 26, 30, 26, 3, and 0% respectively. The renal function was unknown in 15% of the patients.
Assessment of renal function and adjustment of drug doses accordingly and termination some of these treatments because of renal failure is important to prevent medical errors. Serum creatinine level monitorization is essential for whom used metformin . However in some departments, it wasn't monitorized.

Keywords: metformin, renal function, lactic acidosis

C058: Urinary N-acetylated cysteine-disulfides as indicators of kidney disease progression in HIV-infected patients

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N-acetyltransferase 8 (NAT8) has emerged as a candidate for renal regulation and nephrotoxic response. NAT8 catalyzes the last step of mercapturic acid formation, i.e. the N-acetylation of cysteine S-conjugates, enabling the urinary excretion of these conjugates. We aimed to determine NAT8 urinary products, namely N-acetyl-cysteine (uNAC), as surrogate of N-acetylated cysteine-disulfide conjugates, and coenzyme A (uCoA) and their relation with kidney function. We used a bedside-to-bench approach analyzing a population of human immunodeficiency virus (HIV)-infected patients, a condition where chronic kidney disease (CKD) is a high prevalent comorbidity.

A cross-sectional analysis was performed in a HIV+-population, followed by a prospective analysis in a smaller group. The study was approved by the hospital's ethics committee. Informed consent was obtained. uNAC and uCoA and serum cysteine (sCYS) were quantified by HPLC with fluorescence detection. Both sCYS/uNAC ratio and the fractional excretion of phosphorous (FePI) were calculated. Glomerular filtration rate (eGFR) was estimated by CKD-EPI equation. Data are presented as median [IQR] or as coefficient of variation (%).

A total of 200 HIV-infected patients were included (65% men, 75% non-Black, 51

[44-59] years old). uNAC level was 57

[39-83] µM (CV 54%); sCYS level was 226

[195-253] µM (CV 19%); sCYS/uNAC was 4.0

[2.6-6.1] (CV 63%). The uNAC levels were positively related with eGFR (Spearman r=0.298, p<0.001) and negatively correlated with FePI (Spearman r=-0.236, p<0.001). These correlations were strengthened when the ratio sCYS/uNAC was related to eGFR (Spearman r=-0.375, p<0.001) and FePI (Spearman r=0.297, p<0.001). Preliminary data of a prospective analysis show that uNAC decreases alongside with eGFR, highlighting these findings.

uNAC disulfides levels decrease along with progressive kidney loss of function. These data suggest uNAC as a non-invasive indicator of kidney disease progression and might reflect a role for NAT8 in the pathogenesis of CKD in HIV-infection.

This study is sponsorsed by EXPL/DTP-FTO/1792/2013;PD/BD/105892/2014(CGD).

Keywords: chronic kidney disease, disulfides, human immunodeficiency virus infection, N-acetyltransferase 8, urinary N-acetyl-cysteine

C059: How much signal do we get from spontaneous vs. solicited adverse drug reaction (ADR) reports? Defining an ADR-signal index

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Spontaneous reports from healthcare professionals and solicited reports from organized data collection programs are the two major sources of information for monitoring ADRs. Both of these reporting systems have their own superior and inferior properties. In this study, we aim to compare signal detection capabilities and clustering properties of spontaneous and solicited ADR reports.

We analyzed spontaneous and solicited ADR reports that reach to Turkish Pharmacovigilance Center between 2010 and 2013 related to the drugs adalimumab, etanercept and infliximab. We determined the drug-ADR signals seperately in spontaneous and solicited report datasets for each drug. We counted the signals that are detected with both frequentist methods (Reporting Odds Ratio and Proportional ADR Reporting Ratio) and an empirical-bayesian model (Multi-item Gamma Poisson Shrinker). Then, using graph theory we built a model for each dataset and calculated the clustering coefficients. Finally we formulated an "ADR-signal index (ADR-SI)" as an arbitrary measure of the signal detection capability per a unit of cluster.

We observed that ADR-SI's of all datasets were similar, except for etanercept-spontaneous and infliximab-solicited datasets. For etanercept-spontaneous dataset ADR-SI was zero owing to the absence of any signal. Clustering coefficient was also very low in this dataset. ADR-SI for infliximab-solicited dataset was higher compared to other datasets. Number of signals per number of reports was not different in this dataset compared to that of others. However, clustering was lower.

This study suggests that signal detection capabilities of spontaneous and solicited ADR reports may differ on the drug basis. We believe that the factors that could explain the low and high signal detecting capability respectively in etanercept-spontaneous and infliximab-solicited reports may guide future pharmacovigilance actions on effective data collection. We are planning to investigate these underlying factors with further studies.

Keywords: adverse drug reaction, spontaneous reports, solicited reports, ADR-drug signals, ADR-signal index

C060: The knowledge and attitudes of nursing students towards rational drug use

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Because nurses are responsible from the administration of drugs, they play an important role in rational drug use (RDU). Undoubtedly, the education of nurses should begin in the school in order to ensure that they will implement RDU correctly in their work life. Therefore, it will be beneficial to determine the knowledge level of nursing students about this topic.

This study was carried out in order to determine the knowledge and attitudes of nursing students from two different universities in Central Anatolia, Turkey.

This descriptive study was carried out on 1590 nursing students studying at the Nursing Faculty of two universities in Central Anatolia. A total of 961 (60.44%) nursing students filled out the questionnaire form used in this study. The questionnaire form was prepared according to the literature data. Descriptive data were expressed as percentage and mean.

Of the students, 68.80% were studying at the University A, 30.90% were at Class III and 14.00% were graduated from Health Vocational School. The students participating in the study reported that they have well knowledge about the administration form of the drug but have moderate knowledge about the indication, duration of effect, side effects, contraindications, drug interactions, warning, precautions and special situations about the drug use. Of the students, 79.4% have reported that they have reported side effects for the drug with 57.9% are reporting that they have reported the side effects to a nurse. It was determined that 80.50% of the nursing students do not or inadequately get education about RDU at the school.

Results of the present study suggest that nursing students have moderate knowledge about RDU and get inadequate education about RDU at school.

This study is financially supported by Scientific Research Projects Unit of Ahi Evran University,

Project No: TIP.A3.16.001.

Keywords: rational drug use, nurse, education

Oral Presentation Session 5, Hall E

Neuropharmacology / Psychopharmacology (C061-C064)

This session is cancelled.

14:00 C061: Evaluation of worldwide use of idarucizumab in a clinical practice setting: rationale and design of the RE-VECTO surveillance programme
Joanne Van Ryn, Joanne Lee, Fredrik Gruenenfelder, Martin Feuring, Kristina Zint, Nils Schoof, Deborah Reardon, Peter Zilles, Jörg Kreuzer
No registration, abstract will be deleted.

14:15 C062: Potassium channel-independent effects of pinacidil on the isolated human saphenous veins from diabetic patients
Jovana Rajkovic, Miodrag Peric, Radmila Novakovic, Dusko Nezic, Vladimir Djokic, Vladimir Zivanovic, Helmut Heinle, Ljiljana Gojkovic Bukarica
No registration, abstract will be deleted.

14:30 C063: 2beta-hydroxybetulinic acid 3beta-caprylate: an active principle from euryale ferox salisb. seeds with antidiabetic, antioxidant, pancreas & hepatoprotective potential in streptozotocin induced diabetic rats
Danish Ahmed, Vikas Kumar, Harish Kumar Bajaj, Manju Sharma
No registration, abstract will be deleted.

14:45 C064: Clinicopathologic features of methotrexate-induced epidermal necrosis
Shuen Iu Hung, Ting Jui Chen, Wen Hung Chung
No registration, abstract will be deleted.

C061: Evaluation of worldwide use of idarucizumab in a clinical practice setting: Rationale and design of the RE-VECTO surveillance programme

Joanne Van Ryn[1], Joanne Lee[2], Fredrik Gruenenfelder[1], Martin Feuring[1], Kristina Zint[3], Nils Schoof[3], Deborah Reardon[4], Peter Zilles[3], Jörg Kreuzer[1]
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Immediate and rapid reversal of the effects of an oral anticoagulant may be required in patients who have a life-threatening or uncontrolled bleeding event or who require emergency surgery/procedures. Idarucizumab, a humanized monoclonal antibody fragment, was developed as a specific reversal agent for the direct thrombin inhibitor, dabigatran, and is the first specific reversal agent for a non-vitamin K antagonist oral anticoagulant approved in the European Union and United States.

RE-VECTO is a global, drug administration surveillance programme designed to evaluate idarucizumab usage in a clinical practice setting.

This international multi-centre surveillance programme will be available to hospital pharmacies that dispense idarucizumab. Registration programme websites and electronic data capture will facilitate site enrolment. The target population is all adult patients treated with idarucizumab, excluding patients who have participated in dabigatran or idarucizumab clinical trials. The study size will be driven by idarucizumab usage and pharmacist participation. Anonymized information will be collected using electronic Drug Administration Forms.

Information on basic patient characteristics and use of idarucizumab will be entered into the idarucizumab drug administration surveillance web portal. Details of idarucizumab utilization will include department/patient setting, reason for idarucizumab use (e.g. uncontrolled bleeding, emergency surgery), type of surgery/procedure, information on bleeding event, and idarucizumab dosage/timing. All variables will be presented using descriptive statistics.

This global surveillance programme aims to capture and characterize a large proportion of patients treated with idarucizumab and assess idarucizumab usage patterns in clinical practice.

Keywords: idarucizumab, dabigatran, surveillance, bleeding, surgery

C062: Potassium channel-independent effects of pinacidil on the isolated human saphenous veins from diabetic patients

Jovana Rajkovic[1], Miodrag Peric[2], Radmila Novakovic[1], Dusko Nezic[2], Vladimir Djokic[1], Vladimir Zivanovic[3], Helmut Heinle[4], Ljiljana Gojkovic Bukarica[1]
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Pinacidil is well known potassium (K^+) channel opener which relaxes blood vessels trough opening the ATP-dependent K^+ (K_{ATP}) channels located in the plasma membrane of smooth muscle cells. However, numerous *in vitro* studies were reported K^+ channels-independent effects of pinacidil. Therefore, the objective of this study was to investigate K^+ channels-independent component of the pinacidil-induced vasorelaxation in the isolated human saphenous vein (HSV) obtained from the patients with type-2 *diabetes mellitus*.

Rings of HSV, without endothelium, obtained from the patients undergoing coronary bypass surgery, were mounted in an organ bath system and isometric tension was being recorded. The experiments followed a multiple curve design. Pinacidil was used for vasorelaxation of HSV precontracted with phenylephrine or solution with 80 mM K^+.

Glibenclamide, a selective inhibitor of K_{ATP} channels, antagonized significantly the effect of pinacidil on HSV in the presence of normal Krebs-Ringer solution ($P<0.05$). Also, the effect of pinacidil was antagonized significantly in the presence of 80 mM K^+ solution ($P<0.05$). In the presence of normal Krebs-Ringer solution, pretreatment with nickel, Na^+-Ca^{2+} exchanger inhibitor, or with nifedipine, inhibitor of voltage-gated Ca^{2+}channels, did not inhibited pinacidil effect ($P>0.05$, both). However, in the presence of 80 mM K^+, pretreatment with nickel or nifedipine or both at the same time, antagonized significantly the effect of pinacidil ($P<0.001$, all). Interestingly, there was no difference between the maximal vasorelaxation, produced by 0.1 mM of pinacidil in the absence and presence of inhibitors.

Pinacidil induced endothelium-independent vasorelaxation of HSV from diabetic patients. K_{ATP} channels are partly involved in its effect. In the K^+ channel-independent component of the pinacidil-induced vasorelaxation, the Na^+-Ca^{2+} exchanger and voltage-gated Ca^{2+} channels are partly involved. However, we have to investigate further the additional K^+ channel-independent mechanism(s) of pinacidil.

Keywords: pinacidil, human saphenous vein, type-2 *diabetes mellitus*, potassium channels

C063: 2beta-hydroxybetulinic acid 3beta-caprylate: an active principle from euryale ferox salisb. seeds with antidiabetic, antioxidant, pancreas & hepatoprotective potential in streptozotocin induced diabetic rats

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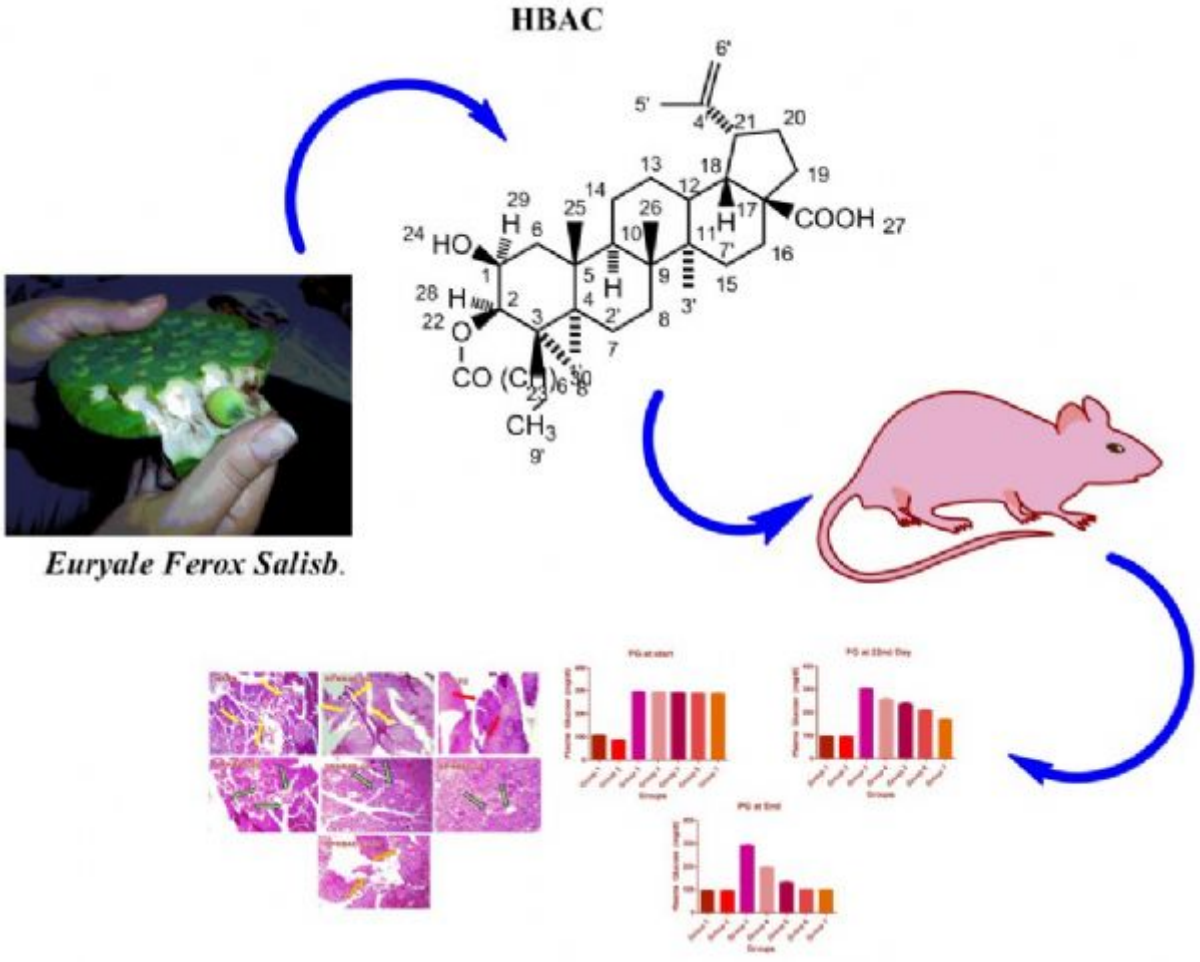
The aim of the present study was to evaluate the glycemic control, antioxidant, pancreas and liver protective effect of 2beta-hydroxybetulinic acid 3beta-caprylate (HBAC) from Euryale ferox Salisb. seeds on streptozotocin induced diabetic rats.

The active principle was isolated from Euryale ferox Salisb. seeds extract by utilizing chromatographic techniques. The rats were divided into seven experimental groups: Gp 1- normal; Gp2- normal + HBAC (60 mg/kg p.o.); Gp3- diabetic control; Gp 4- Diabetic + HBAC (20 mg/kg p.o.); Gp5- Diabetic + HBAC (40 mg/kg p.o.); Gp6- Diabetic + HBAC (60 mg/kg p.o.) and Gp 7- Diabetic + Glibenclamide (10mg/kg p.o.). Biochemical estimation, free radical scavenging examination and histopathological study was performed at the end of experimentation i.e. on 28th day.

The active principle isolated and identified with spectral data as 2beta-hydroxybetulinic acid 3beta-caprylate (HBAC). It was detected for the first time that HBAC has improvised the glycemic control in streptozotocin induced diabetic rats. Furthermore, it is remarkable to note that it exhibited excellent free radical scavenging property and pancreas and hepatoprotective property as well, supported by histopathological examination. One of the mechanisms of action of HBAC appears to be stimulating the release of insulin from pancreatic beta-cells.

HBAC improved the glycemic control, reduced the free radical activity along with corrected glycemic control, lipid profile, and enhanced level of insulin along with improvement in pancreas and hepatoprotective architecture. Considering the above results, HBAC shows potential to develop a medicine for diabetes as combinatorial or mono-therapy.

Keywords: diabetes, euryale ferox, streptozotocin, seeds



Graphical Abstract

C064: Clinicopathologic features of methotrexate-induced epidermal necrosis

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Methotrexate is widely used in the treatment of autoimmune diseases and cancer; however, it can cause a variety of side effects. Methotrexate-induced epidermal necrosis (MEN) is a rare but life-threatening adverse reaction. The clinical presentation of MEN mimics Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), all of which show extensive skin detachment and mucosal ulcers. The objectives of this study are to investigate the risks, prognostic factors, and clinicopathology of MEN.

A total of 24 patients with MEN and 150 tolerant controls were enrolled from Taiwan. The demographics, clinical manifestations, risks, drug dosage, and prognosis of MEN were analyzed. The expression of immune-related proteins and apoptosis molecules in the skin lesions and plasma of MEN patients was investigated and compared to those of SJS/TEN.

Four of 24 patients died from MEN, giving an overall mortality of 16.7%. Risk factors of MEN included old age, chronic renal insufficiency, hypoalbuminemia, and high starting dose. Patients with impaired renal function, increased plasma levels of methotrexate, and leucopenia showed poor prognosis. Immunohistochemistry revealed high levels of active caspase 3 but decreased anti-apoptosis signals, Bcl-2 and Bcl-xl, in the skin lesions of patients with MEN. By comparison, SJS/TEN-associated immune-biomarkers including IL-2, IFN-gamma and granulysin, had no association with MEN.

This is the largest study of methotrexate-induced epidermal necrosis. Risks of MEN included old age, chronic renal insufficiency, hypoalbuminemia, and high starting dose. In contrast to the immune features of SJS/TEN, MEN exhibited characteristics of drug toxicity related to the delayed clearance, increased active caspase 3 and down-regulated anti-apoptosis molecules. This study revealed that despite sharing similarity in clinical presentation, MEN has different clinicopathologic mechanism from SJS/TEN.

Keywords: methotrexate, epidermal necrosis, Stevens-Johnson syndrome, toxic epidermal necrolysis

Plenary Lecture Hall A (15:30-16:30)

miRNAs as potential therapeutic targets in cardiovascular disorders

Stefanie Dimmeler

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In recent year, increasing evidence suggests that non-coding RNAs play important roles in the regulation of tissue homeostasis and pathophysiological conditions. Besides small non-coding RNAs (e.g. microRNAs), >200-nucleotide long transcripts, namely long non-coding RNAs (lncRNAs), can interfere with gene expressions and signaling pathways at various stages. MicroRNAs have been shown to control cardiovascular homeostasis and disease and might be interesting targets for therapy. For example, inhibition of miR-92a promotes neovascularization and repair after myocardial infarction in mice and large animal models. Pre-clinical studies currently assess whether antimiRs directed against miR-92a may be used in patients with acute or chronic ischemic heart disease. In contrast to microRNAs, which are more close to clinical translation, the function of lncRNAs is hardly known. LncRNAs comprise a heterogeneous class of regulatory RNAs and we are currently addressing the regulation and function of intergenic lncRNAs as wells as circular RNAs, which result from back-splicing, and control endothelial cell functions. The presentation will provide insights into the regulatory RNA network and the translational perspective in the cardiovascular system.

Tuesday, 28th June 2016

Main Sessions (09:00-12:00)

Hall A:
How to improve translation from animals to humans

(Joint Section of EPHAR and EACPT)
Organizers and chairs:
Gérard A. Rongen (Radboud University, The NETHERLANDS)
09:00-12:00

09:00 Innovation in cardiovascular pharmacotherapy: Innovation stuck in translation
Gérard A. Rongen
Radboud University, The NETHERLANDS

09:30 Preclinical models in cardiovascular research: its predictive value in human drug development. Limitations and opportunities
Mercè Roqué
Hospital Clínic de Barcelona, SPAIN

10:00 Elevated talk (C013): Effects of niacin and fatty acid dietary supplementation on adipose tissue metabolic and inflammatory responses in a mouse model of metabolic syndrome
Sergio Montserrat De La Paz
Instituto de la Grasa, CSIC, SPAIN
(Authors: Sergio Montserrat De La Paz, Maria C Naranjo, Maria C Millan Linares, Sergio Lopez, Rocio Abia, Erik A.l. Biessen, Francisco J.g Muriana, Beatriz Bermudez)

10:15 Elevated talk (C014): Montelukast attenuates abdominal aortic aneurysm in rats: role on matrix metalloproteinases
Çağlar Macit
İstanbul Medipol University, TURKEY
(Authors: Gözde Tekin, Göksel Şener, Özge Çevik, Şule Çetinel, Çağlar Macit)

10:30 Coffee break

11:00 Improvement of preclinical animal models for autoimmune-mediated disorders via reverse translation of failed therapies
Bert 'T Hart
Biomedical Primate Research Centre, The NETHERLANDS

11:30 Elevated talk (C015): Nebivolol mediates p44/42 MAPK phosphorylation in experimental cardiac hypertrophy model induced by neurohumoral stimulation
Işıl Özakca
Ankara University, TURKEY
(Authors: Isil Özakca, Mesut Çiçek, Vecdi Melih Altan, Arif Tanju Özçelikay)

12:00 End of symposium

Innovation in cardiovascular pharmacotherapy: Innovation stuck in translation

Gérard A. Rongen
Radboud University Medical Center, Department of Pharmacology-Toxicology and Internal Medicine, P.O. Box 9101, 6500 HB, Nijmegen, the NETHERLANDS.

Systematic reviews and meta-analyses of animal studies have revealed serious limitations in internal and external validity strongly affecting the reliability of this research. In addition inter-species differences are likely to further limit the predictive value of animal research for the efficacy and tolerability of new drugs in humans. Important changes in the research process are needed to allow efficient translation of preclinical discoveries to the clinic, including improvements in the laboratory and publication practices involving animal research and early incorporation of human proof-of-concept studies to optimize the interpretation of animal data for its predictive value for humans and the design of clinical trials. Illustrated by recent clinical trials in the area of (cardiac) ischemia-reperfusion injury performed by this author and his colleagues, the impact of translational problems between animals and humans will be discussed on clinical development of therapeutics.

Preclinical models in cardiovascular research: its predictive value in human drug development. Limitations and opportunities

Mercè Roqué
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Preclinical cardiology research is essential for drug and technological development in cardiovascular disease. The use of adequate and clinically relevant models for in vitro and in vivo studies is a central element in this process.

Different animal models in several species have been developed to address cardiovascular disease, including ischemic heart disease, restenosis, heart failure, pulmonary hypertension, thrombotic diseases, arrhythmias, and others.

Human disease is complex and multifactorial. Identifying the physiopathologic mechanisms involved in the etiology and progression of the disease is of the outmost importance. In addition, the influence of genetic background, epigenetics, and environmental factors, needs to be taken into account.

Translational animal models are, therefore, the basis for a successful development of innovative drugs and devices. Experimental models may allow for studying the role of a single gene in a specific process, such as with the use of genetically engineered animal models.

Even though animal studies can significantly improve our knowledge of human disease mechanisms, properly designed clinical trials remain essential, since animal studies do not ensure predictability in the human. Despite a DNA homology ranging between 98-99% in some animal species commonly used in research (such as mice, monkeys and pigs), there are species differences between cellular pathways, such as signaling cascades, receptor subtypes, that can affect the pharmacodynamic and pharmacokinetic properties of a drug.

Using valid and reproducible preclinical models in each disease condition, depending on the pathophysiology, a good study design, and adequate endpoint assessment, are essential to maximize predictability and a possible translation into human studies.

Elevated talk (C013): Effects of niacin and fatty acid dietary supplementation on adipose tissue metabolic and inflammatory responses in a mouse model of metabolic syndrome

Sergio Montserrat De La Paz[1], Maria C Naranjo[1], Maria C Millan Linares[2], Sergio Lopez[1], Rocio Abia[1], Erik A.l. Biessen[3], Francisco J.g Muriana[1], Beatriz Bermudez[4]
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NAD+ is a rate-limiting co-substrate for the sirtuin enzymes; its modulation is emerging as a valuable tool to regulate metabolic diseases. Herein, we aimed the divergent modulation by dietary fats in adipose tissue-associated inflammation.
Niacin-treated MetS mice (Lepob/obLDLR-/-) were fed low-fat diet (LFD) or three high-fat low-cholesterol diets (HFLCDs) of different fatty acid compositions (saturated fatty acids, SFAs; monounsaturated fatty acids, MUFAs; and polyunsaturated fatty acids, PUFAs). Serum cardiometabolic parameters, adipocytes size and mRNA expression of pro-inflammatory biomarkers and macrophage polarization-related genes from adipose tissue were evaluated. HFLCD-SFAs administration increased insulin, triglycerides and pro-inflammatory cytokines in serum compared to HFLCD-MUFAs or HFLCD-PUFAs. The incremented adipocytes size, macrophage accumulation, M1 phenotype and pro-inflammatory biomarkers was higher in mice fed HFLCD-SFAs, whereas those fed HFLCD-MUFAs or HFLCD-PUFAs showed a partial restoration in adipocyte size distribution, a reduction of macrophage content, and to induce M2 polarization in adipose tissue.

These exciting findings open new opportunities for developing novel nutritional strategies with olive oil as the principal dietary source of oleic acid and NA such as nutraceutical complement to prevent development and progression of obesity-related inflammation.

Keywords: olive oil, adipose tissue, macrophages, metabolic syndrome, inflammation

Elevated talk (C014): Montelukast attenuates abdominal aortic aneurysm in rats: role on matrix metalloproteinases

Gözde Tekin[1], Göksel Şener[2], Özge Çevik[3], Şule Çetinel[4], Çağlar Macit[5]
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Oxidative stress and inflammation are the major factors that cause abdominal aortic aneurysm (AAA). The aim of this study is to investigate the possible protective effects of montelukast against abdominal aortic aneurysm in rats.

Forty Sprague-Dawley rats, weighing 250-300g, were equally divided into four groups; Sham operated-control (C), C + montelukast, AAA, and AAA+Montelukast. In order to induce AAA, a CaCl2 model was used. Gauze pre-soaked in 0.5M $CaCl_2$ was directly applied to the adventitia of the infrarenal abdominal aorta for 15 minutes. After the surgery, animals received montelukast intraperitoneally (10 mg/kg/day) for four weeks. At the end of the study, rats were decapitated and infrarenal aorta tissue samples were taken for the measurement of matrix metalloproteinase-2 and 9 (MMP-2 and MMP-9) protein expressions and myeloperoxidase (MPO) activities and 8-hydroxy-2'-deoxyguanesine (8-OHdG) levels. Tissue antioxidant status was evaluated by measuring superoxide dismutase (SOD) activity. Furthermore histological examination was performed. All the data was evaluated with GraphPad Prism v.5 statistic program.

In the AAA group, aortic tissue 8-OHdG levels and MPO activities were found to be increased while antioxidant enzyme, SOD was decreased. Furthermore in rats with AAA, both MMP-2 and MMP-9 protein expressions were increased. Montelukast administration reversed the inflammation and reduced both MPO activities and 8-OHdG levels along with decreses in MMP expressions. Montelukast treatment also reversed the histopathological changes caused by AAA.

These results demonstrate that montelukast reduces $CaCl_2$ induced inflammation and aortic tissue damage. The protective effects of this anti-inflammatory agent can be attributed to its ability to inhibit MMP-2 and MMP-9 expression, to inhibit neutrophil infiltration, and to balance oxidant–antioxidant status, suggesting a future role in the treatment of abdominal aortic aneurysms.

Keywords: abdominal aortic aneurysm, montelukast, matrix metalloproteinases, myeloperoxidase

Improvement of preclinical animal models for autoimmune-mediated disorders via reverse translation of failed therapies

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The ageing western societies are facing an increasing prevalence of autoimmune-mediated inflammatory diseases (AIMID) for which no effective treatment exists. A main reason is the poor translation of scientific concepts developed in AIMID animal models into effective treatments. Improvement of the translational validity of the animal models that are used for the selection of promising drug candidates from the development pipeline is clearly needed. However, how can this be done when the reasons for the high attrition rate, which in some areas are above 90%, are not known. I propose that critical information needed for the highly desired innovation of currently used preclinical models can be obtained from a reverse translation analysis of the mechanistic reasons why certain therapies fail and others succeed.

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't Hart, B. A. 2015. Reverse translation of failed treatments can help improving the validity of preclinical animal models. *Eur J Pharmacol* 759: 14-18.
't Hart, B. A., S. A. Jagessar, Y. S. Kap, K. G. Haanstra, I. H. Philippens, C. Serguera, J. Langermans, and M. Vierboom. 2014. Improvement of preclinical animal models for autoimmune-mediated disorders via reverse translation of failed therapies. *Drug Discov Today* 19: 1394-1401.

Elevated talk (C015): Nebivolol mediates p44/42 MAPK phosphorylation in experimental cardiac hypertrophy model induced by neurohumoral stimulation

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Nebivolol is a member of third-class beta-blockers which has been approved for the treatment of hypertension. We have previously shown nebivolol partially protects the responsiveness of β-adrenoceptor (β-AR) signaling and the development of isoprenaline (ISO)-induced cardiac hypertrophy independent of its β_1-AR blocking effect. Here, we examined the possible effects of nebivolol treatment on the development of cardiac hypertrophy and p44/42 MAPK phosphorylation in Iso- and angiotensin II (ANG)-induced hypertrophic hearts compared with metoprolol, selective β_1-blocker. Rats infused by either isoprenaline (2.4 $mg.kg^{-1}.day^{-1}$, 14 days) or angiotensin II (1 $mg.kg^{-1}.day^{-1}$, 14 days) were randomized into three groups according to treatment with metoprolol (30 $mg.kg^{-1}.day^{-1}$, ISO-M, ANG-M), nebivolol (10 $mg.kg^{-1}.day^{-1}$, ISO-N, ANG-N), or placebo (ISO, ANG). Nebivolol mediated a significant improvement on left ventricle-to-body weight ratio and mRNA expression levels of atrial natriuretic peptide (ANP) compared with metoprolol and placebo in ISO-induced cardiac hypertrophy (Table 1). In this experimental model, the phosphorylation of p44/42 MAPK is decreased in response to Iso infusion, however nebivolol increased the phosphorylation levels to control (Fig1A). In ANG-induced cardiac hypertrophy model, contrary to ISO-induced one, nebivolol failed to attenuate the cardiac mass (Table 1). Although both beta-blockers decreased the ANP mRNA levels significantly (Table 1), nebivolol, but not metoprolol, increased the phosphorylation level of p44/42 MAPK which was slightly reduced in response to ANG infusion (Fig1B). These data suggest that the effect of nebivolol on the phosphorylation of p44/42 MAPK may occur independent from its antihypertrophic and β_1-AR blocking effects in conditions triggered by neurohumoral stimulations.

This study is supported by TUBITAK 114S563.

Keywords: beta-blocker, isoprenaline, angiotensin II, p44/42 MAPK, cardiac hypertrophy

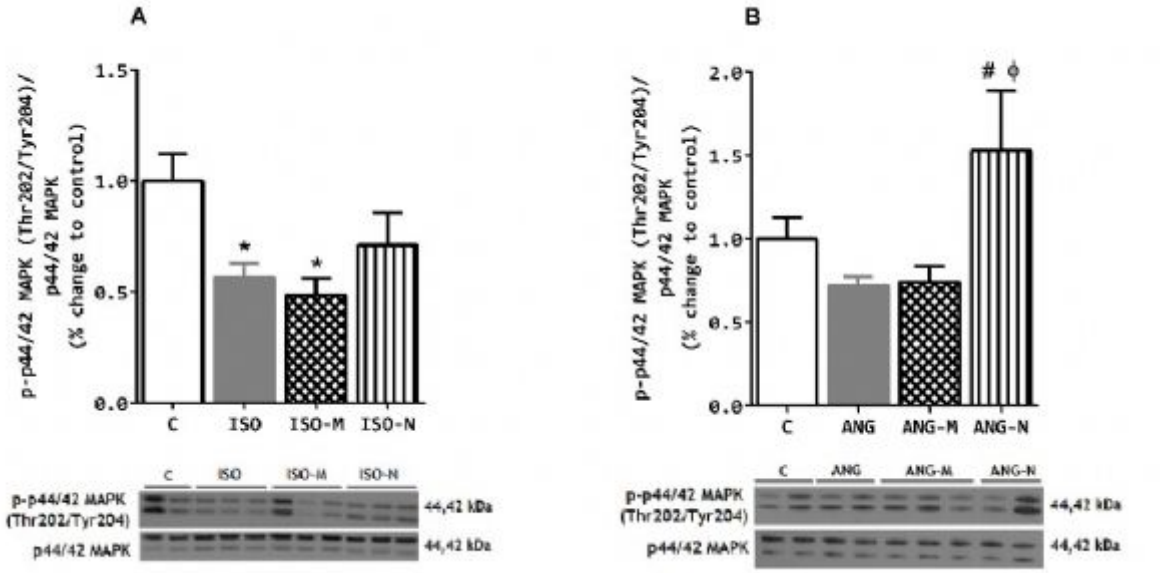

Figure 1. Analysis of p44/42 MAPK phosphorylation in left ventricle samples from ISO- (A) and ANG-induced cardiac hypertrophy (B). Values were normalized to HSP90 as loading control (n=3 to 6 per group). All results are expressed as means±SEM. Statistical analysis was performed using one-way Anova followed by Newman-Keuls multiple comparison test. *, p<0.05 vs C; #, p<0.05 vs Ang; ϕ, p<0.05 vs Ang-M by post hoc analysis.

Table 1. Morphologic and molecular assessment of in vivo cardiac hypertrophy models and the effects of beta-blocker treatments on these parameters.

Hypertrophic agent	**Experimental groups**	**Left ventricle-to-body weight ratio (mg/g)**	**ANP mRNA expression (% change to control)**
Isoprenaline	C	1.90±0.08	1.00±0.03
	Placebo (ISO)	2.25±0.05*	12.95±5.47*
	Metoprolol (ISO-M)	2.15±0.06*	8.12±1.17*
	Nebivolol (ISO-N)	1.93±0.04# ϕ	5.62±1.42*# ϕ
Angiotensin II	C	1.71±0.11	1.00±0.03
	Placebo (ANG)	1.95±0.11*	11.55±1.12*
	Metoprolol (ANG-M)	2.17±0.09**	3.09±1.28#
	Nebivolol (ANG-N)	2.21±0.11*	5.50±2.06#

*Left ventricular weight-to-body weight ratio and ANP mRNA expression levels were evaluated as morphologic and molecular parameters, respectively. (n=3 to 6 per group). All results are expressed as means±SEM. Statistical analysis was performed using one-way ANOVA followed by Newman-Keuls multiple comparison test. *, p<0.05 vs C; **, p<0.01 vs C; #, p<0.05 vs placebo; ϕ, p<0.05 vs metoprolol-treated, by post hoc analysis.*

Hall B:
Emerging therapeutic targets in chronic pain and inflammation

(Organized by Hungarian Society for Experimental and Clinical Pharmacology)
Organizers and chairs:
Beata Sperlagh (Hungarian Academy of Sciences, HUNGARY)
Zsuzsanna Helyes (University of Pecs, HUNGARY)
09:00-12:00

09:00 Central mechanism of botulinum toxin action on pain supersensitivity, allodynia and migraine
Zdravko Lacković
University of Zagreb, CROATIA

09:30 Extracellular vesicles and their therapeutic potential in inflammatory diseases
Agnes Kittel
Hungarian Academy of Sciences, HUNGARY

10:00 Analgesic effects of the novel semicarbazide-sensitive amine-oxidase inhibitor SzV-1287 in animal models of chronic pain
Zsuzsanna Helyes
University of Pecs, HUNGARY

10:30 Coffee break

11:00 Elevated talk (C016): Inhibition of NLRP3 inflammasome prevents lipopolysaccharide-induced Inflammatory hyperalgesia in mice: contribution of NF-kB, caspase-1/11, ASC, NADPH oxidase, and NOS isoforms
Bahar Tunçtan
Mersin University, TURKEY
(Authors: Bahar Tunctan, Abdurrahman Dolunay, Sefika Pinar Senol, Meryem Temiz Reşitoğlu, Demet Sinem Guden, Ayse Nihal Sari, Seyhan Sahan Firat)

11:15 Elevated talk (C017): Investigating the analgesic effect of FAAH inhibitor JNJ-1661010 alone and in combination with COX inhibitors
Nergiz Hacer Turgut
Cumhuriyet University, TURKEY
(Authors: Nergiz Hacer Turgut, Ahmet Altun, Merve Ergul, Muhammed Mucahit Cicek, Bulent Sarac, Ihsan Bagcivan)

11:30 Elevated talk (C018): The analgesic effect of FAAH inhibitor AM-1172 alone and in combination with iNOS inhibitors BYK and SM
Ahmet Altun
Cumhuriyet University, TURKEY
(Authors: Ahmet Altun, Nergiz Hacer Turgut, Merve Ergul, Muhammed Mucahit Cicek, Bulent Sarac, Sahin Yildirim)

12:00 End of symposium

Central mechanism of botulinum toxin action on pain supersensitivity, allodynia and migraine

Zdravko Lackovic

Authors: Zdravko Lackovic[1], Lidija Bach Rojecky[2], Ivica Matak[1], Zsuzsanna Helyes[3], Boris Filipovic[4], Višnja Drinovac[2], Maja Relja[5]

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Experimental and clinical observations suggest beneficial effect of botulinum toxin A (BoNT/A) in different painful conditions. It was assumed that antinociceptive action is associated with BoNT/A enzymatic inactivation of SNAP25 and prevention of neurotransmitter release from the peripheral nerve endings. However BoNT/-A is more potent and faster acting if applied intrathecally, in some experimental condition injected unilaterally acts bilaterally, and its action can be prevented by axonal transport blocker colchicine. Tracing toxin enzymatic activity we found immunohistochemically that it is transported to sensory regions of the CNS. All these observation suggest central action of BoNT/A (review Matak and Lackovic, Prog Neurobiol 2014). However, molecular mechanism is still unknown. Application of receptor blocking drugs revealed association with opioid and GABA system. Because BoNT/A is registered for treatment of chronic migraine recently we concentrated more on its action in trigeminal region. Surprisingly, three different types of pain: infraorbital nerve constriction injury, temporomandibular joint inflammation and facial formalin injection in rats are accompanied by dural neurogenic inflammation characterized by extravasation of plasma proteins and inflammatory cells. Such phenomenon was previously described only in association with migraine. Immunohistochemistry revealed cocalization of BoNT/A enzymatic activity with calcitonin gene related polypeptide (CGRP) in dural nerves, and biochemically we found increase in dural CGRP level. Thus it seems that after peripheral injection BoNT/A is taken up by sensory nerve endings and axonally transported to dural nerves where it is colocalized with CGRP and suppress its action on neurogenic inflammation (Lackovic et al 2016). This previously unknown phenomenon is specific only for trigeminal region, since peripheral types of pain like partial transection of the sciatic nerve and sciatic nerve constriction injury are not associated with extravasation of lumbar or cranial dura.

This work has been supported in part by Croatian Science Foundation under the Project IP-2014-09-4503

External vesicles and their therapeutic potential in inflammatory diseases

Agnes Kittel

Authors: Agnes Kittel[1], Xabier Osteikoetxea[2], Tamás Géza Szabó[2], Katalin Szabó-Taylor[2], Tamás Baranyai[3], Zoltán Giricz[3], Edit Irén Buzás[2]

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Although the story of extracellular vesicles (EVs), these cell-secreted phospholipid bilayer-bound structures started about 70 years ago. By now it has been proved that the release of membrane vesicles is a process conserved in both prokaryotes and eukaryotes and compelling piles of evidence support the significance of the three extracellular vesicle populations namely exosomes, microparticles/microvesicles (MV/MP) and apoptotic bodies in a broad range of physiological-pathological conditions. Not surprisingly, the possibility of EV-related biomarker and therapeutic applications made this research field attractive for clinical and pharmaceutical research, too.

Among pathological conditions EVs have long been suspected to promote inflammation. This presumption was in line with our finding when we demonstrated changes in the localization and distribution of purinergic receptors P2X7 and P2Y12 in BV2 cell-derived MVs and exosomes following LPS treatment of the cells.

Recently we hypothesized that the effect of EVs on cells must be a combined effect of EVs and cytokines, also present in the medium or bodily fluids. In a detailed study we proved, that indeed, the effects of EVs, TNF, or their combination on gene expression of recipient cells are substantially different. Our results propose to test the combined effects of soluble mediators and extracellular vesicles since this may model the in vivo effects more accurately than testing them on cells separately. Furthermore, uncoupling the interaction of EVs and soluble mediators may help forward to more effective therapeutic intervention.

Analgesic effects of the novel semicarbazide-sensitive amine-oxidase inhibitor SzV-1287 in animal models of chronic pain

Zsuzsanna Helyes

Authors: Zsuzsanna Helyes[1,2], Bálint Scheich[1], Ádám Horváth[1,2], Bálint Botz[1,2], Valéria Tékus[1,2], Dóra Bogdán[3], Erika Pintér[1,2], Janka Csepregi[4], Attila Mócsai[4], János Szolcsányi[1,2], Péter Mátyus[3]

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[2]MTA-PTE NAP B Chronic Pain Research Group, Hungary;

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Semicarbazide-sensitive amine oxidase (SSAO) is a multi-functional enzyme involved in the oxidative deamination of primary amines. Its products, such as formaldehyde and methylglyoxal, are important mediators in pain, possibly via the activation of the Transient Receptor Potential Ankyrin 1 (TRPA1) ion channel localized on primary afferent neurones, immune cells and the central nervous system. We studied the effects of our novel SSAO inhibitor, SzV-1287 (3-(4, 5, -dipheyl-1, 3-oxazol-2-yl) propanoloxime), in chronic pain models, as well as the involvement of TRPA1 in its mechanism of action.

Traumatic mononeuropathy was induced by sciatic nerve ligation and arthritis evoked by complete Freund's adjuvant or K/BxN arthritogenic serum transfer. The effects of SzV-1287 was investigated in male C57Bl/6 wildtype mice (WT) and also in TRPA1 gene-deleted (TRPA1-/-) ones (N=6-8/group). The mechanonociceptive threshold was measured by aesthesiometry, thermonociception by hot plate, cold sensitivity by paw withdrawal latency from 0°C water, paw volume by plethysmometry, clinical severity by scoring, grasping on a grid, myeloperoxidase activity by luminescence and vascular leakage by fluorescence imaging.

Both a single and repeated daily injections of 20 mg/kg i.p. SzV-1287 resulted in similar, approximately 50% significant reduction of neuropathic mechanical hyperalgesia involving central sensitization in WT mice 7 days after nerve ligation, but cold hyperalgesia was not altered. It did not exert any effect in TRPA1-/- mice. SzV-1287 significantly decreased mechanical hyperalgesia in both arthritis models without affecting thermo-nociception, but the inflammatory components (oedema, vascular leakage and neutrophil accumulation) were only moderately reduced in the adjuvant model. These inhibitory actions of SzV-1287 were similar in WT and TRPA1-/- mice.

SzV-1287 exerts potent analgesic effect, which seem to be primarily centrally mediated via TRPA1 in neuropathy, but not in arthritis. These results can open promising perspectives for drug development.

Support: KTIA_NAP_13-2014-0022 (888819), OTKA-NN 114458.

Elevated talk (C016): Inhibition of NLRP3 inflammasome prevents lipopolysaccharide-induced Inflammatory hyperalgesia in mice: contribution of NF-kB, caspase-1/11, ASC, NADPH oxidase, and NOS isoforms

Bahar Tunctan, Abdurrahman Dolunay, Sefika Pinar Senol, Meryem Temiz Reşitoğlu, Demet Sinem Guden, Ayse Nihal Sari, Seyhan Sahan Firat
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The nucleotide binding and oligomerization domain-like receptor (NLR) family protein NLR family pyrin domain-containing 3 (NLRP3), an intracellular signaling molecule that senses many environmental- and pathogen/host-derived factors, has been implicated in the pathogenesis of several diseases associated with inflammation. It has been suggested that NLRP3 inflammasome inhibitors may have a therapeutic potential in the treatment of NLRP3-related inflammatory diseases. The aim of this study was to determine whether inhibition of NLRP3 inflammasome prevents inflammatory hyperalgesia induced by lipopolysaccharide (LPS) in mice as well as changes in expression/activity of cardiac nuclear factor kB (NF-kB), caspase-11, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and endothelial/neuronal/inducible nitric oxide synthase (eNOS/nNOS/iNOS) that may regulate NLRP3/apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC)/pro-caspase-1 inflammasome formation and activity by using MCC950, a selective NLRP3 inflammasome inhibitor.

Male mice received saline (10 ml/kg; ip), LPS (10 mg/kg; ip), and/or MCC950 (3 mg/kg; ip). Reaction time to thermal stimuli within 1 minute was evaluated after 6 hours. The mice were killed and brain, hearts, and lungs were collected for measurement of NF-kB, caspase-1, caspase-11, NLRP3, ASC, NADPH oxidase subunits (gp91phox and p47phox), nitrotyrosine, eNOS, nNOS, iNOS, and β-actin protein expression, NOS activity, and interleukin (IL)-1β levels.

LPS-induced hyperalgesia was associated with a decrease in eNOS, nNOS, and iNOS protein expression and activity as well as an increase in NF-kB p65, caspase-1 p20, caspase-11 p20, NLRP3, ASC, gp91phox, p47phox, and nitrotyrosine protein expression in addition to IL-1β levels in the tissues. The LPS-induced changes were prevented by MCC950.

The results suggest that inhibition of NLRP3/ASC/pro-caspase-1 inflammasome formation and activity prevents inflammatory hyperalgesia induced by LPS in mice as well as changes in NF-kB, caspase-11, NADPH oxidase, and eNOS/nNOS/iNOS expression/activity.

This study is supported by grants from TUBITAK (SBAG-S215047) and Mersin University (BAP-2016-1-TP2-1427).

Keywords: lipopolysaccharide, inflammatory hyperalgesia, NLRP3 inflammasome, MCC950

Elevated talk (C017): Investigating the analgesic effect of FAAH inhibitor JNJ-1661010 alone and in combination with COX inhibitors

Nergiz Hacer Turgut[1], Ahmet Altun[2], Merve Ergul[1], Muhammed Mucahit Cicek[2], Bulent Sarac[2], Ihsan Bagcivan[2]
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Fatty acid amide hydrolase (FAAH) is an enzyme responsible for the metabolism of fatty acid amides (FAA). Recent studies suggest that FAAH enzyme inhibition may result in analgesic effect. We aimed to determine the analgesic effect profiles of JNJ-1661010, a FAAH enzyme inhibitor and cyclooxygenase (COX) enzyme inhibitor drugs (meloxicam and SC-58125) in acute pain models. We also aimed to perform combinations with effective concentration of JNJ-1661010 and effective and/or ineffective concentrations of COX inhibitory drugs to observe the changes on analgesic effect.

JNJ-166101 (4 mg/kg, 1 mg/kg, 0.25 mg/kg), meloxicam (6 mg/kg, 1.5 mg/kg, 0.375 mg/kg) and SC-58125 (4 mg kg, 1 mg/kg, 0.25 mg/kg) were applied intraperitoneally to adult male Wistar rats alone and in combinations. Hot-plate test was used to examine both the central and peripheral mechanisms, while tail flick test was used to examine the results of central mechanisms at spinal level. Additionally rota rod performance test was performed to observe locomotor coordination mediated side effects. Latencies were measured at 0, 15, 30, 45, 60, 90 and 120 minutes. The obtained results were expressed in % MPE (Maximal potent effect) and compared by ANOVA test.

JNJ-1661010 produced a strong dose-dependent analgesic effect. When combined with low and high analgesic doses of both COX inhibitors, significantly stronger analgesic effect occurred than alone applications ($p < 0.05$).

Although there are many agents used in the treatment of pain, limitations related to both cost and side effect profile of available agents, requires the development of new analgesic agents. The results of our study have revealed that JNJ-1661010 can perform strong analgesic effect and when combined with drugs targeting classic pathways stronger effect can be observed.

This study is supported by Cumhuriyet University Scientific Research Project T-593 (CUBAP, Sivas, Turkey).

Keywords: pain, fatty acid amide hydrolase (FAAH), cyclooxygenase (COX), tail-flick, hot-plate

Elevated talk (C018): The analgesic effect of FAAH inhibitor AM-1172 alone and in combination with iNOS inhibitors BYK and SM

Ahmet Altun[1], Nergiz Hacer Turgut[2], Merve Ergul[2], Muhammed Mucahit Cicek[1], Bulent Sarac[1], Sahin Yildirim[1]

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Fatty acid amide hydrolase (FAAH) is an enzyme responsible for the catabolism of endogen cannabinoids which are the main modulators of pain in human body. Indirect management of cannabinoids may prevent unexpected results and provide stronger analgesic effect. We aimed to investigate the analgesic effects of AM-1172, a FAAH enzyme inhibitor, in acute pain models alone and in combination with (S)-metilizotiyoüre sülfat (SM) and BYK-191023, inducible nitric oxide synthase (iNOS) inhibitors AM-1172 (2 mg/kg, 0.25 mg/ kg, 0.15 mg/kg), BYK-191023 (8 mg/ kg, 1.6 mg/kg, 0.32 mg/kg) and SM (1 mg/kg, 0.25 mg/kg, 0.0625 mg/kg) were administrated to adult male Wistar rats intraperitoneally alone and in combinations. Hot-plate test was used to examine both the central and peripheral mechanisms, while tail flick test was used to examine the results of central mechanisms at spinal level. Additionally rota rod performance test was performed to observe locomotor coordination mediated side effects. Latencies were measured at 0, 15, 30, 45, 60, 90 and 120 minutes. The data was expressed as % MPE (maximal potent effect) and compared by ANOVA test.

AM-1172 produced a strong dose-dependent analgesic effect. When combined with low and high analgesic doses of both iNOS inhibitors, significantly stronger analgesic effect occurred than alone applications ($p<0.05$).

Although there are many non-steroidal anti-inflammatory agent used in pain management, there are not many aiming endo-cannabinoid system since it is hard to control strong analgesic effect and risky side effects. Our indirect approach to increase endo-cannabinoid levels seems to be working and combination applications which allows us to use agents in lower concentrations proves that this approach may be a promising one.

This study is supported by Cumhuriyet University Scientific Research Project T-600 (CUBAP, Sivas).

Keywords: analgesia, fatty acid amide hydrolase (FAAH), iNOS, tail-flick, hot-plate

Hall C:
Calcium signalling induced by sphingosine-1-phosphate and other agonists in urogenital tract

(Organized by Turkish Pharmacological Society)
Organizers and chairs:
Nezahat Tuğba Durlu-Kandilci (Hacettepe University, TURKEY)
09:00-12:00

09:00 The roles of sphingosine in lysosomal calcium signalling and Niemann-Pick disease type C
Doris Höglinger
University of Oxford, UK

09:30 Sphingosine-1- phosphate and carbachol induced secondary messengers in detrusor
Nezahat Tuğba Durlu-Kandilci
Hacettepe University, TURKEY

10:00 New sights in calcium signalling in myometrium
Özgür Öktem
Koç University, TURKEY

10:30 Coffee break

11:00 Elevated talk (C020): Sphingosine-1-phosphate (S1P) induced contractile responses in detrusor smooth muscle of rats having cyclophosphamide induced cystitis
Irfan Anjum
Hacettepe University, TURKEY
(Authors: Irfan Anjum, Merve Denizalti, N. Tugba Durlu Kandilci, Inci Sahin Erdemli)

11:15 Elevated talk (C021): Fine tuning control of cholinergic nerve activity by excitatory P2X2/3 and inhibitory P2Y12 receptors in men with prostatic bladder obstruction
Isabel Silva
Universidade do Porto, PORTUGAL
(Authors: Isabel Silva, Miguel Silva Ramos, Fátima Ferreirinha, Julie Pelletier, Jean Sévigny, Paulo Correia De Sá)

11:30 Elevated talk (C022): Investigation of the functional responses of myometrium smooth muscle in secondhand smoking and chronic alcohol consuming rats
Semil Selcen Göçmez
Kocaeli University, TURKEY
(Authors: Semil Selcen Gocmez, Zeynep Ece Korun, Tugce Demistas Sahin, Tijen Utkan)

11:45 Elevated talk (C023): Endothelial dysfunction in the human umbilical artery due to preeclampsia can be prevented by sildenafil
Edibe Minareci
Akdeniz University, TURKEY
(Authors: Edibe Minareci, Nurten Kayacan, Gulay Sadan, Bora Dinç)

12:00 End of symposium

The roles of sphingosine in lysosomal calcium signalling and Niemann-Pick disease type C

Doris Höglinger

Authors: Doris Höglinger[1,3], Per Haberkant[1], Forbes D. Porter[2], Frances M. Platt[3], Antony Galione[3], Carsten Schultz[1]

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Several distinct lipid species have been shown to actively participate in intracellular signal transduction events and to influence central cellular processes, however the bioactive actions of most lipids remain unexplored. This lack of knowledge is mainly due to a shortage of tools to manipulate lipid levels within living cells in a non-invasive way and to identify their protein interactors. Here, two novel methods to overcome these drawbacks applied to the signalling lipid sphingosine (Sph) are presented.

A 'caged' variant of sphingosine enables a precise elevation of Sph levels in single living cells within seconds using light. This acute increase in Sph concentration led to an immediate release of lysosomal calcium through the actions of the two-pore channel 1 (TPC1). Cells derived from patients suffering from the lysosomal storage disease Niemann-Pick type C (NPC) exhibited reduced calcium signals upon Sph uncaging. Sph-induced calcium release also initiated the nuclear translocation of transcription factor EB, which positively regulates the expression of autophagic and lysosomal biogenesis genes, further underlining the importance of lysosomal calcium release in direct lysosome-to-nucleus signalling pathways.

In order to capture Sph-interacting proteins, a trifunctional Sph (TFS) was developed. TFS facilitates the release and immediate crosslinking of Sph to its interacting partners within the living cell. Mass-spectrometric analyses identified known Sph-binding proteins such as the ceramide synthase, as well as novel putative Sph-interactors. TFS was further employed in investigations of the subcellular localization and transport of Sph through the cell. NPC patient fibroblasts showed a striking accumulation of Sph in late endosomes and lysosomes. Sph transport out of these vesicles was severely hindered in the NPC condition. The kinetics of Sph efflux correlated with the severity of symptoms in different NPC patients, so this assay could potentially be used for monitoring and prognosis of NPC disease severity.

Sphingosine-1- phosphate and carbachol induced secondary messengers in detrusor

Nezahat Tuğba Durlu-Kandilci
Hacettepe University, Faculty of Pharmacy, Department of Pharmacology

Sphingosine-1-phosphate (S1P) that is a bioactive sphingolipid metabolite plays a role in smooth muscle contraction via G-protein coupled receptors ($S1P_{1-5}$ receptors). $S1P_2$ and $S1P_3$ receptors are known to activate smooth muscle contraction via RhoA/ROK pathways. Besides some physiological events, S1P production also takes place in some chronic pathological situations as in overactive bladder syndrome and partial urethral obstruction[1]. Interstitial cystitis, a syndrome that is characterized with chronical inflammation which causes overactive bladder, enhances both S1P-induced calcium release from intracellular stores and calcium sensitization[3] in rat detrusor smooth muscle through activation of Rho kinase and protein kinase C pathways. Agonists such as carbachol are normally thought to induce contraction in smooth muscle through inositol 1,4,5-triphosphate (IP_3) production and release of Ca^{2+} from the main intracellular Ca^{2+} store, sarcoplasmic reticulum[2]. However, pyridine nucleotides that are cyclic adenosine diphosphate ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP) may also be involved in Ca^{2+} mobilizing processes. cADPR has been shown to induce Ca^{2+} release via ryanodine receptors from sarcoplasmic reticulum[3]. The other Ca^{2+} mobilizing agent NAADP was found to activate another Ca^{2+} release mechanism different from those activated by IP_3 and cADPR, known to be acting on lysosome-related acidic compartments in various tissues including mammalian cells. Cross-talk between IP_3, cADPR and NAADP induced Ca^{2+} release has been shown in many cells. Two-pore channels (TPCs) that are novel members of the superfamily of voltage-gated cation channels, have been shown to function as NAADP receptors. cADPR and NAADP both have a role in Ca^{2+} signalling in guinea-pig detrusor while NAADP mainly mobilizes Ca^{2+} in relation with TPC2 in mice detrusor[4]. Thus, key differences in Ca^{2+} signalling mechanisms are apparent between detrusor smooth muscles from different species.

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[2] *Nature 1993;361:315–325*
[3] *Science 1991;253:1143– 1146*
[4] *J Biol Chem 2010;285(32):24925–24932*

New sights in calcium signalling in myometrium

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A better understanding of the molecular mechanisms regulating myometrial contractility in human uterus is vital to understand and provide more effective treatment options for premature labour and postpartum uterine atony, two important adverse obstetric outcomes with significant fetal and maternal morbidity and mortality. Myometrial contractions are generated by periodic synchronous rises in intracellular calcium concentration as a result of spontaneously generated action potentials propagating throughout the entire myometrium. Controling a myogenically active muscle, such as the myometrium, is closely connected to expression of ion channels. Since it is their properties and expression levels that will drive or dampen the firing of action potentials, and thus the changes of intracellular Ca, channel components and their mechanisms of action are now become more of an issue. Unlike nerve action potentials, although Na channels are present, Ca was found to be the major inward charge carrier rather than Na, as voltage-sensitive Ca channels open as the membrane started to depolarize. The predominant inward current in myometrium is the L-type (CaV1.2) calcium channel. Inhibition of L-type Ca channels by organic and inorganic Ca antagonists blocks myometrial action potentials, Ca transients and spontaneous contractions of the uterus. These recent findings hold true the pivotal role of voltage- gated Ca channels in the control of Ca signalling and contraction emphasizes the importance of the action potential in uterine smooth muscle.

Elevated talk (C020): Sphingosine-1-phosphate (S1P) induced contractile responses in detrusor smooth muscle of rats having cyclophosphamide induced cystitis

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Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid metabolite that causes smooth muscle contraction via G-protein coupled receptors (S1P1-5 receptors)(1). S1P production is known to take place both in physiological states and some pathological situations, as in overactive bladder syndrome (2). Interstitial cystitis is a syndrome characterized with chronical inflammation which causes overactive bladder (3). The intracellular mechanism of S1P-induced contractile responses was investigated in β-escin permeabilized detrusor smooth muscle of rats having cyclophosphamide induced cystitis.

The study protocol was approved by the University Animal Ethics Committee (2014/34-6). Cyclophosphamide (150 mg/kg) was injected to rats (Sprague-Dawley, female, 200-250 g) intraperitoneally once a day on days 1, 4 and 7 to induce cystitis. Control groups were injected with saline (NaCl 0.9 %), in respectively. Detrusor smooth muscle strips were mounted in 1 ml organ baths containing Hepes buffered modified Krebs' solution. Tissues were permeabilized with 40 µM β-escin for 30 min. Isometric contractions were expressed as % of 80 mM K^+. Data are given as mean±S.E.M. $P<0.05$ was accepted as significant.

S1P (50 µM)-induced contractions were significantly increased from 20.1±1.5% (control, n=11) to 42.9±6% in cystitis (n=8). These contractions were significantly inhibited by protein kinase C inhibitor GF-109203X (5 µM) in both control (12.2±1.4%, n=6) and cystitis group (8.8±1.7%, n=6). S1P-induced contractions were also significantly inhibited by Rho kinase inhibitor Y-27632 (1 µM) in both control (13.4±2.4%, n=6) and cystitis group (12.5±1.4%, n=6).

According to the present data, interstitial cystitis triggers S1P-induced intracellular calcium release in permeabilized detrusor smooth muscles. Since this contractile response is inhibited approximately the same proportion by Rho kinase and protein kinase C inhibitors, we may suggest that the augmentation in S1P-induced contractions in cystitis involves both pathways.

1.Watterson et al. (2007) FASEB J.21, 2818–2828.
2.Aydin et al. (2010) BJU Int.106,562–572.
3.Masago et al. (2009) Int.J.Uro.16, 842-847

Keywords: cyclophosphamide, sphingosine-1-phosphate, permeabilization, detrusor smooth muscle, rat

Elevated talk (C021): Fine tuning control of cholinergic nerve activity by excitatory P2X2/3 and inhibitory P2Y12 receptors in men with prostatic bladder obstruction

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Purines are important regulators of urinary bladder activity. Besides ATP release from mechanically-stimulated uroepithelial cells driving normal and abnormal sensations during bladder filling, the nucleotide acts as a co-transmitter with acetylcholine (ACh) to initiate detrusor contractions during voiding. We demonstrated that uroepithelial cells and cholinergic nerves from men with bladder outflow obstruction (BOO) due to benign prostatic hyperplasia release 3-5 fold more ATP than controls. Here, we show that the kinetics of the extracellular ATP and ADP catabolism is 1.4 and 1.9 times slower in the detrusor of BOO patients than controls. This difference was attributed to decreases in NTPDase1 (ATP diphosphohydrolase) activity converting ATP directly into AMP, with subsequent dephosphorylation to adenosine by ecto-5'-nuleotidase/CD73 in smooth muscle fibres. Minor changes were detected in NTPDase2 (ATPase) expression bound to cholinergic nerves, which might favour transient ADP accumulation at the neuromuscular synapse. Differential inhibition of NTPDase1 and 2 with ARL67156 (100µM) and POM-1 (100µM) decreased evoked [^{3}H]ACh release by 13±8% (n=5) and 24±6% (n=5) in BOO patients, respectively, but the two compounds were equipotent in reducing (by 30%) evoked transmitter release in control individuals. The P2X3 receptor antagonist, A317491 (100nM), decreased [^{3}H]ACh release by 20±2% (n=4) and 24±6% (n=5) in control and BOO patients, respectively, while the P2X2/3 receptor blocker, TNP-ATP (10nM), was more effective in BOO patients (43±16%, n=4) than in controls (27±9%, n=4). Selective antagonist of ADP-sensitive $P2Y_{12}$ receptor with AR-C66096 (100nM), but not of $P2Y_1$ and $P2Y_{13}$ receptors with MRS2179 (300nM) and MRS2211 (10µM), increased [^{3}H]ACh release only by 23±9% (n=5). Data suggest that transient accumulation of ADP leading to inhibition of cholinergic neurotransmission via $P2Y_{12}$ receptors is not enough to counteract neuronal excitation mediated by ATP through ionotropic P2X2/3 receptors in men with BOO.

Isabel Silva was in receipt of a PhD fellowship by FCT (SFRH/BD/88855/2012).

Keywords: human bladder, purines, bladder outflow obstruction (BOO), $P2Y_{12}$ receptors, NTPDases

Elevated talk (C022): Investigation of the functional responses of myometrium smooth muscle in secondhand smoking and chronic alcohol consuming rats

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Smoking during pregnancy is a major risk factor for preterm delivery, but the underlying mechanism by which smoking stimulates uterine contractions is still poorly understood. Maternal consumption of ethanol is also a risk factor for birth complications. The aim of this study was to investigate the effects of chronic smoking and alcohol consumption on the contractile and relaxant responses of preterm myometrial strips isolated from rats.

Twenty one Wistar albino rats were divided into three groups: Control (n=7), received tap water ad libitum; smoking group, rats exposed to smoke for 12 weeks (n=7); alcohol group, were fed with ethanol for 12 weeks (n=7). The reactivities of myometrial strips obtained from term labor rats of the smoking, alcohol and the control groups were mounted in organ chambers for recording of isometric tension. The effects of cumulative concentrations of carbachol (10^{-8}-10^{-4} M), oxytocin (10^{-9}-10^{-3} M) and diltiazem (10^{-8}-10^{-4} M) on myometrial spontaneous contractions were measured.

Carbachol contractile responses were decreased the amplitude and frequency of spontaneous contractions in alcohol group, whereas significantly increased in smoking group ($p<0.05$). Oxytocin contractile responses were decreased the amplitude and frequency of spontaneous contractions in alcohol group ($p<0.05$). The frequency of myometrial contraction of oxytocin were significantly increased by smoking ($p<0.05$), while the amplitude of contractions were not changed. The amplitude and frequency of myometrial relaxations were significantly decreased by alcohol beginning from the concentration of 10-5 M ($p<0.05$). There were no significant differences between relaxant responses to diltiazem of smoking and control group.

These findings suggest that smoking and alcohol consumption impaired contractile and relaxant responses in the isolated myometrial smooth muscles and thereby increases the risk of birth complications.

Keywords: smoking, alcohol consumption, pregnancy, rat, myometrium

Elevated talk (C023): Endothelial dysfunction in the human umbilical artery due to preeclampsia can be prevented by sildenafil

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We aimed to determine the effects of sildenafil in human umbilical artery preparation taken from preeclamptic or normal pregnant women, also to investigate underlying mechanisms in these effects. Eighteen pregnant women with preeclampsia and 18 healthy pregnant women were involved. Relaxation responses of sildenafil in presence and absence of nitric oxide (NO) synthase inhibitor, N-[omega]-nitro-L-arginine methyl ester (L-NAME), and soluble guanylyl cyclase inhibitor, 1H-[1,2,4] oxadiazolo [4,3-a]quinoxalin-1-one (ODQ), were compared between the preeclampsia group and control group. Sildenafil-induced relaxation responses were significantly attenuated in the presence of preeclampsia, L-NAME or ODQ, but not totally abolished. Interestingly, except with ODQ incubation, in all set of experiments maximal relaxation response was achieved by sildenafil. These data indicate that sildenafil might effect vascular responsiveness of human umbilical artery through the involvement of NO/cyclic guanosine monophosphate (cGMP)-dependent and -independent pathways. Further investigations are needed to clarify the exact mechanisms.

Keywords: preeclampsia, sildenafil, nitric oxide, phosphodiesterase

Hall D:
Signalling pathways in vascular function and dysfunction

(Organized by Turkish Pharmacological Society)
Organizers and chairs:
Günay Yetik Anacak (Ege University, Faculty of Pharmacy, Department of Pharmacology)
B. Sönmez Uydeş Doğan (İstanbul University, Faculty of Pharmac. Department of Pharmacology)
09:00-12:00

09:00 Hsp90: A regulator of vascular relaxation
Günay Yetik Anacak
Ege University, TURKEY

09:30 Control of human vascular tone by prostanoid pathway
Gökçe Topal
İstanbul University, TURKEY

10:00 Dysregulated NO/cGMP signalling in priapism
F. Sena Sezen
Karadeniz Technical University, TURKEY

10:30 Coffee break

11:00 Vascular role of adipocytokines in health and diseases
Johan Van De Voorde
Ghent University, BELGIUM

11:30 Elevated talk (C024): The effect of PVAT on vascular tone regulation of human coronary vessels: Role of H2S and PGE2
Gülsev Özen
İstanbul University, TURKEY
(Authors: Gulsev Ozen, Ingrid Gomez, Larissa Kotelevets, Eric Chastre, Catherine Deschildre, Lilia Boubaya, Dan Longrois, Sonmez Uydes Dogan, Gokce Topal, Jean Baptiste Michel, Xavier Norel)

11:45 Elevated talk (C025): The role of voltage-dependent potassium channels in the relaxation of renal artery of diabetic rats
Ljiljana Gojkovic Bukarica
University of Belgrade, SERBIA
(Authors: Ljiljana Gojkovic Bukarica, Jasmina Markovic Lipkovski, Helmut Heinle, Sanja Cirovic, Jovana Rajkovic, Vladimir Djokic, Radmila Novakovic)

12:00 End of symposium

Hsp90: A regulator of vascular relaxation

Günay Yetik Anacak
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Heat shock protein 90 (Hsp90) is a ubiquitous chaperone protein, involved in proper folding, stability and maturation of numerous client proteins. Hsp90 is associated with all forms of nitric oxide synthases (NOS); endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS) and important for NOS functions including vascular tonus, angiogenesis and inflammation. Binding of HSP90 ensures recruitment of activated Akt to the eNOS–HSP90 complex and phosphorylation of eNOS.

We found that hsp90 interacts with eNOS in penile tissue and disruption of this interaction by hsp90 inhibitors radicicol or 17-AAG leads to reduction of Acetylcholine, bradykinine or sphingosine induced relaxation of corpus cavernosum. However hsp90 inhibitors does not inhibit acetylcholine-induced relaxation in hypercholesterolemic rabbit penile tissue. Hsp90-eNOS association found to be decreased by high glucose in diabetes, pulmonary hypertension and sickle cell anemia-induced priapism. Thermal stimulation, calcium ionophore, shear stress, VEGF, exercise and hypoxia are known stimulators of hsp90. We showed that fluvastatin also induce hsp90 expressions and restore decreased endothelial relaxation through hsp90 activation in hypercholesterolemia-induced erectile dysfunction in rabbits.

Hydrogen sulfide (H2S) is another gasotransmitter in regulation of vascular tonus. NaHS induces hsp90 expression and hsp90 inhibitors decrease its antioxidant effect in cardiomyocytes. hsp90 also interacts with CSE in rat aorta and hsp90 inhibitor Geldanamycine inhibits the effect of testosterone on relaxation and H2S formation.

Other interactions of hsp90 with sGC and inhibitor-κB kinase-β (IKKB) also contribute to the regulation of vascular tonus by hsp90.

While activation of hsp90 can beneficial in erectile dysfunction or pulmonary hypertension, hsp90 inhibitors may provide double protection in inflammatory vascular diseases by inhibiting both eNOS and iNOS. Thus Hsp90 appears as a multitarget to control vascular tonus.

Acknowledgement: We thank Turkish Scientific Research Council TUBITAK for the grant #114s448 and the COST action BM1005 (ENOG: European Network on Gasotransmitters).

Control of human vascular tone by prostanoid pathway

Gökce Topal
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Prostanoids (prostaglandin (PG) and thromboxane (TxA2)) are produced from arachidonic acid in response to various stimuli by the sequential actions of cyclooxygenases and the respective synthases. Prostanoids are involved in control of vascular tone and remodeling of the vascular wall as well as in platelet aggregation and thrombosis. The different effects of prostanoids are also dependent on the activation of specific receptors, namely, the eight classical prostanoid receptors (DP, EP1–4, FP, IP, and TP) as well as the recently described chemoattractant receptor CRTH2 and nuclear receptors (PPARα,δ,γ). When the receptors are localized on the smooth muscle, the activation of IP, EP2, EP4, or DP receptors by prostanoids induces vasodilatation, while the activation of TP, EP1, EP3, or FP receptors is responsible for vasoconstriction. The role of the classical prostanoid receptors stimulated by PGI2 and TxA2 in the control of the human vascular tone has been largely documented. However, there are few studies concerning the involvement of other prostanoids (PGE2, PGF2α and PGD2) and their receptors in the control of the vascular tone. Accumulating evidences also suggest the key cardiovascular role for PGE2. The four EP receptor subtypes activated by PGE2 are present on the cells of the vascular wall during vascular inflammation. Their activation and, more specifically, the EP3 and EP4 receptor activation appears associated in most human physiological or pathophysiological responses of the vascular wall. Moreover, perivascular adipose tissue derived PGE2 has been also shown to involve in the control of human vascular tone. Therefore, these classical prostanoid receptors are also promising cardiovascular therapeutic targets. Consequently, characterization of prostanoid receptors in human vascular wall and perivascular adipose tissue will be presented in order to highlight the role of prostanoid pathway in the control of vascular tone under physiological and/or pathophysiological conditions.

Dysregulated NO/cGMP signalling in priapism

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Nitric oxide (NO) is the primary mediator of normal erectile function. Neuronal nitric oxide synthase (nNOS)-induced release of NO from cavernous nerves initiates erection, whereas both nNOS and endothelial nitric oxide synthase (eNOS)-induced release of NO from endothelial cells cause relaxation of cavernosal smooth muscle and maintain erectile response. NO acts primarily by stimulating production of cyclic guanosine monophosphate (cGMP) via guanylate cyclase activity in cavernosal smooth muscle cells and this signaling pathway is terminated by phosphodiesterase type 5 (PDE5) hydrolysis of cGMP in the penis. Priapism is an erectile disorder of abnormally prolonged supraphysiological erection, without sexual stimulation and with episodes often lasting longer than 4 h. Men with sickle cell disease (SCD) are particularly at risk, about 45% of men with SCD developing a urological emergency. Previously, SCD- associated priapism was considered as a consequence of thrombosis and vascular blockage, however, it is now acknowledged as a vasculopathy with a dysfunctional NO signalling. Several studies using transgenic SCD mouse models, which mimic priapic phenotypic changes seen in humans, have demonstrated a chronically impaired NO bioavailability causing a defect in the NO/cGMP/PDE5 signaling pathway in the cavernousal smooth muscle and endothelial cells. More recently, priapism in SCD mice has also been shown to be associated with dysfunctional signaling of RhoA/Rho kinase, dysregulation of opiorphin and adenosine signaling, as well as increased oxidative stresss in the penis. This presentation critically discusses the recent proposed contributory mechanisms and potential new treatments using animal models of priapism and also recent findings at human tissue level.

Vascular role of adipocytokines in health and diseases

Johan Van de Voorde
Vascular Research Unit, Department of Pharmacology, Gent University, BELGIUM

Adipocytes are no longer considered just as cells related to storage of energy and thermoregulation. Now we know that they release a huge number of paracrine and endocrine biologically active molecules, the so called adipo(cyto)kines. There is growing evidence that these adipo(cyto)kines may link obesity to cardiovascular diseases. The excessive adipocyte hypertrophy in obesity induces hypoxia in adipose tissue. This leads to adiposopathy, the process that converts “healthy” adipose tissue to “sick” adipose tissue. This is accompanied by a change in profile of adipo(cyto)kines released, with less production of the “healthy” adipo(cyto)kines such as adiponectin and omentin and more release of the “unhealthy” adipo(cyto)kines, ultimately leading to the development of cardiovascular diseases. Also perivascular adipose tissue (PVAT) that surrounds almost all blood vessels in the organism secretes adipo(cyto)kines that, because of its proximity, can easily influence vascular smooth muscle cells. The role of PVAT on vascular function can be both protective and deleterious. Normal healthy PVAT, as present in lean subjects, helps to keep the blood vessels dilated as its presence diminishes the effect of vasocontractile agents. Obesity is associated with an increased mass in PVAT. Excessive adipocyte hypertrophy may result in “adiposopathy” in which PVAT attracts macrophages and becomes a more inflammatory phenotype. This leads to a change in profile of the released adipo(cyto)kines, resulting in a decreased vasorelaxing effect of PVAT, which may be linked to obesity-induced hypertension. It also results in smooth muscle cell migration and proliferation and the development of atherosclerotic lesions. The increased knowledge of the functions of adipo(cyto)kines brings up new targets that can be useful to develop novel therapeutic and preventive strategies for obesity related cardiovascular diseases.

Elevated talk (C024): The effect of PVAT on vascular tone regulation of human coronary vessels: Role of H_2S and PGE_2

Gulsev Ozen[1], Ingrid Gomez[2], Larissa Kotelevets[3], Eric Chastre[3], Catherine Deschildre[4], Lilia Boubaya[4], Dan Longrois[5], B. Sonmez Uydes Dogan[6], Gokce Topal[6], Jean Baptiste Michel[4], Xavier Norel[4]

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Most of the human blood vessels are surrounded by perivascular adipose tissue (PVAT). Prostaglandin E_2 (PGE_2) and hydrogen sulphide (H_2S) released by PVAT could control vascular tone. We aimed to determine the effects of PGE_2 and H_2S on vascular tone of human coronary vessels (HCV) and interaction with PVAT.

Atherosclerotic HCV were obtained after heart transplantation for myocardial ischemia while healthy HCV were dissected after non-ischemic cardiomyopathy. Using an organ bath system, cumulative dose-response curves were established with various PGE_2 receptor (EP receptors) agonists, in the presence of different treatments (EP receptor antagonists or an inhibitor of CSE, enzyme responsible for H_2S synthesis), in both HVC preparations with or without PVAT. The release of H_2S and the density of CSE were measured using polarographic and western blot methods, respectively. EP3 receptor mRNA has been determined in HCV and their PVAT.

In HCV without PVAT, PGE_2 and the EP3receptor agonists induced concentration-dependent contractions. A selective EP3 receptor antagonist (L-826266) inhibited significantly the contraction induced by PGE_2 (control: E_{max}=133±17% *vs* L-826266: E_{max}=77±15%, n=5-13) while EP1-antagonists were ineffective in healthy HCV (similar result was obtained in atherosclerotic HCV). In the presence of PVAT, the contractile response to PGE_2 was significantly reduced (E_{max} = 73±06%, n=14) only in healthy HCV, this reduction was abolished after incubation with the EP3 receptor antagonist or CSE inhibitor. The EP3 receptor mRNA and CSE expression were detected in HCV and PVAT. H_2S production from PVAT were significantly decreased after EP3 receptor antagonist.

These results suggest that PGE_2 is responsible for HCV contraction via EP3 receptors present in vascular wall. In addition, PGE_2 could stimulate PVAT-vasorelaxant factor through the release of H_2S via EP3 receptor expressed in PVAT. This vasorelaxant effect is abolished in atherosclerotic HCV and could accelerate atherosclerosis and plaque rupture.

Keywords: perivascular adipose tissue, prostanoid, hydrogen sulphide, vascular tone, human coronary vessels

Elevated talk (C025): The role of voltage-dependent potassium channels in the relaxation of renal artery of diabetic rats

Ljiljana Gojkovic Bukarica[1], Jasmina Markovic Lipkovski[2], Helmut Heinle[3], Sanja Cirovic[2], Jovana Rajkovic[1], Vladimir Djokic[1], Radmila Novakovic[1]

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It has been documented that wine polyphenol, resveratrol reduced hyperglycemia and improved metabolic parameters in animal models of diabetes. The aim of our study was to investigate the role of voltage-sensitive potassium (Kv) channels in relaxation of renal artery (RA) of diabetic rats by resveratrol. Diabetes in male Wistar rats was induced by alloxan. Rings of RA were mounted in an organ bath for recording isometric tension. Contractions of RA were provoked by phenyephrine. The experiments followed a multiple curve design. Expression of different Kv channels (Kv1.1-6 and Kv4.2) in vascular wall of RA was evaluated by immunohistochemistry. Resveratrol relaxed RA of normal rats more potently than RA of rats with diabetes (EC_{50} were 8 and 50 microM, respectively). A nonselective blocker of Kv channels, 4-aminopyridine partly inhibited the relaxation of RA of normal as well as of diabetic rats. However, margatoxin, a selective antagonist of Kv1.3 channels, completely antagonized the relaxation of RA of diabetic rats only. In contrast, a selective antagonist of Kv4.2 channels, phrixotoxin antagonized the effect of resveratrol in the RA of normal rats only. In endothelium and media of healthy RA and in the media of diabetic RA, Kv1.1., Kv1.2, Kv1.6 and Kv4.2 channels were present. On the endothelium of diabetic rats Kv1.3 channel was present only. We have shown that resveratrol induces a stronger relaxation of RA of normal rats than diabetic rats. In endothelium of RA of diabetic rats almost all tested Kv channels are missing. It seems that resveratrol induced relaxation of RA of diabetic rats by an interaction with Kv1.3 channels.

Keywords: resveratrol, diabetes, rat renal artery, potassium channels

Plenary Lecture, Hall A (13:30-14:30)

Purinergic signaling in adult neural progenitor cell functions: Experimental basis and possible therapeutic significance

Peter Illes

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Sponsor: Hungarian Society for Experimental and Clinical Pharmacology

Purine receptors may be classified to the P1 und P2 types, responding to extracellular adenosine and ATP/ADP, respectively. P2 receptors consist of two subtypes termed P2X (ligand-gated cationic channels) and P2Y (G protein-coupled receptors). Adult neural progenitor cells (NPCs) are located at the so called neurogenic niches of the subventricular zone below the wall of the lateral ventricle and the subgranular zone of the hippocampal dentate gyrus. These NPCs pass through transient developmental states, and eventually give rise to neurons, which become integrated into existing neuronal circuits of the olphactory bulb and hippocampus. Whereas in case of neurodegenerative diseases newly formed NPCs replace e.g. damaged hippocampal neurons and are thereby beneficial, pathological seizure activity may have deleterious consequences. Status epilepticus causes the excessive proliferation NPCs, their migration to ectopic loci e.g. in the hilus hippocampi and consequently the chronic manifestation of a one-time epileptic fit. It is noteworthy that any type of damage afflicted onto neurons including metabolic limitation during epilepsy may release large quantities of ATP from neurons, glial cells and endothelium. The subsequently stimulated P2Y1 receptors will facilitate proliferation, migration and differentiation of NPCs, while P2X7 receptors will cause necrosis and apoptosis. Thus, the same ATP/ADP molecule might cause opposing effects, albeit at a different time scale, by both increasing the number of NPCs via P2Y1 receptor activation and decreasing this number by P2X7 receptor stimulation. We suggest that P2Y1 receptors after a status epilepticus may increase the ATP-induced proliferation/ectopic migration of NPCs; the P2X7 receptor mediated necrosis/apoptosis might counteract these effects, which would otherwise lead to a chronic manifestation of recurrent epileptic fits.

Oral Presentation Sessions (15:00-16:30)

Oral Presentation Session 6, Hall A

Neuropharmacology / Psychopharmacology(C065-C069)

Chairs: Aletta D Kraneveld (Utrecht University, The Netherlands)
Filippo Drago (University of Catania, Italy)

15:00 C065: Dietary interventions that reduce mTOR activity rescue autistic-like behavioral deficits in mice
Jiangbo Wu, Caroline Gm De Theije, Sofia Lopes Da Silva, Suzanne Abbering, Hilma Van Der Horst, Laus Broersen, Martien J Kas, Johan Garssen, Aletta D Kraneveld

15:15 C066: The effect of early life supplementation of non-digestible oligosaccharides on brain development and function in healthy mice
Kirsten Szklany, Cindy de Waard, Nienke G. van Staveren, Thecla A. van Wageningen, Monika Verdouw, Kees van Limpt, Harm Wopereis, Lucianne Groenink, Raish Oozeer, Leon M. J. Knippels, Johan Garssen, Aletta D. Kraneveld

15:30 C067: Investigation of the involvement of dopaminergic receptor subtypes in the gallic acid-induced antidepressant-like activity
Nazlı Turan, Özgür Devrim Can, Ümide Demir Özkay, Yusuf Öztürk

15:45 C068: Dopaminergic receptor subtypes mediated antidepressant-like activity of quercetin
Feyza Alyu, Ümide Demir Özkay, Özgür Devrim Can, Yusuf Öztürk

16:00 C069: From pharmacogenetics to personalized medicine: Regulatory perspectives and Implications for Latin-American countries
Diadelis Diade Remirez

C065: Dietary interventions that reduce mTOR activity rescue autistic-like behavioral deficits in mice

Jiangbo Wu[1], Caroline Gm De Theije[1], Sofia Lopes Da Silva[2], Suzanne Abbering[1], Hilma Van Der Horst[1], Laus Broersen[2], Martien J Kas[3], Johan Garssen[1], Aletta D Kraneveld[1]
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Enhanced mammalian target of rapamycin (mTOR) signaling pathway in the brain has been implicated in the pathogenesis of autism spectrum disorder (ASD). Inhibition of the mTOR signaling pathway improves behavior and neuropathology in mouse models of ASD containing mTOR-associated single gene mutations. The aim of the current study was to assess the effects of dietary interventions with an mTOR-targeting amino acid diet (Active 1 diet) or a multi-nutrient supplementation diet (Active 2 diet) on autistic-like behavior and mTOR signaling in food allergic mice and in inbred BTBR T+ Itpr3tf/J (BTBR) mice.

Male BTBR or C3H mice were fed a Control, Active 1, or Active 2 diet for 8 consecutive weeks, followed by behavioral tests. After two weeks of diet, C3H mice were orally sensitized and challenged with whey protein to induce cow's milk allergy (CMA). Brain samples were collected for western blotting analysis of mTOR signaling pathway.

CMA mice showed reduced social interaction and increased self-grooming behavior. Both diets reversed behavioral impairments in CMA mice and inhibited the mTOR activity in the prefrontal cortex and amygdala of CMA mice. In BTBR mice, Active 1 diet reduced repetitive self-grooming behavior and attenuated the mTOR activity in the prefrontal and somatosensory cortices.

The current results suggest that activated mTOR signaling pathway in the brain may be a convergent pathway in the pathogenesis of ASD bridging genetic background and environmental triggers (food allergy) and that mTOR over-activation could serve as a potential therapeutic target for the treatment of ASD.

Keywords: mammalian target of rapamycin (mTOR), food allergy, autism, mice, neuro-immmune interaction

C066: The effect of early life supplementation of non-digestible oligosaccharides on brain development and function in healthy mice

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Non-digestible oligosaccharides have immunomodulatory properties by changing the intestinal microbiome composition. Recent studies have shown that changes in microbiota composition also affect brain development and function. In a dietary intervention study with short chain galacto- and long chain fructo-oligosaccharides (scGOS:lcFOS), we investigated the effects of changes in microbiome metabolism on brain development and function of healthy BALB/c mice.

Dietary supplementation with or without 3% scGOS:lcFOS (9:1) was tested in male BALB/c mice starting from day of birth (n=10 per group). Social, anxiety-like, and stereotypic behaviors were studied by conducting social interaction, marble burying and self-grooming tests, respectively. Furthermore, cecal fatty acids, monoamine levels and mRNA expression of several brain markers in the brain have been assessed.

Male offspring receiving scGOS:lcFOS from day of birth and onwards showed changes in the serotonergic system. These neurological modulations were associated with behavioral changes: scGOS:lcFOS fed mice showed less anxious and repetitive behavior during development and increased social interest in adulthood. Alternated mRNA expression of astrocytic glial fibrillary acidic protein (GFAP) and microglial integrin alpha M (ITGAM) and brain-derived neurotrophic factor (BDNF) was detected in the brain of the scGOS:lcFOS group. Relatively increased levels of butyric acid and decreased levels of valeric, isobutyric and isovaleric acid were observed in cecal content of the scGOS:lcFOS fed mice.

Dietary supplementation with scGOS:lcFOS changes the serotonergic system and behavior in healthy mice. These neurological changes were accompanied by a suppression of microglia and astrocyte activation in the brain. In addition to this immune modulation in the brain, levels of the neuroprotective BDNF were enhanced. As SCFA have been shown to be crucial for microglia maturation, these neurological changes may be induced by the altered microbiota metabolism that was observed in these mice.

Keywords: non-digestible oligosaccharides, short-chain fatty acids, brain development and function, immune modulation, healthy mice

C067: Dopaminergic receptor subtypes mediated antidepressant-like activity of quercetin

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Antidepressant-like activity of quercetin has been reported by our research group, previously. Moreover, we exhibited that antidepressant-like activity of this flavonoid is mediated through an increase in serotonin and catecholamine levels in the synaptic cleft as well as through interactions with alpha-1 and beta-adrenergic receptors. Due to the lack of studies investigating the relationship between dopaminergic receptors and antidepressant-like activity of quercetin, in this study, we aimed to investigate the probable contribution of dopaminergic receptors to this pharmacological effect.

The possible contribution of the D_1 dopaminergic receptors to the anti-depressant-like effect of quercetin was evaluated using SCH 23390 (0.05 mg/kg, s.c.), whereas probable involvement of the D_2/ D_3 dopaminergic receptors was examined using sulpiride (50 mg/kg, i.p.). 15 min after the administrations of these antagonists, BALB/c strain mice were treated with quercetin or saline. Then, 60 min later, tail suspension tests were performed to examine the immobility time of animals. Spontaneous locomotor activities were assesed in an activity cage apparatus.

The experimental protocol was approved by the Local Ethical Committee on Animal Experimentation of Anadolu University, Eskişehir, Turkey.

In this study, as expected, quercetin significantly shortened the immobility time of mice in tail suspension test and did not induce any significant change in the locomotor activities. Additionally, SCH 23390 and sulpiride pretreatments significantly reversed the observed antidepressant-like activity of quercetin.

Data obtained from this mechanistic study exhibited that all D_1, D_2, and D_3 receptors are involved in the anti-depressant-like effect of quercetin. However, possible contribution of other systems (such as GABAergic, glutaminergic, and nitrergic systems) to the observed pharmacological effect of quercetin should also be investigated with further detailed studies.

Keywords: quercetin, antidepressant, tail suspension, dopaminergic receptors

C068: Investigation of the involvement of dopaminergic receptor subtypes in the gallic acid-induced antidepressant-like activity

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Our research group exhibited the antidepressant-like effect of gallic acid (GA) in mice, recently. In addition, in our previous studies, we demonstrated that the antidepressant-like effect of GA is dependent on increase in serotonin and catecholamine levels in the synaptic cleft as well as interaction with serotonergic 5-$HT_{2A/2C}$, 5-HT_3 and alpha-adrenoceptors. However, possible contribution of dopaminergic receptor to this pharmacological effect have not been studied, yet. This knowledge prompted us to investigate the role of dopaminergic receptors in the antidepressant-like effect of GA.

All experiments were carried out using adult BALB/c mice. Possible involvement of the dopaminergic receptor subtypes in the antidepressant-like effect of GA was investigated using SCH 23390 (a dopamine D_1 receptor antagonist, 0.05 mg/kg, s.c.), and sulpiride (a dopamine D_2/D_3 receptor antagonist, 50 mg/kg, i.p.). Mice were pre-treated with SCH 23390, sulpiride, or vehicle 15 min before the saline or GA administrations. 60 min after these administrations, immobility behavior was assesed in tail suspension test. Spontaneous locomotor activities of mice were registered by the activity cage apparatus. The experimental protocol was approved by the Anadolu University Animal Experiments Local Ethics Committee.

By supporting our previous findings, GA administrations caused significant decrease in the immobility time of mice without any change in spontaneous locomotor activities. The anti-immobility effect of GA was reversed with both SCH 23390 and sulpiride pre-treatments.

Data obtained from this study supported the previous findings demonstrating the antidepressant-like effect of GA. In addition, this study provides evidence that anti-immobility effect of GA occurs at least partly by activating dopaminergic D_1, D_2, and D_3 receptors. However, other mechanisms, for example mechanisms involving the opioidergic, GABAergic, glutaminergic, and nitrergic systems, may also be contributed to the antidepressant action of GA. Future studies are needed to clarify additional underlying mechanisms.

Keywords: gallic acid, antidepressant, tail suspension, dopaminergic receptors

C069: From pharmacogenetics to personalized medicine: Regulatory perspectives and Implications for Latin-American countries

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The science of pharmacogenomics has advanced significantly in the last five years, but it is still in infancy and is mostly used on research basis. The Pharmacogenomics helps identify interindividual variability's in drug response (both toxicity and effectiveness). This information will make it possible to individualize therapy with the intent of maximizing effectiveness and minimizing risk. The aims of this work are to present the bases of pharmacogenetic, the advantage and challenges of this specialty, the main enzymes characterized for the genetic polymorphism and the world and Cuban regulatory perspective about this subject. We will show the main advantages and disadvantages of this type of research for Latin American countries.

The hope for the future is that through personalized medicine, doctors and patients will be able to make better-informed choices about treatment. This treatment will avoid the adverse drug reaction to the medication and will improve the diagnosis diseases as well as the prevention and treatment of diseases.

Keywords: pharmacogenetics, regulations, Latin-america

Oral Presentation Session 7, Hall B

Cardiovascular Pharmacology (C070-C075)

Chairs: Fatma Akar (Gazi University, Turkey)
Pınar Yamantürk Çelik (İstanbul University, Turkey)

15:00 C070: Activation of PPARβ/δ improves endothelial dysfunction and protects kidney in a mouse model of systemic lupus erythematosus
Miguel Romero, Marta Toral, Iñaki Robles Vera, Rosario Jimenez, Francisco O'valle, Alba Rodriguez Nogales, Julio Galvez, Juan Duarte

15:15 C071: Effect of high glucose on vascular function and eNOS/Akt pathway in Isolated rat aorta: Modulatory roles of resveratrol and juglone
Fatma Akar, Mehmet Bilgehan Pektas, Ozge Turan, Gozde Ozturk, Gokhan Sadi

15:30 C072: Effects of taurine in a model of oxidative stresss induced by glutathione depletion in rabbit carotid arteries
Gonen Ozsarlak Sozer, Gulnur Sevin, Gunay Yetik Anacak, Hakan Ozgur, Zeliha Kerry

15:45 C073: Effects of psychoactive drugs on antihypertensive treatment
Sibel Akbilek Batmaz, Iliriana Alloqi Tahirbegolli, Bernard Tahirbegolli, Berrin Umman, Pınar Yamantürk Çelik

16:00 C074: Functional effects of P2X7 receptors in human internal thoracic arteries
Zeliha Bayram, Ikbal Ozen Kücükcetin, Sebahat Ozdem, Cahit Nacitarhan, Cengiz Türkay, Sadi S. Ozdem

16:15 C091: Investigation of the effects of vitamin U on ceacal flora in rats with liver and renal ischemia/reperfusion injury
Satı Zeynep Tekin, Özlem Öztopuz, Hakan Türkön, Aslı Kiraz, Muhammet Kasım Arık, Ufuk Demir, Sait Elmas, Mehmet Akif Ovalı, Uğur Altınışık*

16:30 C075: Prevention of progressive cardiac remodelling by histamine 2 receptor antagonism – a novel approach
Ajay Godwin Potnuri, Lingesh Allakonda, Arul Velan Appavoo, Renuka R Nair

* Transferred from Hall E.

C070: Activation of PPARβ/δ improves endothelial dysfunction and protects kidney in a mouse model of systemic lupus erythematosus

Miguel Romero[1], Marta Toral[1], Iñaki Robles Vera[1], Rosario Jimenez[2], Francisco O'valle[3], Alba Rodriguez Nogales[1], Julio Galvez[1], Juan Duarte[1]
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We tested whether GW0742, a peroxisome proliferator activator receptor beta/delta (PPARβ/δ) agonist, ameliorates hypertension, endothelial dysfunction and renal injury in a female mouse model of lupus.

Thirty-week-old NZBWF1 (lupus) and NZW/LacJ (control) mice were treated with GW0742, 5 mg/kg/day by oral gavage, or with GSK0660 1 mg/kg/day intraperitoneally plus GW0742 orally, for 5 weeks. At the end of the experiment, systolic blood pressure, plasma anti-dsDNA antibodies and cytokines, morphological indices, and proteinuria were measured. Histopathological evaluation of kidneys was also performed. Endothelial function, reactive oxygen species (ROS) levels and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity were tested in aorta. Flow cytometry was used to measure B and T cells from spleens. Protein and mRNA expression were measured by Western blotting analysis and RT-PCR analysis, respectively. Statistical analyses were performed using Graph Pad Prism 5 software.

GW0742 treatment did not alter lupus disease activity (assessed by plasma double-stranded DNA autoantibodies), but significantly ($P<0.05$) lowered blood pressure, cardiac and renal hypertrophy, splenomegaly, proteinuria and renal injury in lupus mice, but not in control. GW0742 did not modify the elevated Treg and B cells, whereas reduced Th1 cells in spleens from lupus mice. In addition, GW0742 lowered ($P<0.05$) the higher plasma concentration of interferon-γ and interleukin-21 observed in lupus mice. Aortae from lupus mice showed reduced endothelium-dependent vasodilator responses to acetylcholine, which were normalized by GW0742 treatment. Vascular ROS content and mRNA levels of NADPH subunits NOX-1, NOX-2 and p22phox were increased in lupus mice and reduced by GW0742. All these effects of GW0742 were inhibited by PPARβ/δ blockade with GSK0660.

PPARβ/δ may be an important target to control hypertension, endothelial dysfunction, and protect organ damage in severe lupus.

Keywords: systemic lupus erythematosus, PPARβ/δ, endothelial dysfunction, kidney

C071: Effect of high glucose on vascular function and eNOS/Akt pathway in isolated rat aorta: Modulatory roles of resveratrol and juglone

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Hyperglycemia causes cardiovascular disturbances. The aim of the present study was to investigate the potential effects of resveratrol and juglone on the acute high glucose-induced vascular endothelial dysfunction and to elucidate molecular mechanism underlying.

Aortic rings from 12-week-old male Wistar rats were incubated with high glucose (30 mM) for 30 min with or without resveratrol (10µM) or juglone (5µM). Endothelial relaxations to acetylcholine (ACh, 10^{-9}-10^{-5} M) were determined in the aortas. Western blotting was used to measure aortic levels of p-Akt and p-eNOS protein, whereas Real Time-PCR was utilized to quantify Akt and eNOS mRNA expressions.

Glucose incubation caused to impaired endothelial relaxation to Ach in the aorta, which is partially improved by resveratrol. Contrary, ACh-induced relaxation is further decreased by juglone treatment. The levels of p-Akt and p-eNOS protein were diminished after glucose incubation, which are promoted by resveratrol, but minimized by juglone treatment. mRNA expression of eNOS showed a similar profile to corresponding protein measurements. However, Akt mRNA levels did not change after the treatments.

High glucose-induced vascular dysfunction was positively correlated with the attenuation of the concentrations of aortic p-Akt, p-eNOS protein and mRNA expression. Resveratrol improved high glucose-induced vascular dysfunction possibly via activating eNOS/Akt pathway. Importantly, juglone seems to be a potential risk factor for vascular disorder.

Keywords: resveratrol, juglone, eNOS, Akt, endothelial and vascular function

C072: Effects of taurine in a model of oxidative stresss induced by glutathione depletion in rabbit carotid arteries

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Oxidative stress occurs when free radical formation and the antioxidant system is unbalanced. A reduction in glutathione (GSH) accompanies an increase in free radicals in inflammatory diseases. Taurine protects cells from the cytotoxic effects of inflammation. There have been limited studies to date evaluating the effect of taurine in oxidative stresss-induced vascular dysfunction and its role in vascular inflammatory diseases. Therefore, we aimed to investigate the effect of taurine on the regulation of vascular tonus and vascular inflammatory markers in rabbit aortae and carotid arteries in oxidative stress-induced by GSH depletion.

Rabbits were treated subcutaneously with buthionine sulfoximine (BSO), GSH-depleting compound and/or taurine. Cumulative concentration–response curves for acethylcholine (Ach), phenylephrine and 5-hydroxytriptamine (5-HT) were constructed with or without L-nitroω arginine (LNA) in the carotid artery and aorta rings. Immunohistochemical staining was perfomed for TNF-alpha and IL-1β.

BSO increased ACh-induced NO-dependent relaxations, phenylephrine-induced and 5-HT induced-contractions in the carotid artery. ACh-induced NO-dependent relaxations and augmented contractions were normalized by taurine (see Table). BSO increased TNF-alpha and IL-1β expressions. The BSO-induced increase in TNF-alpha was reversed by taurine.

Treatment with BSO resulted in vascular reactivity changes and increased immunostaining of TNF-alpha in mainly carotid arteries in this model of oxidative stresss. The inhibition of the increase in contraction and TNF-alpha expression by taurine in carotid arteries supports the proposal that taurine has a beneficial effect in the treatment of inflammatory diseases such as atherosclerosis.

This study is supported by grants from Ege University Scientific Research Fund, Project No: 06ECZ008 and 08ECZ019.

Keywords: buthionine sulfoximine, atherosclerosis, taurine, inflammation, rabbit

Table 1. Effects of BSO and taurine on relaxation and contraction responses.

Acetylcholine (precontracted with phenylephrine) E_{max} (%)	**Control group**	**BSO group**	**BSO+TAU group**	**Taurine group**
NO+	85,94± 1,73	92,81± 1,38*	85,50± 2,57+	84,35± 3,41
NO-	21,26± 5,22	20,92± 5,00	20,58 ± 4,60	23,68 ± 4,96
Phenylephrine E_{max}	**Control group**	**BSO group**	**BSO+TAU group**	**Taurine group**
NO+	2,62±0,44	4,51± 0,69*	3,69± 0,46	4,98± 0,46*
NO-	2,95± 0,47	5,38± 0,50**	3,83± 0,44*+	5,89± 0,63**
5-HT E_{max}	**Control group**	**BSO group**	**BSO+TAU group**	**Taurine group**
NO+	2,74± 0,64	5,67± 0,88*	3,33± 1,04+	4,89± 1,59
NO-	2,87± 1,79	5,68± 0,69*	3,78 ± 0,51	6,19 ± 1,33*

*ANOVA: *p<0.05 vs Control, **p<0.01 vs Control, +p<0.05 vs BSO, n=4-7*

C073: Effects of psychoactive drugs on antihypertensive treatment

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It is well-known that emotional states may affect blood pressure. In this regard, the purpose of this study was to assess the effects of psychoactive drug use on the success of hypertension treatment. A total of 131 hypertensive patients treated in outpatient clinic were enrolled in this observational study. Two study groups were formed, with one randomly selected group assigned from the patients treated only with antihypertensive drugs and the other assigned from the patients treated with the combination of antihypertensive drugs and psychoactive drugs. Body weights, heights and arterial blood pressures of the patients were measured. Their recent biochemical parameters of kidney and liver functions were recorded. The questions related with therapeutic and adverse effects of the drugs used were asked to them. Potential drug interactions were identified by the database Micromedex. SF-12 health survey was used to assess quality of life in patients. The sex, age and body mass index of the patients did not differ significantly between groups. The rate of successive blood pressure regulation in combination treatment group was significantly higher than in the group treated only with antihypertensive drugs. There were no significant difference in elevated values of BUN-kreatinin, AST-ALT parameters between the groups. Nevertheless, polypharmacy and major potencial drug interactions were significantly higher in the patients treated with both antihypertensive and psychoactive medications. Possible drug-related complaints has been found higher in this group, as well. On the other hand, while patients in combination treatment group had higher SF-12 physical compound score, there were no significant difference between groups in mental compound score. Obtained results suggest that psychoactive drug use in hypertensive patients may be of benefit in the control of blood pressure in case of needed, but the risk of potentially harmful drug interactions and adverse effects of drugs should be kept in mind.

Keywords: treatment of hypertension, psychoactive drugs

C074: Functional effects of P2X7 receptors in human internal thoracic arteries

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Temporary stasis of arteries during surgical procedures may result in increases in local blood levels of ATP and its metabolites especially during coronary bypass surgery. Therefore, we investigated the effects of selective P2X7 receptor activation on relaxation responses of human internal thoracic artery (ITA) preparations together with the levels of ischemia marker; ischemia-modified albumin (IMA) and the markers of endothelial function; von willebrand factor (vWF), thrombomodulin and soluble intracellular adhesion molecule-1(sICAM-1) in blood samples from ITA prone to temporary stasis by clamping during coronary bypass surgery.

Samples of ITA obtained during coronary bypass surgery were suspended in organ baths and the isometric tension responses were recorded by a computer-based data acquisition system. Blood levels of thrombomodulin and sICAM-1were determined with ELISA, IMA with spectrophotometry and vWF with immunoturbidimetric method.

Selective P2X7 receptor agonist Bz-ATP (10^{-10}-10^{-5} M) did not cause a significant alteration in tension of ITA preparations pre-contracted by phenylephrine (10^{-6} M). Relaxation responses of ITA preparations to cumulative concentrations of ATP, ADP, UTP, adenosine, isoprenaline, 8-bromo cAMP (10^{-8}- 10^{-4} M; for all), acetylcholine (10^{-9}- 10^{-5} M) and sodium nitroprusside (10^{-11}- 10^{-4} M) did not differ significantly following incubation of preparations with selective P2X7 receptor agonist Bz-ATP for 1 min. On the other hand, Bz-ATP incubation for 1 h caused significant reductions in vasodilatory effects of ATP and acetylcholine that were reversed by selective P2X7 receptor antagonists Brilliant Blue G or AZ11645373. Levels of thrombomodulin, sICAM-1, vWF and IMA in blood samples obtained before (pre-stasis) and after (post-stasis) clamping of ITA during coronary bypass surgery did not differ significantly.

Findings of the present study suggested that selective P2X7 receptor antagonists may be useful in preventing deterioration of vascular functions induced by P2X7 receptor activation for long-term following stasis that might increase local levels of ATP and its metabolites.

Keywords: ATP, human internal thoracic artery, purinergic system, P2X7 receptors, vasodilation

C075: Prevention of progressive cardiac remodelling by histamine 2 receptor antagonism – a novel approach

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Hypertension induced left ventricular hypertrophy (LVH) is an independent risk factor for cardiac failure. Histamine is found to be elevated in the failing hearts. However, the role of histamine-2 receptor (H2R) antagonism in prevention of LVH is unknown. We therefore aimed to investigate the effect of H2R antagonist famotidine (FAM) on prevention of LVH.
Six-month-old Spontaneously Hypertensive Rat (SHR) was treated with FAM (30 mg.kg-1.day-1 n = 8) or Metaprolol (MET) (50 mg.kg-1.day-1 n = 8) for 60 days. BP was measured noninvasively. Cardiac function was evaluated by 2D echocardiography. LVH was assessed by hypertrophy index (HI), myocyte cross-sectional area (MCSA), Sirius red staining, BNP and Procollagen type 1 pro-fibre (PCT1PF) levels, Hydroxyproline (HP) and calcineurin (CN) and Akt / p Akt expression. Lipid Peroxidation and GSH levels were measured. Data was presented as mean ± S.D and analysed by One-way ANOVA followed by Bonferroni post-hoc test.

All variables were significantly different in SHR compared to WST. In response to treatments, BP and cardiac function was improved (Table 1). Markers of LVH like HI, MCSA, BNP and Calcineurin A were normalised with treatment (Figure 1). Reduction in HP content (µg. g tissue-1: 224.16±12.84 [FAM], 204.16±9.13 [MET] Vs 298.5±17.55 [SHR]), PCT1PF (pg.mL-1:2418±319.4 [FAM], 2350.16±278 [MET] Vs 3589±296.5 [SHR]) and also normalized AKt / p Akt ratio(ratio: 1.91 ± 0.19 [FAM], 1.54±0.24 [MET] Vs 2.98±0.25 [SHR]). Both Treatments preserved glutathione (nMole.mg protein -1: 8.74 ± 0.99 [FAM], 9.98±1.65 [MET] Vs 5.67±1.22 [SHR]), Peroxiredoxin 3 levels (Fold change: 0.88 ± 0.09 [FAM], 1.1±0.09 [MET] Vs 0.4±0.05 [SHR] n=8) and reduced lipid peroxidation (nMole.mg protein -1: 24.54 ± 3.78 [FAM], 22.36±2.99 [MET] Vs 36.98±5.98 [SHR]).

In summary, the observations of this study identify H2R antagonism as a novel pharmacological approach for prevention of LVH.

Keywords: left ventricular hypertrophy, histamine 2 receptor, famotidine, spontaneously hypertensive rat

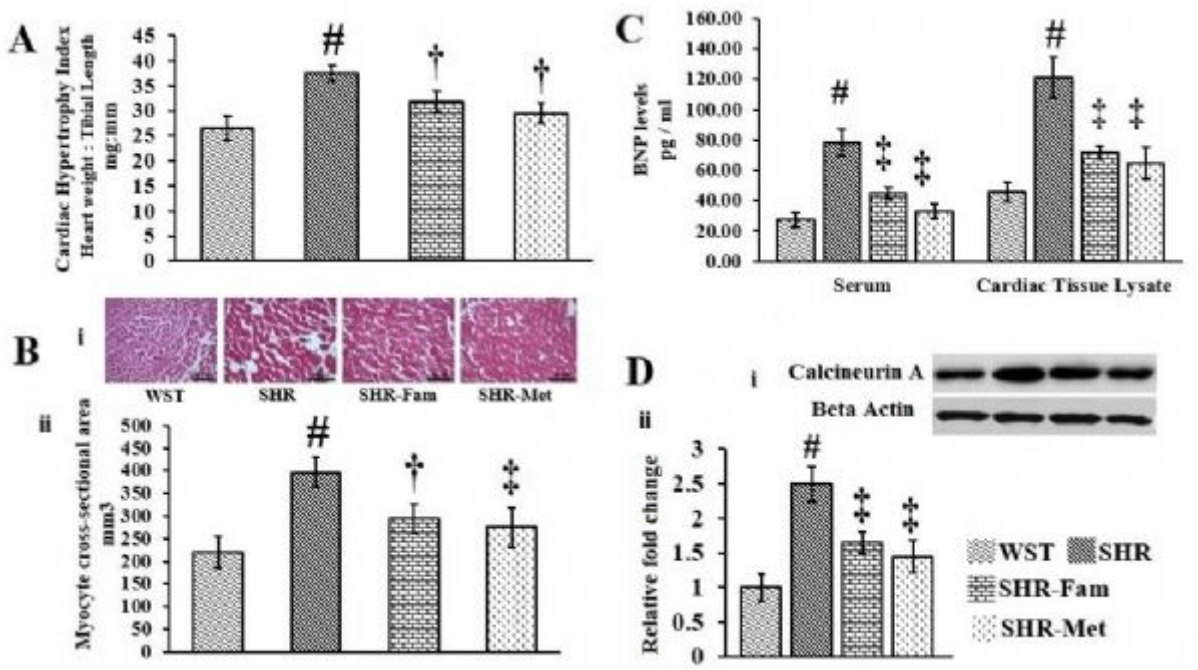

Figure 1. Effect of 60 days treatment with famotidine (30 mg.kg-1.day-1) and metoprolol (30 mg.kg-1.day-1) treatments on Cardiac Hypertrophy in 6 month old SHR. Metoprolol treated group served as positive control. (A) Hypertrophy index represented as heart weight (mg)/ tibia length (mm). (B i)Representative pictograph of histological sections of different treatment groups stained with haematoxylin and eosin for calculation of Myocyte cross-sectional area, B (ii) Graphical representation of effect of the treatments on myocyte cross sectional area (C) Graphical representation of the effect of the treatments on serum and cardiac tissue lysate levels of BNP, (D) Western blot analysis for determination of tissue Calcineurin A levels (i) Representative blots (ii) Graphical representation of the results. Data is represented as mean ± SD. Variation between groups was analysed by one way ANOVA followed by Bonferroni post hoc test.# p<0.01 *vs* WST, ‡ p<0.01 *vs* SHR and † p<0.05 *vs* SHR. ANOVA Fig. A: p<0.05, Fig B: p<0.05 Fig C: Serum: p<0.01 and for Cardiac tissue Lysate: p<0.001, and Fig D: p< 0.05.

Table 1. Effect of FAM and MET on BP and cardiac function in SHR.

Variable	WST (n=8)	SHR (n=8)	SHR- Fam (n=8)	SHR – Met (n=8)
MAP (mm of Hg)	98.8±6.62	137.5±9.56*	109.8±9.35‡	100.1±7.65‡
HR(beats.min-1)	345.8 ± 10.8	344.7 ±14	350 ± 10	354 ± 7.3
LVDD (cm)	0.616 ± 0.03	0.545 ±0.025#	0.591 ± 0.01 ‡	0.590 ± 0.021‡
LVSD (cm)	0.367 ± 0.02	0.283±0.02#	0.285±0.01	0.289±0.009
FS (%)	40.5 ± 0.9	47.9 ±3.3*	49.6±3.6#	49.4±2.7#
RWT	0.75 ± 0.002	0.84 ±0.04*	0.77±0.013#	0.76±0.005#
LVEDV (ml)	0.81 ± 0.07	0.63 ±0.025*	0.71±0.017#	0.713±0.02#
LVESV (ml)	0.040 ± 0.01	0.036 ±0.01*	0.0358±0.009#	0.0358 ±0.006#
E wave	63.8 ± 2.9	56.8 ±3.2*	56.3±1.7*	56.3 ±1.7*
A wave	24.5 ± 2.2	33.7 ±1.9*	28.8±2.26#	28.9 ±1.74#
IVRT	19.2 ±1.829	22.7±1*	19.9 ±1.9#	18.7 ±1#

** p<0.05 Vs WST, # p < 0.01 Vs WST, and ‡ p < 0.01 Vs SHR*

Oral Presentation Session 8, Hall C

Pharmacokinetics and Drug Metabolism (C076-C081)

Chairs: Abdullah Tuncay Demiryürek (University of Gaziantep, Turkey)
Selim Kortunay (Pamukkale University, Denizli, Turkey)

15:00 C076: Deprivation of the essential nutrient choline: the impact on drug metabolism
Charis Liapi, Dimitrios Segos, Hussam Al Humadi

15:15 C077: Evidence for the association between TRPM7 gene polymorphisms and preeclampsia
Belgin Alaşehirli, Zekiye Doğantürk, Elif Oğuz, Serdar Öztuzcu, Şeniz Demiryürek, Reyhan Gündüz, Mete Gürol Uğur, Abdullah Tuncay Demiryürek

15:30 C078: Evaluation of cytochrome P450 2C9 enzyme activity in patient with ankylosing spondylitis
Mustafa Tuğrul Göktaş, Halil Kara, Erdem Kamil Özer, İlknur Albayrak Gezer, Ümit Yaşar

15:45 C079: Determination of CYP2C9 activity in patients with systemic lupus erythematosus
Mustafa Tuğrul Göktaş, Erdem Kamil Özer, Ahmet Müderrisoğlu, Said Kalkışım, Emel C. Emlakcıoglu, Ümit Yaşar

16:00 C080: Pharmacokinetics of S-ketamine and S-norketamine after racemic or S-ketamine IV bolus administration in dogs during sevoflurane anaesthesia
Andrea Barbarossa, Noemi Romagnoli, Rima Bektas, Annette Kutter, Paola Roncada, Regula Bettschart Wolfensberger

16:15 C081: Pharmacokinetics of S-ketamine and S-norketamine following racemic or S-ketamine IV bolus administration in dogs premedicated with medetomidine
Noemi Romagnoli, Andrea Barbarossa, Rima Bektas, Annette Kutter, Paola Roncada, Regula Bettschart Wolfensberger

C076: Deprivation of the essential nutrient choline: The impact on drug metabolism

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Drug metabolism can be affected by various factors, either endogenous (such as genetic polymorphism and several pathologies) or exogenous (such as diet, exposure to drugs and life style factors, i.e. tobacco smoking and alcohol consumption)

Choline (Ch) is an important nutrient that is involved in many physiological functions (i.e., as metabolism of methyl groups and lipid transport) but also an essential component of various important biological compounds including the membrane. Deficiency of Ch, observed in various pathological (alcoholism and malnutrition) or physiological states (pregnancy and lactation) may lead to hepatocellular modifications and induction of fatty liver. In particular, dietary choline deprivation (CD) causes decreased tissue S-adenosyl-methionine levels, global DNA hypomethylation, hepatic steatosis, cirrhosis and hepatic tumorigenesis. Thus CD may modify the metabolic profile of the liver, in particular when combined to a concomitant drug administration.

The effect of CD upon biochemical, histological and metabolic alterations induced by two drugs that affect hepatic functional integrity and various drug metabolizing systems via distinct mechanisms, namely acetaminophen (ACET) and phenobarbital (PB), was studied in CD rats and normaly fed (FD) rats; ACET is a widely used antipyretic and analgesic drug which in overdose may cause centrilobular hepatic necrosis while PB is an antiepileptic drug widely used on an experimental basis as a prototype inducer of many CYP isozymes.

ACET down-regulated CYP1A2 and CYP2B1 expression in CD rats, while up-regulating them in NF rats; PB suppressed CYP1A2 apoprotein levels in CD rats, whereas it had no effect on NF rats. The PB-induced up-regulation of CYP2B, CYP2E1 and CYP1A1 isozymes was markedly higher in CD than in NF rats.

Choline deprivation caused modifications of the drug metabolizing pattern after exposure to ACET or PB. This event is of paramount significance as CD may alter the effectiveness of drug therapy and modify drug-drug interactions and toxicity.

Keywords: choline, paracetamol, phenobarbital, drug metabolism

C077: Evidence for the association between TRPM7 gene polymorphisms and preeclampsia

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Preeclampsia is a pregnancy-specific disorder characterized by de novo development of concurrent hypertension, proteinuria, and placental oxidative stresss. Preeclampsia is a major contributor to maternal and perinatal morbidity and mortality, and affects 7-10% of pregnancies. Transient receptor potential melastatin 7 (TRPM7) has been characterized as a magnesium-permeable ion channel involved in cellular magnesium homeostasis. Preeclampsia has been associated with impaired maternal mineral homeostasis, particularly related to magnesium. The aim of this study was to investigate a possible association between *TRPM7* gene polymorphisms and preeclampsia in a Turkish population.

A total of 93 patients with preeclampsia and 96 healthy controls with similar age and sex were enrolled to this study. Genomic DNA from the participants was analyzed by a BioMark 96.96 dynamic array system (Fluidigm, South San Francisco, CA, USA). For calculation of the significance of differences in genotype and allele frequencies, the chi-square test or Fisher's exact test was used.

There were marked changes in the genotype (TT, 36.7%; TC, 60.0%; CC, 3.3%) and allele (T, 66.7%; C, 33.3%) frequencies for the *TRPM7* gene rs62021060 polymorphism in patients when compared to the controls (TT, 60.7%; TC, 37.2%; CC, 2.1%, p=0.0051; T, 79.3%; C, 20.7%, p=0.0092). Genotype distribution (CC, 100%; CG, 0%; GG, 0%) and allele frequencies (C, 100%; G, 0%) of rs77165588 polymorphism in patients were significantly different compared to the controls (CC, 88.5%; CG, 0%; GG, 11.5%; p=0.0007, and C, 88.5%; G, 11.5%, p<0.0001). However, no association was found with the *TRPM7* rs8042919 (Thr1482Ile) polymorphism.

To the best of our knowledge, these results are the first to demonstrate that *TRPM7* gene polymorphisms may modify individual susceptibility to preeclampsia.

Keywords: polymorphism, preeclampsia, TRPM7

C078: Evaluation of Cytochrome P450 2C9 enzyme activity in patient with Ankylosing Spondylitis

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Ankylosing spondylitis (AS) is a systemic autoimmune disease characterized with chronic inflammation and ossification of the sacroiliac articulation and enthesis of the tendon. Approximately 0.2% of the general population suffers from AS, and its incidence is 0.1–1.4% in European population. Prevalence of AS was found as 0.49% in Turkish population. In previous study, we found that CYP2C9 activity was lower in Turkish Behçet's disease patients compared to healthy subjects. This study aims to compare the phenotype of CYP2C9 in patients with AS and healthy volunteers.

A total of 32 patient with AS and 97 healthy volunteers were included the study. Phenotyping of CYP2C9 was performed by using a HPLC method in urine collected for 8 hours after administration of a 50-mg single oral dose of losartan. Metabolic ratio (MR) of losartan to its metabolite E-3174 was calculated.

The average of age of AS group was 37.4±11.3 and 13 female and 19 male AS patients' losartan MR was calculated. The losartan MRs of AS group and healthy volunteers deviated from normal distribution according to the D'Agostino normality test ($p < 0.0001$). The median losartan MR was 1.4 in the AS patients and 0.8 in healthy volunteers ($p = 0.006$; t-test).

CYP2C9 enzyme activity was lower in AS group compared healthy volunteers. Our theory is that inflammatory markers related to AS might have reasoned the down-regulation of the CYP2C9 enzyme activity because of immune cytokine reactions. This study supports the findings that we obtained from patients with Behcet disease.

Keywords: ankylosing spondylitis, losartan, CYP2C9, HPLC

C079: Determination of CYP2C9 activity in Patients with Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE) is an autoimmune disease which is characterised by cellular and humoral immunity to self-antigens. Environmental, genetic and immunologic factors have roles in the pathogenesis of SLE. Plasma levels of pro-inflammatory cytokines (TNF-alpha, IL-6, and IL-18) were found higher in SLE patients than healthy controls. It has been reported that higher levels of pro-inflammatory cytokines tend to inhibit activities of CYP enzymes. The aim of this study was to evaluate CYP2C9 activity in patients with SLE.

Eighteen patients with SLE and 97 healthy volunteers were evaluated in the study. The activity of CYP2C9 was determined by using high pressure liquid chromatography in urine samples collected for 8 hours after administration of a 50-mg single oral dose of losartan. The losartan metabolic ratio (losartan/ E-3174 metabolite) in SLE patients and healthy controls were compared using Mann-Whitney U test.

Losartan / E-3174 ratio (as median and range) was 1.20 (0.34-6.16) and 0.86 (0.21-160.7) for SLE patients and healthy controls, respectively (Figure 1. p=0.12).

In this preliminary study, CYP2C9 activity seemed to be similar in both SLE patients and healthy controls. Currently, we recruit more SLE patients into the study.

Keywords: CYP2C9, HPLC, losartan, SLE

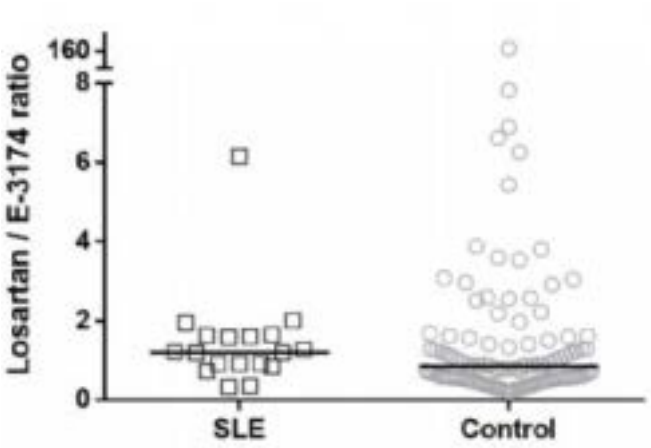

Figure 1. Comparison of the losartan / E-3174 metabolic ratios in SLE patients and in the healthy control

C080: Pharmacokinetics of S-ketamine and S-norketamine after racemic or S-ketamine IV bolus administration in dogs during sevoflurane anaesthesia

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The aim of the study was to investigate the pharmacokinetics of Ketamine enantiomers, and their metabolites, in dog anesthetised with sevoflurane.

Six adult healthy beagle dogs were used in a blinded randomised crossover study design. Anaesthesia was induced and maintained using 1.5 MAC sevoflurane for 240 minutes. An intravenous bolus of 4 mg/kg racemic ketamine (RS-KET) or 2 mg/kg S-ketamine (S-KET) was administered, with a three-weeks washout period between treatments. Venous blood samples were collected at fixed times (0, 1, 2, 5, 10, 20, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, 300, 360, 450, 540, 630, 720, 810 and 900 min) and S- and R-ketamine as well as S- and R-norketamine plasma levels determined by liquid chromatography coupled with tandem mass spectrometry. Cardiovascular parameters were recorded during the anesthesia until 240 min. Wilcoxon signed rank test was used to detect differences between the groups, significance level $p<0.05$.

All dogs recovered well from anaesthesia. No statistical differences between groups were detected in any cardiovascular parameter. A 2-compartment model best described the plasma concentrations. The pharmacokinetic parameters did not show any relevant difference. The area under the curve of R-norketamine was statistically higher than the one of S-norketamine in both groups (Table 1).

The disposition of racemic ketamine in dogs anesthetised with sevoflurane seems not to be stereoselective, leading to similar (not statistically different) concentrations of S- and R-norketamine.

The authors thank Prof. Wolfgang Thormann and Dr Regula Theurillat, from the Department of Clinical Pharmacology and Visceral Research, University of Bern, Switzerland, for the scientific support within the analytical part of the project.

Keywords: S-ketamine, S-norketamine, sevoflurane, pharmacokinetics, dog

Table 1. Means and standard deviations (SD) of pharmacokinetic variables for ketamine and norketamine enantiomers. p<0.05: difference between (a) and within (b) the groups.

Group	**S-KET**	**RS-KET**	**RS-KET**
Compound	S-ketamine	S-ketamine	R-ketamine
T1/2el (min)	28.48±5.74	28.64±5.41	30.27±4.84
T1/2el (min)	13.07±6.19	15.56±6.98	15.42±5.46
ClB (mL/min/kg)	72.68±14.72	71.82±12.80	67.48±10.68
Vss (L/kg)	0.68±0.37	0.75±0.37	0.75±0.33
	S-norketamine	S-norketamine	R-norketamine
AUC0→∞ (µg min/mL)	31.74±22.66 [a]	30.11±14.12 [b]	39.25±12.00 [a,b]
C_{max} (µg/mL)	0.37±0.14	0.40±0.13	0.37±0.12
T_{max} (min)	9.17±2.04	11.67±4.08	11.67±4.08

C081: Pharmacokinetics of S-ketamine and S-norketamine following racemic or S-ketamine IV bolus administration in dogs premedicated with medetomidine

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The current study investigated the pharmacokinetics of S-ketamine and racemic ketamine and their metabolites after medetomidine sedation in dogs.

Six adult healthy beagle dogs were used in a blinded, randomized, crossover study. After insertion of two venous catheters the dogs were sedated with intramuscular medetomidine (450 µg/m2). Twenty minutes (min) later 2 mg/kg S-ketamine (S-KET) or 4 mg/kg racemic ketamine (RS-KET) was administered intravenously, with a three-weeks washout period between the two treatments. Three mL of venous blood were collected at fixed times (-20, -5, 1, 2, 5, 10, 15, 20, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, 300, 360, 450, 540, 630, 720, 810 and 900 min) and plasma levels of S- and R-ketamine as well as S- and R-norketamine were determined by liquid chromatography/tandem mass spectrometry. Wilcoxon signed rank test was used to detect differences between the groups with a significance level of $p<0.05$.

A 2-compartment model best described the plasma concentrations following IV bolus administration of racemic ketamine and S-ketamine. There were no differences between the groups, except for a lower AUC for R-norketamine compared to the S-enantiomer (Table 1).

In dogs sedated with medetomidine following S-ketamine at 50% dose rate of racemic ketamine only differences in metabolite formation were found.

The authors thank Prof. Wolfgang Thormann and Dr Regula Theurillat, from the Department of Clinical Pharmacology and Visceral Research, University of Bern, Switzerland, for the scientific support within the analytical part of the project.

Keywords: S-ketamine, S-norketamine, enantiomers, pharmacokinetics, dog

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Table 1. Means and standard deviations (SD) of pharmacokinetic variables for ketamine and norketamine enantiomers. $p<0.05$: difference between (a, c) and within (b) the groups.

Group	**S-KET**	**RS-KET**	
Compound	**S-ketamine**	**S-ketamine**	**R-ketamine**
$AUC_{0\rightarrow\infty}$ (µg min/mL)	37.28±5.28	36.83±12.99	35.58±12.15
$T_{1/2el}$ (min)	43.77±20.12	46.02±18.51	46.74±18.07
Cl_B (mL/min/kg)	54.62±7.72	59.81±20.94	61.77±21.75
V_{ss} (L/kg)	1.79±0.69	1.94±0.52	2.03±0.56
	S-norketamine	**S-norketamine**	**R-norketamine**
$AUC_{0\rightarrow\infty}$ (µg min/mL)	3.27±0.94[a]	3.29±0.86[b]	1.45±0.25[a,b]
C_{max} (µg/mL)	0.05±0.01	0.05±0.01	0.04±0.01
T_{max} (min)	6.00±2.24	7.40±5.13	8.40±6.14

Oral Presentation Session 9, Hall D

Receptors and Cell Signalling (C082-C087)

Chairs: Mojca Krzan (University of Ljubljana, Slovenia)
Hakan Gürdal (Ankara University, Turkey)

15:00 C082: High glucose-induced SRC kinase activity causes 5-hydroxytryptamine receptor subtypes mediated EGFR transactivation in A7R5 cell
Sahika Guner, Tamila Akhayeva, Hakan Gurdal

15:15 C083: Deuteration afffects the binding but not functional characteristic of glial histamine H2 receptor
Mojca Krzan, Nika Jurisevic, Anze Zorc, Janez Mavri

15:30 C085: A novel proteolytic cleavage of ROCK 1 by caspase-2
Burçin İbişoğlu, Aysun Özdemir, Yaprak Dilber Şimay, Mustafa Ark

15:45 C086: Functionalized hyaluronic acid based biomaterial and assessment of its safety and wound healing activity in acute and diabetic skin wound models
Baiba Jansone, Joanna Jenina, Katrina Jukevica, Martins Boroduskis, Zane Dzirkale, Elga Poppela, Liene Patetko, Jelena Eglite, Elvira Hagina, Anna Ramata Stunda

16:00 C087: The reversal effect of ondansetron on bupivacaine induced sciatic nerve block in rats
Ali Ozgul Saltali, Seza Apiliogullari, Sengal Bagci Taylan, Mustafa Fevzi Sargon, Jale Bengi Celik, Ibrahim Ozkan Onal

16:15 C090: Antithrombotic effect of new cyclohexilammonium salt 2-[1-ethyl-3-methyl-7-(dioxothietanyl-3)xanthinyl-8-thio]acetic acid on experimental venous thrombosis
Aleksandr V. Samorodov, Felix Kh. Kamilov, Ferkat A. Khaliullin, Yuliya V. Shabalina, Almaz R. Khalimov, Daniyar Z. Murataev*

* Transferred from Hall E.

C082: High glucose-induced src kinase activity causes 5-Hydroxytryptamine receptor subtypes mediated EGFR transactivation in A7R5 cell

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5-Hydroxytryptamine (5-HT) receptors induce vasocontraction and have an important role in vascular system. We previously showed that epidermal growth factor receptor (EGFR) transactivation contributes to 5-HT-induced vasocontraction. Furthermore, we demonstrated that 5-HT2A and 5-HT1B receptors subtypes mediated EGFR transactivation are dependent on PI3-kinase and/or Src kinase in rat aorta. In this study, we investigated 5-HT receptor subtypes dependent EGFR transactivation mechanism in A7R5 rat vascular smooth muscle cell. Interestingly, 5-HT receptor subtypes mediated EGFR transactivation is only dependent on Src kinase in A7R5. These results are different from our previous findings. It has been shown that Src kinase activity can be induced by supraphysiological glucose concentration. High-glucose (25 mM) cell culture media is standardly used for culturing and growing vascular smooth muscle. Thus, we also investigated glucose-dependent Src kinase phosphorylation in A7R5 cell.

We first investigated non-selective agonist 5-HT, selective 5HT2A agonist α-Methyl-5HT and selective 5HT1B agonist Sumatriptan-mediated EGFR, PI-3 kinase, Src kinase phosphorylation in the absence and presence of EGFR inhibitor AG1478, Src kinase inhibitor PP2 and PI-3 kinase inhibitor LY 294002. Secondly, the effects of low and high-glucose on Src kinase and EGFR phosphorylation were studied in A7R5 cell. Statistical comparison was performed in 4- to 5 experiments using two-way ANOVA following Bonferroni post hoc test.

5-HT, α-Methyl-5HT and Sumatriptan approximately increased 2-to-3 fold EGFR, Src and PI-3 kinase phosphorylation. AG 1478 and PP2 decreased all-agonists dependent EGFR and PI-3 kinase phosphorylation. However, LY 294002 did not affect EGFR and Src kinase phosphorylation. Baseline and stimulated Src kinase and EGFR phosphorylation were increased by high glucose concentration in A7R5 cell.

High-glucose increased Src kinase activity in A7R5. Thus, Src kinase might be the only mechanism that has an effect on 5-HT receptor subtypes mediated EGFR transactivation.

Keywords: A7R5, transactivation, high-glucose, EGFR, serotonin receptors

C083: Deuteration affects the binding but not functional characteristic of glial histamine H_2 receptor

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A crucial step in the binding of histaminergic ligand to H_2 receptor represents a formation of three hydrogen bonds between amino acid residues of H_2 receptor and three nitrogen atoms of the ligand. In order to estimate the relevance of hydrogen bonds for the functional activity of receptor we compared the binding and functional properties of histamine and cimetidine to glial H_2 receptor in control and deuterated medium.

We performed inhibition binding studies using ^{3}H-tiotidine as a biomarker and histamine and cimetidine as displacers of radioligand binding in cultured glial cells in control and deuterated medium. In the next step we measuring cAMP production induced by histamine or inhibited by cimetidine in cultured neonatal astrocytes in control and deuterated medium.

Deuteration significantly increased the affinitiy of histamine towards H_2 recepotor binding sites - pIC50 of histamine changed from 7.25 ± 0.11 (control) to 7.80 ± 0.16 (D20 medium), $p < 0.05$; whereas cimetidine affinity (pIC50 7.7 ± 0.48 (control) changed to 7.6 ± 0.21 (D2O). Deuteration did not significanly affect either histamine-induced increase of cAMP formation or cimetidine-mediaded fall in cAMP production within astrocytes.

Deuteration results in attenuated strengths of hydrogen bonds involved in binding of ligands to receptor binding sites and in ligand-water interaction, where binding affinity is the difference between those two values. The opposing effects substantially change binding of an agonist (histamine) but not antagonist. Functional activity of H_2 receptor was not changed which can be rationalized by too short experiment to allow D2O to cross the cell membrane, and therefore the effects remained limited to the extracellular part of H_2 receptor.

This study is supported by Slovenian Research Agency grants, Project No: P3-067, J1-2014, P1-012.

Keywords: histamine H_2 receptor, histamine, cimetidine, deuteration, astrocytes

C085: A novel proteolytic cleavage of ROCK 1 by caspase-2

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Aspartate-specific cysteine proteases called caspases have roles in activating the protease cascade during apoptosis leading to the activation/inactivation of the key structural proteins and cellular signalings. Caspase-2 is one of the first discovered caspases and the most highly conserved caspase among many species. Although, caspase-2 structurally belongs to initiator caspases, its substrate specificity is similar to effector caspases. On the other hand, it has been shown that small G protein Rho and its downstream effector Rho kinases (ROCK 1 and ROCK 2) are involved in several pathophysiologic mechanisms such as cell contraction, proliferation, apoptosis and cell migration. It has been previously demonstrated that both ROCK 1 and ROCK 2 are cleaved by caspase-3 and caspase-2, respectively. In the current work, we also observed that ROCK 1 was cleaved in oxaliplatin-induced cell death (300 µM, 48h) in caspase-3-deficient MCF-7 cells and that cleavage was sensitive to caspase inhibitors. Oxaliplatin-induced ROCK 1 cleavage was totally prevented by the preincubation of pan caspase inhibitor, z-VAD-fmk (50 µM, 2h), and partially inhibited by the preincubation of caspase-3/7 inhibitor, z-DEVD-fmk (50 µM, 2h) and caspase-2 inhibitor, z-VDVAD-fmk (50 µM, 2h). This finding indicates that ROCK 1 might be also cleaved by caspase 2. To confirm whether caspase-2 cleaves ROCK 1, we incubated three different cell lysates (HeLa, MCF-7 and MDA-MB-231) with recombinant active caspase-2. Cleavage of ROCK 1 was also detected after active caspase-2 incubation in all three cell lysates. To the best of our knowledge, this is the first demonstration of caspase-2 induced ROCK 1 cleavage in cell death. Our findings also indicate that ROCK 1 could have a new cleavage site for caspase-2 and this cleavage process might be a new activation/inactivation mechanism for ROCK 1.

Keywords: apoptosis, caspase, rho-rho kinase

C086: Functionalized hyaluronic acid based biomaterial and assessment of its safety and wound healing activity in acute and diabetic skin wound models

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Ageing of the general population and increasing incidence of chronic diseases, leads to growing demand for efficient wound healing products. The objective of this study was to evaluate the safety and skin healing efficacy of a new biomaterial where the matrix of hyaluronic acid (HA) is functionalized with natural immunomodulatory glycoprotein. Evaluation was aimed to affirm that improved HA matrix structure together with added immunomodulatory activity is efficient in wound healing.

To evaluate wound healing efficacy experimental acute skin wounds where created on dorsal area of Wistar male rats. In case of diabetic wound model diabetes was induced by streptozotocin prior to wounding. Wound photographs where taken on days 0, 2, 4, 6, 8, 12 and wound area measured to analyse healing dynamics. Plasma levels of TNF-alpha, IL-1β, IL-6, VEGF, IFNγ, IL-10, IL-18, IP-10, MIP-1α, GRO/KC on days 6 and 12 were detected. In diabetic animals wound fluid was collected on days 2 and 4 and CTFG levels evaluated. Skin tissue samples were taken on days 6 and 12 and stained with hematoxilin-eosin and Massson's trichrome.

Positive dynamics of wound healing was observed in all groups compared to control, besides the functionalized biomaterial proved to be more effective promoter of reepitelization. Application of the new biomaterial on wounds did not affect plasma levels of cytokines and growth factors. Minor changes of CTGF levels in wound fluids were observed in diabetic wounds. Histological analysis revealed normal skin structure and typical deposition of collagen in dermis.

HA based biomaterial functionalized with natural immunomodulator proved to be safe and efficient for local applications – it promotes reepitelization without evoking systemic effects. Findings point to the potential of the biomaterial to be effectively used in clinical practice.

This study is supported by ERDF, Project No. 2014/0044/2DP/2.1.1.1.0/14/APIA/VIAA/046.

Keywords: natural immunomodulator, wound healing, rats

C087: The reversal effect of ondansetron on bupivacaine induced sciatic nerve block in rats

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There is evidence that intravenous ondansetron, a 5HT3 receptor antagonist, shortens sensory but not motor block durations of local anesthetics. The reverse of the motor block is essential for hospital discharge criteria after regional anesthesia. The rapid regression of sensory block in the presence of motor block is a disadvantage for the patient throughout the perioperative period. This study was designed to test the hypothesis that perineural ondansetron, would decrease the duration of motor block, sensory block, and proprioceptive block in a dose-dependent fashion in bupivacaine-induced sciatic nerve blockade.

After Institutional approval, forty-nine Wistar rats were randomized into seven experimental groups (each group n=7): Group B received a perineural injection of 1000µg (0.2ml) of bupivacaine; Group BO200 received perineural 1000µg of bupivacaine+200µg (0.4ml) of ondansetron 10min later; Group BO400 received perineural 1000µg of bupivacaine and 400µg (0.4ml) of ondansetron 10min later; Group BO800 received perineural 1000µg of bupivacaine and 800µg(0.4ml) of ondansetron 10min later; Group BO800IP received a perineural 1000µg of bupivacaine and an intraperitoneal injection of 800µg (0.4ml) of ondansetron 10min later; Group O800 received perineural 800µg of ondansetron (0.4ml); and Group S was sham operated. A blinded investigator assessed motor sensory and proprioceptive function every 10min until the return of normal function. The sciatic nerves were checked for neurotoxicity with electron microscopy. Statistical comparisons were made using Kruskal-Wallis H test followed by Man Whitney U (with Bonferroni correction). $p<0.05$=significant.

Block properties are in Table 1. Intraperitoneal ondansetron has no reversal effect. Perineural ondansetron had no local anesthetic effect. Perineural ondansetron injection was not neurotoxic.

This is the first experimental study to show that perineural ondansetron decreases the duration of motor, sensory, and proprioception blockade in rats, in dose dependent fashion. These findings are an essential first step in encouraging future efficacy studies in humans.

Keywords: antagonist, local anesthetics, ondansetron, rat

Table 1. Durations of motor block, sensory block and proprioceptive block of the groups.

	Motor block	Sensory block	Proprioceptive block
Group B (n=7)	95.5±10.3	91.2±8.0	90.7±8.9
Group BO200 (n=7)	95.7±7.2	90.2±5.9	80.4±23.3
Group BO400 (n=7)	87.1±12.9	89.8±12.3	63.4±30.4
Group BO800 (n=7)	67.4±2.3[a,b,c]	67.4±2.3[a,b,c,d]	48.4±8.2[a]
Group BO800ip (n=7)	97.8±22.9	97.8±22.9	85.5±23.4

Results are minutes, Mean±SD. Grup B= bupivacaine alone, Grup BO200= bupivacaine+perineural 200µg ondansetron, Grup BO400= bupivacaine+perineural 400µg ondansetron, Grup BO800= bupivacaine+perineural 800µg ondansetron, Group BO800ip= bupivacaine+intraperitoneal 800µg ondansetron. [a]p<0.05 compared to Group B, [b]p<0.05 compared to Group BO200, [c]p<0.05 compared to Group BO800ip, [d]p<0.05 compared to Group BO400.

Oral Presentation Session 10, Hall E

Drug Discovery, Development and Evaluation (C088-C092)

This session is cancelled.

C088: In-vivo evaluation of the preventive effect of the ethanolic leaf extract of Hibiscus rosa-sinensis (Malvaceae) against calcium oxalate crystals in ethylene glycol- and ammonium chloride-induced hyperoxaluria in adult male albino Swiss mice

Rhemcee Pascual, Monica Del Rosario, Jarrah Patacsil, May Pleños, Prescilla San Pedro, Shannen Suñga, Jose Villanueva, Aleth Dacanay, May Magtoto, Gregory Martin

No registration, abstract will be deleted.

C089: Dimethoxyflavone isolated from the stem bark of Stereospermum kunthianum possesses antidiarrhoeal activity in rodents

Fidelis Poh Ching, Otokiti Abrahim Nosahare

No registration, abstract will be deleted.

C090: Antithrombotic effect of new cyclohexilammonium salt 2-[1-ethyl-3-methyl-7-(dioxothietanyl-3)xanthinyl-8-thio]acetic acid on experimental venous thrombosis

Aleksandr V. Samorodov, Felix Kh. Kamilov, Ferkat A. Khaliullin, Yuliya V. Shabalina, Almaz R. Khalimov, Daniyar Z. Murataev

Transferred to Hall B, 16:15

C091: Investigation of the effects of vitamin U on ceacal flora in rats with liver and renal ischemia/reperfusion injury

Satı Zeynep Tekin, Özlem Öztopuz, Hakan Türkön, Aslı Kiraz, Muhammet Kasım Arık, Ufuk Demir, Sait Elmas, Mehmet Akif Ovalı, Uğur Altınışık

Transferred to Hall B, 16:15

C092: Inhibitory effect of hydroalcoholic extract of green tea on cognitive impairment and oxidative stresss induced by streptozocin in rats

Amin Ataee, Ramin Ataee

No registration, abstract will be deleted.

C088: In-vivo evaluation of the preventive effect of the ethanolic leaf extract of Hibiscus rosa-sinensis (Malvaceae) against calcium oxalate crystals in ethylene glycol- and ammonium chloride-induced hyperoxaluria in adult male albino Swiss mice

Rhemcee Pascual, Monica Del Rosario, Jarrah Patacsil, May Pleños, Prescilla San Pedro, Shannen Suñga, Jose Villanueva, Aleth Dacanay, May Magtoto, Gregory Martin
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Urolithiasis, the formation of stones that is caused by the supersaturation of urine. *Hibiscus rosa-sinensis* Linn. (Malvaceae), known as Gumamela in the Philippines, is known for having numerous pharmacologic uses. Having the same genus as *Hibiscus sabdariffa,* a proven anti-urolithiatic agent, the researchers choose Gumamela as the plant to be evaluated for its anti-urolithiatic activity. Thirty-six male Albino Swiss mice were randomly divided into 6 equal groups. Group I served as the normal group. Groups II, III, IV, V, and VI received ethylene glycol (0.75% v/v) and ammonium chloride (2% w/v) from day 4 up to day 10. Group II served as the negative control. Alongside, group III (positive control) received 25mg/kg Hydrochlorothiazide; groups IV, V, and VI (preventive groups) received 250mg/kg, 500mg/kg, and 1000mg/kg of ethanolic leaf extract respectively from day 1 up to day 10, considering the first three days as prophylaxis. The 24-hour urine samples were collected on day 0, 3 and 10 have been examined for the presence of calcium oxalate (CaC_2O_4) crystals. On the 10th day, sub-mandibular blood collection was used to collect for testing of biochemical parameters. The urine crystals of the group I ($p=0.105$), group III ($p = 0.368$), group IV ($p =0.097$) and group V ($p =0.174$) did not have significant changes. However, the urine crystals of the group II significantly increased ($p=0.022$), specifically at day 10, while the urine crystals of the group VI had continuous decrease ($p=0.016$) from baseline to day 10. The decrease in calcium oxalate crystallization was due to the ability of the ethanolic leaf extract to prevent urinary supersaturation of calcium oxalate in a dose dependent manner. To conclude, *Hibiscus rosa-sinensis* Linn. (Malvaceae) ethanolic extract has a significant therapeutic potential for the prevention and inhibition of the formation of CaC_2O_4 induced crystals.

Keywords: urolithiasis, calcium oxalate, blood urea nitrogen, serum creatinine, Swiss albino mice

C089: Dimethoxyflavone isolated from the stem bark of Stereospermum kunthianum possesses antidiarrhoeal activity in rodents

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This study was undertaken to evaluate the antidiarrhoeal activity of 3, 7, 4/-trihydroxy-3/-(8//-acetoxy-7//-methyloctyl)-5,6-dimethoxyflavone, a flavonoid isolated from the stem bark of Stereospermum kunthianum.
The antidiarrhoeal activity was evaluated using rodent models with diarrhoea. The normal intestinal transit, castor oil-induced intestinal transit and castor oil-induced diarrhoea tests in mice as well as castor oil-induced intestinal fluid accumulation in rats were employed in the study. The animals were pretreated with distilled water (10 ml/kg for mice, 5 ml/kg for rats), dimethoxyflavone (25 mg/kg or 50 mg/kg), morphine (10 mg/kg), or indomethacin (10 mg/kg) before induction of diarrhoea with castor oil (0.2ml for mice and 2ml for rats).

Dimethoxyflavone dose dependently and significantly reduced ($P<0.05$) castor oil-induced intestinal motility. Its antimotility effect at the dose of 50 mg/kg was higher compared to that of morphine (10 mg/kg). Dimethoxyflavone (25 mg/kg and 50 mg/kg) caused a delay in the onset of diarrhoea, reduction in the number and weight of wet stools and total stools in mice with castor oil-induced diarrhoea compared to the distilled water treated mice. Treatment with dimethoxyflavone (25 mg/kg or 50 mg/kg) did not produce any remarkable effect on castor oil-induced intestinal fluid accumulation in rats and normal intestinal transit in mice. The results indicate that dimethoxyflavone possesses antidiarrhoeal activity due to its intestinal antimotility effect and inhibition of other diarrhoeal pathophysiological processes.

Our results taken together indicate that dimethoxyflavone isolated from Stereospermum kunthianum stem bark reduced the frequency and severity of diarrhoea in the diarrhoeal models studied.

Keywords: dimethoxyflavone, stereospermum kunthianum, antidiarrhoeal activity, rodents

C090: Antithrombotic effect of new cyclohexilammonium salt 2-[1-ethyl-3-methyl-7-(dioxothietanyl-3)xanthinyl-8-thio]acetic acid on experimental venous thrombosis

Aleksandr V. Samorodov, Felix Kh. Kamilov, Ferkat A. Khaliullin, Yuliya V. Shabalina, Almaz R. Khalimov, Daniyar Z. Murataev
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The previous findings in vitro show high antiaggregation activity of newly synthesized cyclohexilammonium salt of 2-[1-ethil-3-methyl-7-(dioxotiethanyl-3)xantinyl-8-thio]acetic acid. The current findings are received in vivo on the model of thrombosis IVC (vena cava inferior). Male rats (200-250 g) were used for all studies and all protocols were approved by the University of Bashkirian State Medical (BSM) Animal Care Committee. Thrombosis was induced by IVC ligation to produce a stasis thrombus as described. Briefly, a laparotomy with ligation of the IVC below the renal veins and all visible side branches was performed. Rats received treatment beginning 24 hours after a stasis venous. The survival analysis was carried out with the help of Kaplan-Meier method. The difference in survival between the groups was assessed with the help of Wilkonson criterion. Intraperinoneal injection of the new secondary 1-ethylxantin reduced mortality in lab rats by 1,6 times ($p<0.01$) in comparison with the camparator agents. Thus, cyclohexilammonium salt of 2-[1-ethil-3-methyl-7-(dioxotiethanyl-3)xantinyl-8-thio]acetic acid is a potential antithrombotic agent.

Keywords: 1-ethylxanthine derivatives, hemostasis, antiaggregation activity

C091: Investigation of the effects of vitamin U on ceacal flora in rats with liver and renal ischemia/reperfusion injury

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Perfusion of the bowel is one of the most affecting factors in intestinal bacterial flora. Bacteria in intestinal flora may cross the intestinal wall and cause infections in other organs in case of ischemia/reperfusion(I/R) injury. Vitamin U is a substance with anti-inflammatory, antioxidant properties affecting the gastrointestinal system. We aimed to investigate the effects of Vitamin U on ceacal flora in rats with liver and renal I/R injury.

After ethical approval, 45 adult, male Wistar albino rats were randomly divided into six groups (Table 1). After procedures lasting for 15 days, laparotomy was performed in all rats and one gram of ceacal contents were collected. Sterile physiological saline solution of 9 mL was added to all ceacal contents and consecutive 10-fold serial dilutions were prepared with a final concentration of 10^{-8} M. The diluted samples were inoculated on Plate Count agar and Violet Red Bile Glucose agar to determine the total number of enteric bacteria. After incubation at 37 °C for 48 hours, isolated bacterial counts from the plates were recorded as CFU/g (colony forming unit/gram).

Total aerobic bacterial counts of groups given vitamin U were significantly lower than groups given distilled water in all plates inoculated with ceacal samples in all dilutions.

Vitamin U reduced the bacterial count isolated by conventional aerobic culture methods we used, however other bacteria that could not be isolated with this method were not counted and this is a limitation of our study. It is known that damages induced by I/R injury may result in bacterial translocation (BT). Critically ill patients are at risk to develope BT. We think that, addition of vitamin U on enteral nutrition solutions may be helpful in recovery of critically ill patients predisposing to BT by reducing enteric bacterial count.

Keywords: enteric bacteria, bacterial translocation, vitamin U, rat

Table 1. Groups and applied procedures

Groups	**Procedures**
Group 1	Intragastric gavage with vitamin U for 15 days + I/R injury in liver + ceacal sample collection with laparotomy
Group 2	Intragastric gavage with vitamin U for 15 days + renal I/R injury + ceacal sample collection with laparotomy
Group 3	Intragastric gavage with vitamin U for 15 days + exploration of hepatic artery and portal vein (without I/R injury) + ceacal sample collection with laparotomy
Group 4	Intragastric gavage with distilled water for 15 days + renal I/R injury + ceacal sample collection with laparotomy
Group 5	Intragastric gavage with distilled water for 15 days + I/R injury in liver + ceacal sample collection with laparotomy
Group 6	Intragastric gavage with distilled water for 15 days + exploration of hepatic artery and portal vein (without I/R injury) + ceacal sample collection with laparotomy

C092: Inhibitory effect of hydroalcoholic extract of green tea on cognitive impairment and oxidative stresss induced by streptozocin in rats

Amin Ataee[1], Ramin Ataee[2]
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Alzheimer's disease (AD) is the most common kind of progressive neurodegenerative dementia of the aged brain, characterized by disturbance of cognitive functions. Many promising chemicals have failed because of therapeutic limitations i.e. only making symptomatic relief.The antioxidant system, is known to contribute to the etiology of some conditions affecting neurodegenerative disorders, notably AD.This imbalance may originate from an overproduction of free radicals or from a reduction in antioxidant defenses. Streptozotocin (STZ), is administered in lateral ventricles, that is characterized by long-term and progressive deficits in learning, memory, and cognitive behavior. Green tea has been proven as anti-oxidative herbal agent which its' use in prevention of some neurodegenerative disease as parkinson, Alzheimer and depression. Antioxidative properties of green tea belong to polyphenol compounds as cathechin and epi gallo- cathechin, according to these background we planned to study preventive role of green tea extract on streptozocin related brain -oxidative stresss and cognitive impairment.

We administered 3mg/kg i.c.v twice in 48 hr interval between each injection to wistar male rats,green tea hydro alcoholic extract administered for 3 weeks by gavage (100 mg/kg/1ml), after than animals have gone for behavioral cognitive studies through passive avoidance test by shutle box and after killing their brain tissue have been assayed for oxidative stresss.

Cognitive impairment observed in animals which received streptozocin as increased their latency time and green tea could have decreased their latency time and green tea can prevent this impairment. Also green tea extract could ameliorate oxidative stresss parameter as MDA, Glutation, Catalase and SOD significantly.

According to results of this experiment, we can suggest green tea extract for old people who are at risk of Alzheimer and also may involve diabetes.

Keywords: Alzheimer, streptozocin, green tea, passive avoidance, oxidative stresss

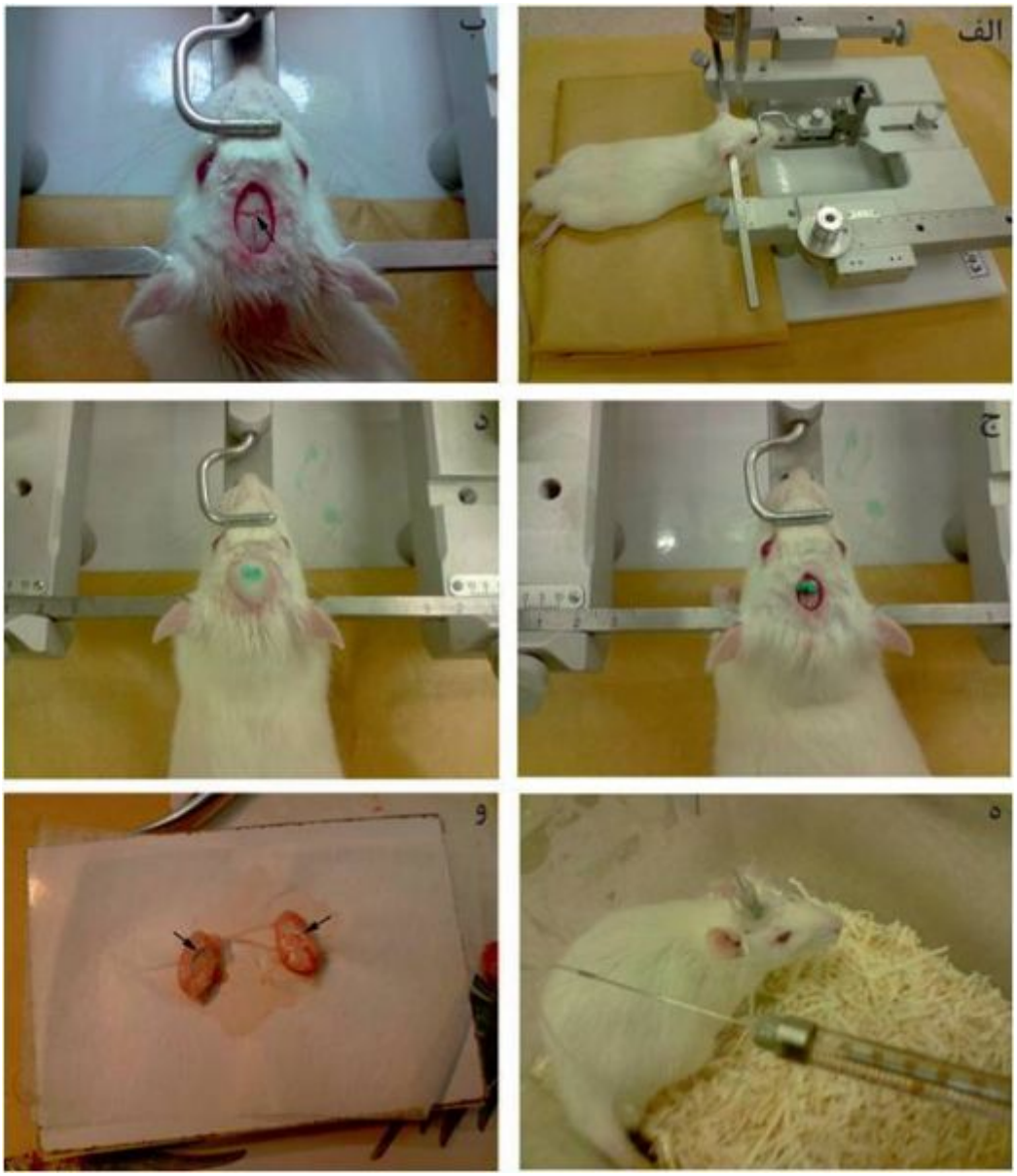

Figure 1. Animal cerebroventicular drug administration.

Wednesday, 29th June 2016

Main Sessions (09:00-12:00)

Hall A:
Dopamine signaling in health and disease

(Organized by Danish Society of Pharmacology)
Organizers and chairs:
Claus Juul Loland (Kopenhag University, DENMARK)
09:00-12:00

09:00 Development and binding characterization of high-affinity modafinil analogous as potential antagonizers of cocaine subjective effects
Claus Juul Loland
Kopenhag University, DENMARK

09:30 Modulation of dopaminergic pathways to improve erectile function
Ulf Simonsen
Aarhus Universitet, DENMARK

10:00 The dopamine transporter as target for new psychoactive substances: a mechanism of action
Harald Sitte
Medical University of Vienna, AUSTRIA

10:30 Coffee break

11:00 Modulating dopamine neurons in visual attention using pharmacogenetics
David P. Woldbye
Kopenhag University, DENMARK

11:30 Elevated talk (C026): Ropinirole prevents the addiction and withdrawal symptoms of morphine induced conditioned place preference in rats
Andleeb Shahzadi
Istanbul University, TURKEY
(Authors: Enes Karabulut, Oruc Allahverdiyev, Andleeb Shahzadi, Zeliha Yazici)

11:45 Elevated talk (C027): Antidepressant-like effects of Baijin Capsule in the olfactory bulbectomized rat
Jianning Sun
Beijing University of Chinese Medicine, CHINA
(Authors: Rong Zhang, Yaoyue Liang, Shifen Dong, Jian Ni, Jianning Sun)

12:00 End of symposium

Development and binding characterization of high-affinity modafinil analogous as potential antagonizers of cocaine subjective effects

Claus Juul Loland
Kopenhag University, DENMARK

Cocaine abuse and addiction is a growing worldwide problem to which there exists no medical treatment. Cocaine act as an inhibitor of the dopamine transporter (DAT) causing a rapid and potent rise in extracellular dopamine levels. This effect is proposed to be directly linked to the stimulant and rewarding effects of cocaine. Modafinil is also a DAT inhibitor but with about 10-fold lower DAT inhibition potency than cocaine. Even though modafinil binds competitively to cocaine it does not seem to possess the stimulant and rewarding effects. Accordingly, modafinil could serve as a possible lead for the development of a cocaine antagonist. Here, we investigate the differences in binding properties between cocaine and modafinil. We show that modafinil binds to a DAT conformation that is more occluded to the extracellular environment. Dialysates from rat nucleus accumbens (NAc) show that the rate of dopamine increase by modafinil is markedly slower than for cocaine. Also the maximal achieved effect is ~50% of cocaine. We propose that this atypical binding mode for modafinil could constitute for the pharmacological differences to cocaine. Accordingly, modafinil analogues with increased potency but with similar atypical binding properties were screened. We found a modafinil analogue JJC8-016 with atypical inhibition properties but possessing higher affinity for DAT. In addition, JJC8-016 administration inhibited cocaine self-administration and reinstatement in rats. Taken together, atypical DAT inhibitors are possible candidates as a substitute therapy with the potential benefit of treating psychostimulant addiction.

Modulation of dopaminergic pathways to improve erectile function

Ulf Simonsen

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The currently recommended first line treatments of erectile dysfunction, phosphodiesterase type 5 inhibitors (PDEi), sildenafil, vardenafil, tadalafil, and avanafil are efficacious in many patients with erectile dysfunction of vascular origin, but this therapy is insufficient in approximately 30-40% of men with erectile dysfunction where there is also a neuronal affection. In addition, medications of central nervous system disorders, like antidepressants, antipsychotics and anxiolytics, have also negative impact on the erectile function. Therefore, we here review the possibility of modulating the dopaminergic pathways to improve erectile function. Dopaminergic neurons in the paraventricular and medial preoptic area are involved in erectile function as well as spinal dopaminergic neurons and dopaminergic pathways within the erectile tissue. Dopamine D1-and D2 (D2-D4)-like receptors in the paraventricular area are involved in erection, and several agonists developed for treatment of Parkinsons disease are associated with increased libido. A therapeutic window for treatment of erectile dysfunction was found by sublingual administration of the general dopamine agonist apomorphine, but it failed mainly due less efficacy on erectile function compared to PDE5i. To avoid the dose limiting side effects mediated by D2 receptors, nausea and emesis, dopamine D4 receptor agonists, ABT724 and ABT670 were developed and found to induce erection in rodents, but these drugs were less potent on D4 receptors than apomorphine. The antibiotic clavulanic acid increases dopamine and serotonin and was found to increase sexual arousal and erections in non-human primates and rats, but the dose-response curve is bell-shaped. Drugs may increase dopamine in the synaptic clefts either by inhibition of dopamine degradation or by inhibiting dopamine reuptake. Recently, we have observed a selective inhibitor of dopamine reuptake induces spontaneous erections in rats both by a central and peripheral mechanism of action. In summary, modulation of the dopaminergic pathways provides a possibility to improve treatment of erectile dysfunction.

The dopamine transporter as target for new psychoactive substances: a mechanism of action

Harald Sitte
Medical University of Vienna, AUSTRIA

Dopamine transporters are clinically relevant target of new psychoactive substances; they can inhibit the reuptake of dopamine by competitively blocking the transporters' action or trigger non-exocytotic neurotransmitter release (efflux) by reversing the transport direction. Thereby, these compounds enhance the extracellular concentration of dopamine, which is relevant for their recreational success as illicit drugs. Importantly, these interactions are not only relevant for the dopamine transporter but also for the closely related transporters for norepinephrine and serotonin, also members of the SLC6-family.

Recent advancement in the understanding of the structural and molecular mechanisms of drug binding to and the induction of efflux via these transporters will be discussed in light of the transport cycle. Furthermore, the impact of constituents of the plasma membrane on the functional properties of SLC6-transporters will be highlighted.

Modulating dopamine neurons in visual attention using pharmacogenetics

David P. Woldbye
Kopenhag University, DENMARK

Authors: Fitzpatrick C[1,2], Christiansen SH[2], Navntoft C[2], Habekost T[3], Runegaard A[2], Gether U[2], Andreasen JT[1] & Woldbye DP[2]
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Attention deficit/hyperactivity disorder (ADHD) is a common neuropsychiatric disorder associated with inattention. The pathophysiology of ADHD is unclear, but dopamine input from the ventral tegmental area (VTA) and noradrenaline input from the locus coeruleus (LC) to the prefrontal cortex appear to be centrally involved in mediating attentional processes. To further determine the roles of dopaminergic and noradrenergic neurotransmission in visual attention, we trained TH-CRE mice in the 5-choice serial reaction time task (5-CSRTT). Once the mice were fully trained, they were injected with a viral vector encoding inhibitory DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) into the VTA or LC in order to respectively exert selective control over dopaminergic and noradrenergic input to the prefrontal cortex. We found that particularly inhibition of VTA dopaminergic activity strongly and consistently reduced performance in the 5-CSRTT, indicating a prominent role for dopamine in visual attention.

Elevated talk (C026): Ropinirole prevents the addiction and withdrawal symptoms of morphine induced conditioned place preference in rats

Enes Karabulut, Oruc Allahverdiyev, Andleeb Shahzadi, Zeliha Yazici
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Ropinirole is a selective dopamine D2/D3 receptor agonist, used to treat Parkinson disease and restless legs syndrome. Morphine withdrawal results in low concentration of dopamine in brain reward centers. We hypothesized that increased dopaminergic activity may reduce the withdrawal symptoms. The experiment was designed to study the effect of ropinirole on addiction and withdrawal symptoms of morphine induced conditioned place preference (CPP) rats.

Morphine dependent rats were used to evaluate the expression, extinction and reinstatement by CPP. Morphine 10mg/kg (i.p.) was administered for 8 days to induce CPP 1, 2 or 5mg/kg ropinirole (i.p.) injection was given to rats on 9th day, 15 minutes later expression phase was evaluated followed by locomotor activity. Ropinirole was injected on daily basis and CPP was extinguished by repeated testing with intervals of three days. Reinstatement was established by administrating single dose of morphine (2mg/kg) to rats of extinction group. Morphine 10-50mg/kg was given for 5-days, on 6th day ropinirole was injected after 4 hours of administration of 50mg/kg morphine, 15 minutes later 2mg/kg naloxone was given subcutaneously to assess the withdrawal symptoms.

Ropinirole 1-5mg/kg decreased the expression 20-30%, relapse 23-33% ($p<0.05$-0.01) while accelerated the extinction rate by 22-47%. Non-significant effect of ropinirole on locomotor activity was observed. Naloxone-precipitated morphine withdrawal symptoms including wet dog shakes and weight loss were attenuated while escape attendance was increased by single ropinirole injection.

Ropinirole was found to be effective in extinction of CPP, reduced the acquisition, relapse and withdrawal symptoms of morphine. These findings showed that ropinirole can be effective to treat dependence and withdrawal symptoms of morphine and other opiate-addiction.

Keywords: morphine, conditioned place preference, dopamine, ropinirole

Elevated talk (C027): Antidepressant-like effects of Baijin Capsule in the olfactory bulbectomized rat

Rong Zhang, Yaoyue Liang, Shifen Dong, Jian Ni, Jianning Sun
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Depression has become one of the world public disease. However, recent clinical depression drugs such as TCAs, SSRIs and SNRIs, have some limitations, such as slow efficacy, poor stability and high prices. Therefore, these problems remain to be solved. This study was aimed to investigate the effectiveness and pharmacological mechanism of Baijin Capsule for treatment of depression. The present study was designed to confirm the antidepressant effect of Baijin Capsule using an olfactory bulbectomy (OB) model. The OB model was established in rats, and the antidepressant effect of Baijin Capsule (12.6, 4.2, and 1.4 g/kg/day, given by gastric gavage for 4 weeks) was tested using the open field test, locomotor activity and Novelty-Suppressed Feeding test. At the end of the experiment, the levels of the neurotransmitters in the cerebral cortex were analyzed by method of Ultra high performance liquid chromatography. The concentrations of tumor necrosis factor-α (TNF-alpha), Interleukin-6 (IL-6), IL-1β in the serum were measured by radioimmunoassay. OB model caused a significant increasing in OFT, locomotor activity and NSFT in rats and these behaviors were significantly improved by Baijin Capsule (12.6 and 4.2 g/kg/day). Meanwhile, the content of dopamine (DA), 5-hydroxytryptamine (5-HT) and Ornithine (Orn) in the cortex were significantly increased with the treatment of Baijin Capsule (12.6 and 1.4 g/kg/day). The concentrations of TNF-alpha and IL-6 in the serum were obviously decreased in rats of the Baijin Capsule (12.6 g/kg/day). The results suggested that Baijin Capsule may improve the behavioral disturbances in depression rat model, which were related to enhancement of the concentration of neurotransmitters in the cortex, and reduce the levels of inflammatory cell factors of serum.

This study is supported by National Natural Science Foundation of China (Grants 81173563); National “Major Drug Discovery” Science and Technology Major Projects of China, Grants No:2012ZX09103201-026.

Keywords: depression, olfactory bulbectomy, Baijin capsule, neurotransmitters, inflammatory

Hall B:
Basic and translational pharmacology of hydrogen sulfide: molecular targets and targeted diseases

(Joint Section of Italian Society of Pharmacology and Hellenic Society of Basic and Clinical Pharmacology)
Organizers and chairs:
Giuseppe Cirino (University of Naples)
Andreas Papapetropoulos (University of Athens)
09:30-12:00

09:30 Current status of H_2S inhibitors
Andreas Papapetropoulos
University of Athens, GREECE

10:00 CBS inhibition in cancer
Csaba Szabo
University of Texas Medical Branch, USA

10:30 Coffee break

11:00 The “two-pharmacophore” approach: improving the safety of NSAIDs with the help of H_2S
John L. Wallace
Antibe Therapeutics, CANADA

11:30 Elevated talk (C028): The interaction of L-cysteine/hydrogen sulfide pathway and muscarinic acetylcholine receptors (mAChRs) in mouse corpus cavernosum
Fatma Tuğçe Dalkır
Çukurova University, TURKEY
(Authors: Fatma Tuğçe Dalkır, Fatma Aydınoğlu, Nuran Öğülener)

11:45 Elevated talk (C029): H2S donors increase cystathionine-γ lyase expression
Sofia Iris Bibli
National and Kapodistrian University of Athens, GREECE
(Authors: Sofia Iris Bibli, Csaba Szabo, Andreas Papapetropoulos)

12:00 End of symposium

Current status of H_2S inhibitors

Andreas Papapetropoulos
Faculty of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis, Zografou, 15771, Athens, GREECE.

For many decades, hydrogen sulfide (H_2S) was considered a biohazardous gas that was of interest only to toxicologists. Following the discovery that mammalian cells are capable of producing H_2S, this molecule underwent a dramatic metamorphosis from a dangerous pollutant to a biologically relevant molecule. H_2S is now considered as the newest member of the gasotransmitter family that also includes nitric oxide and cardon monoxide, and is recognized as an endogenous mediator with important roles in homeostasis, physiology and disease. H_2S is generated through the action of three enzymes, namely cystathionine-β synthase (CBS), cystathionine-γlyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3-MST). CBS and CSE operate in the transsulfuration pathway (the conversion of methionine to cysteine), use cysteine as a substrate to generate H_2S and require pyridoxal-5'-phosphate (PLP) as a cofactor. CBS is the predominant H_2S-producing enzyme in the nervous system, while CSE is believed to be the main enzyme in the cardiovascular system. H_2S controls fundamental mammalian cellular responses, including growth, differentiation, migration and cell death and regulates the activity of kinases, ion channels, transcription factors, as well as cellular bioenergetics. Overproduction of H_2S has been reported to occur in a number of pathophysiological conditions and inhibition of its production has been proposed as a therapeutic target for non-alcoholic fatty liver disease, stroke and cancer. Herein, we will review the literature on the pharmacology of H_2S synthesis inhibitors and discuss their translational potential.

CBS inhibition in cancer

Csaba Szabo
Department of Anesthesiology, University of Texas Medical Branch, Galveston, TX, USA

In various forms of cancer (including colorectal and ovarian cancer), increased production of hydrogen sulfide (H_2S) from cystathionine-β-synthase (CBS) plays an important role in promoting cellular bioenergetics, proliferation and migration. Pharmacological inhibition or genetic silencing of CBS exerts antitumor effects *in vitro* and *in vivo*, and potentiates the efficacy of current standard-of-care anticancer therapeutics. In the current presentation, recently published studies will be overviewed documenting a critical role for CBS-dependent H_2S production in cancer cell proliferation and tumorigenesis, and molecular mechanisms will be presented by which H_2S provides a pro-tumor-growth environment. Next, the state-of-the-art of CBS inhibition will be discussed, including the complex pharmacology of aminooxyacetic acid, which includes the inhibition of CBS, as well as the inhibition of several other PLP (pyridoxal phosphate) dependent enzymes. Finally, a novel pharmaceutical strategy will be presented, which enhances the cellular uptake, the cell-based antiproliferative efficacy and the in vivo antitumor potency of AOAA via the prodrug approach.

The "two-pharmacophore" approach: improving the safety of NSAIDs with the help of H_2S

John L. Wallace
University of Calgary, Calgary, Alberta, Canada & Antibe Therapeutics, Toronto, Ontario, CANADA

There is a rapidly expanding body of evidence for important roles of hydrogen sulfide in protecting against tissue injury, reducing inflammation, and promoting repair. There is also growing evidence that H_2S can be successfully exploited in drug development. H_2S synthesis and degradation are regulated in circumstances of inflammation and injury so as to promote repair and re-establish homeostasis. Novel H_2S-releasing drugs exhibit enhanced anti-inflammatory and pro-restorative effects, while having reduced adverse effects in many tissues. H_2S is a pleiotropic mediator, having effects on many elements in the inflammatory cascade and promoting the resolution of inflammation and injury. It also contributes significantly to mucosal defence in the gastrointestinal tract, and in host defence against infection. There is strong evidence that novel, H_2S-based therapeutics are safe and effective in animal models, and several are progressing through human trials. A better understanding of the physiological and pathophysiological roles of H_2S continues to be restrained by the lack of simple, reliable methods for measurement of H_2S synthesis, and the paucity of highly selective inhibitors of enzymes that participate in endogenous H_2S synthesis. On the other hand, H_2S donors show promise as therapeutics for several important indications.

Elevated talk (C028): The interaction of L-cysteine/hydrogen sulfide pathway and muscarinic acetylcholine receptors (mAChRs) in mouse corpus cavernosum

Fatma Tuğçe Dalkır[1], Fatma Aydınoğlu[2], Nuran Öğülener[1]
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The relaxant effect of hydrogen sulfide (H_2S) has been demonstrated in mouse, rat, rabbit, primate and human corpus cavernosum (CC) tissues. However, the mechanism of this relaxant effect is still unclear. The aim of this study was to investigate the possible interaction of L-cysteine/H_2S pathway and muscarinic acetylcholine receptors (mAChRs) in the mouse CC.

The relaxant responses to L-cysteine (endogenous H_2S substrate; 10^{-3}M), sodium hydrogen sulfide (NaHS; exogenous H_2S; 10^{-6}–10^{-3}M) and acetylcholine (10^{-6}M) were obtained in isolated mouse CC tissues. Firstly, the effects of propargylglycine (PAG, 10^{-2}M) or aminooxyacetic acid (AOAA, 10^{-3}M), inhibitors of H_2S synthase enzymes cystathionine-gamma-lyase (CSE) and cystathionine-β-synthase (CBS) respectively, on the relaxant response to L-cysteine and acetylcholine were investigated. The role of non-selective mAChr antagonist atropine ($5x10^{-5}$M), selective M_1 receptor antagonist pirenzepine ($5x10^{-6}$M), selective M_2 receptor antagonist AFDX-116 (10^{-6}M) and selective M_3 receptor antagonist 4-DAMP (10^{-6}M) were investigated on the relaxant responses to L-cysteine, NaHS and acetylcholine in isolated mouse CC tissues which were pre-contracted by phenylephrine ($5x10^{-6}$M).

The relaxant responses to L-cysteine were significantly reduced by PAG, but not by AOAA. On the other hand, the relaxant responses to acetylcholine were not influenced by PAG, but were significantly increased by AOAA. Atropine, pirenzepin and 4-DAMP significantly reduced the relaxant response to L-cysteine. However, AFDX-116 did not cause a significant inhibition on the relaxant response to L-cysteine. NaHS-induced relaxant response was significantly reduced by atropine, pirenzepin, 4-DAMP and AFDX-116. Furthermore, atropine, pirenzepin and 4-DAMP significantly inhibited the relaxant response to acetylcholine. Whereas AFDX-116 did not affect these relaxant responses.

We conclude that L-cysteine induced relaxant response is mediated via activation of CSE, and mAChRs M_1 and M_3 but not M_2 receptors contributes to relaxant effect of L-cysteine in mouse CC. Also, mAChRs plays role in exogenous H_2S-induced relaxation in this tissue.

Keywords: acetylcholine, corpus cavernosum, hydrogen sulfide, L-cysteine, muscarinic receptors

Elevated talk (C029): H2S donors increase cystathionine-γ lyase expression

Sofia Iris Bibli[1], Csaba Szabo[2], Andreas Papapetropoulos[1]
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Sporadic evidence in the literature has indicated that treatment with H_2S releasing agents leads to an increase in CSE expression. In addition, it has been observed that the effects of H_2S donors are in some instances blocked by CSE inhibitors. The aim of the present study was to systematically investigate whether administration of H_2S donors alters the levels of H_2S producing enzymes. H9c2 differentiated cardiomyocytes were exposed to vehicle, Na_2S (100μM), GYY (100μM), thiovaline (1μM), AP-39 (1nM) for 24hr. Such treatment resulted in elevation of mRNA and protein CSE levels. In contrast, no changes in the expression of CBS or 3MST were noted. Moreover, when C57BL/6 male mice were treated with H_2S donors we observed that CSE expression in the aorta, carotid and the heart was increased, confirming our in vitro observations. Our findings raise the possibility that a feed-forward mechanism exists during which exposure to H_2S donors drives CSE expression leading to generation of uncontrolled H_2S amounts that could potentially reach toxic levels. PKG was recently demonstrated to phosphorylate CSE on Ser377 restricting its activity. H9c2 cardiomyocytes were transfected with human CSE and exposed to Na_2S. After such treatment, immunoprecipitated CSE was found to interact with PKG. Using a phospho-Ser Ab we found an increase in CSE serine phosphorylation following treatment of cells with Na_2S. We next raised a phospho-specific Ab against Ser377 and tested for CSE phosphorylation on this residue in animals treated with Na_2S for 24h. Indeed, H_2S donor-treated mice exhibited an increase of CSE phosphorylation on Ser377 compared to vehicle-treated mice. We conclude that H_2S donors up regulate CSE expression in a PKG-dependent manner and that uncontrolled production of H_2S is prevented by phosphorylation of CSE on Ser377.

Keywords: H_2S, phosphorylation

Hall C:
Trace amine associated receptors (TAARs): a promising target for pharmacotherapies?

(Organized by British Pharmacological Society)
Organizers and chairs:
Clare Stanford (Dept Neuroscience Physiology & Pharmacol, UCL, UK)
Stefano Espinoza (Italian Institute of Technology, ITALY)

09:00 Trace amine receptors: their role in psychosis and its treatment
Stefano Espinoza
Italian Institute of Technology, ITALY

09:30 TAAR1 ligands as new generation pharmacotherapies for addiction treatment
Juan Canales
University of Leicester, UK

10:00 TAAR1-mediated modulation of presynaptic dopaminergic neurotransmission: role of D2 dopamine receptors
Damiana Leo
Italian Institute of Technology, ITALY

10:30 Coffee break

11:00 Non-classical amine recognition evolved in a large clade of olfactory trace amine-associated receptors
Qian Li
Harvard Medical School, USA

11:30 Elevated talk (C030): Discovery of novel TAAR1 ligands through the use of in silico screening on a homology model of the trace amine associated receptor 1
Vincent M. Lam
University of Toronto, CANADA
(Authors: Lam VM, Rodríguez D, Zhang T, Koh EJ, Carlsson J, Salahpour A)

12:00 End of symposium

Trace amine receptors: their role in psychosis and its treatment

Stefano Espinoza
Italian Institute of Technology, ITALY

Trace amine-associated receptor 1 (TAAR1) is a G protein-coupled receptor belonging to the TAAR family. Discovered in 2001, TAARs have been found in several tissues, ranging from the central nervous system to the olfactory epithelium. The best studied receptor, TAAR1, is activated by a class of endogenous compounds named trace amines (TAs). TAs are structurally related to classic monoaminergic neurotransmitters and found at low concentrations in the mammalian brain. Although TAs levels have been associated with many neuropsychiatric disorders, only the discovery of TAAR1 validated their physiological role. TAAR1 can modulate monoamine neurotransmission and, in particular, the dopamine system. Several studies have demonstrated that TAAR1 knockout (TAAR1-KO) mice display a supersensitive dopaminergic system, while activation of TAAR1 can reduce dopaminergic hyperactivity. For these reasons, TAAR1 has been proposed as a novel therapeutic target for neuropsychiatric disorders such as schizophrenia, bipolar disorder, and addiction.

TAAR1 ligands as new generation pharmacotherapies for addiction treatment

Juan Canales
University of Leicester, UK

Addiction to psychoactive drugs is a disease of the brain for which new medications are needed. Psychomotor stimulants, such as cocaine and methamphetamine, are highly addictive substances which consume a tremendous amount of resources to aid in recovery and treatment worldwide. The strategic anatomical location of the trace amine-associated receptor 1 (TAAR1), and its unique ability to regulate monoamine neurotransmission, suggests that this receptor may be a promising target for developing more effective, new generation therapies for addictive disorders. To study the therapeutic potential of TAAR1 agonists, we used clinically relevant animal (rat) models of addiction, including stimulant sensitization, intracranial self-stimulation, drug self-administration and relapse models. To examine TAAR1 regulation of stimulant-induced changes in dopamine transmission we used fast-scan cyclic voltammetry. The findings showed that TAAR1 activation with specific agonists (i) blocked the acquisition and expression of methamphetamine sensitization, (ii) prevented the cocaine-induced lowering of intracranial self-stimulation thresholds, (iii) reduced the motivation to self-administer cocaine and methamphetamine, (iv) blocked relapse to drug seeking after chronic self-administration and (v) prevented stimulant-induced changes in dopamine transmission in the nucleus accumbens. Collectively, these observations indicate that TAAR1 activation has a unique ability to regulate stimulant-induced behaviours, thus support the candidacy of TAAR1-based pharmacotherapies as potential substitute treatments in drug addiction.

TAAR1-mediated modulation of presynaptic dopaminergic neurotransmission: role of D2 dopamine receptors

Damiana Leo

Authors: Damiana Leo[1], L. Mus[1], S. Espinoza[1], M.C. Hoener[2], T.D. Sotnikova[1], R.R. Gainetdinov[1,3,4]

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Trace Amine-Associated Receptor 1 (TAAR1) is a G protein-coupled receptor (GPCR) expressed in several mammalian brain areas and activated by “trace amines” (TAs). TAs role is unknown; however, discovery of their receptors provided an opportunity to investigate their functions. In vivo evidence has indicated an inhibitory influence of TAAR1 on dopamine (DA) neurotransmission, presumably via modulation of dopamine transporter (DAT) or interaction with the D2 DA receptor and/or activation of inwardly rectifying Kþ channels. To elucidate the mechanisms of TAAR1-dependent modulation, we used TAAR1 knockout mice (TAAR1-KO), a TAAR1 agonist (RO5166017) and a TAAR1 antagonist (EPPTB) in a set of neurochemical experiments. Analysis of the tissue content of TAAR1-KO revealed increased level of the DA metabolite homovanillic acid (HVA), and in vivo microdialysis showed increased extracellular DA in the nucleus accumbens (NAcc) of TAAR1-KO. In fast scan cyclic voltammetry (FSCV) experiments, the evoked DA release was higher in the TAAR1-KO NAcc. Furthermore, the agonist RO5166017 induced a decrease in the DA release in wild-type that could be prevented by the application of the TAAR1 antagonist EPPTB. No alterations in DA clearance, which are mediated by the DAT, were observed. To evaluate the interaction between TAAR1 and D2 autoreceptors, we tested the autoreceptor-mediated dynamics. Only in wild type mice, the TAAR1 agonist was able to potentiate quinpirole-induced inhibitory effect on DA release. Furthermore, the short-term plasticity of DA release following paired pulses was decreased in TAAR1-KO, indicating less autoinhibition of D2 autoreceptors. These observations suggest a close interaction between TAAR1 and the D2 autoreceptor regulation.

Non-classical amine recognition evolved in a large clade of olfactory trace amine-associated receptors

Qian Li
Department of Cell Biology, Harvard Medical School, Boston, MA 02115, USA

Biogenic amines are important signaling molecules, and the structural basis for their recognition by G Protein-Coupled Receptors (GPCRs) is well understood. Amines are also potent odors, with some activating olfactory trace amine-associated receptors (TAARs). Here, we report that teleost TAARs evolved a new way to recognize amines in a non-classical orientation. Chemical screens de-orphaned eleven zebrafish TAARs, with agonists including serotonin, histamine, tryptamine, 2-phenylethylamine, putrescine, and agmatine. Receptors from different clades contact ligands through aspartates on transmembrane α-helices III (canonical $Asp^{3.32}$) or V (non-canonical $Asp^{5.42}$), and diamine receptors contain both aspartates. Non-classical monoamine recognition evolved in two steps: an ancestral TAAR acquired $Asp^{5.42}$, gaining diamine sensitivity, and subsequently lost $Asp^{3.32}$. Through this transformation, the fish olfactory system dramatically expanded its capacity to detect amines, ecologically significant aquatic odors. The evolution of a second, alternative solution for amine detection by olfactory receptors highlights the tremendous structural versatility intrinsic to GPCRs.

Elevated talk (C030): Discovery of novel TAAR1 ligands through the use of in silico screening on a homology model of the trace amine associated receptor 1

Vincent M. Lam[1], David Rodríguez[2], Thomas Zhang[1], Eun Jee Koh[1], Jens Carlsson[2], and Ali Salahpour[1]

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Trace amines are biogenic amines that are by-products of the synthesis of classical neurotransmitters within the brain. Trace amine associated receptor 1 (TAAR1) is a GPCR that can be activated by several TA's. Studies on animals have shown that TAAR1 acts as a negative regulator of dopamine transmission. Therefore TAAR1 antagonists could be beneficial in conditions of reduced dopamine transmission such as Parkinson's disease, which arises from the loss of dopamine neurons. Unfortunately, no antagonist for TAAR1 with in vivo activity has been identified to date.

We aimed to discover novel TAAR1 ligands with a focus on TAAR1 antagonists with good pharmacological profile.

Using a TAAR1 homology model based on the crystal structure of other GPCRs, we carried out an in silico screen of over 3 million commercially available compounds. The top 42 hits predicted to have the highest affinities by the modelling were ordered and their pharmacological activity on TAAR1 assessed using a BRET cAMP EPAC biosensor.

A homology model of TAAR1 was generated and over 3 million commercially available compounds were screened against the orthosteric site using molecular docking. Among the 42 top-ranked compounds that were tested in functional assays, three partial agonists with EC_{50} values ranging from 1 to 52 µM were discovered. In addition, four potentially weak antagonists were identified. Ten analogs of the two most potent agonists from the screen were also evaluated and three of these displayed equal or greater activity compared to the parent compound. Several of the discovered ligands represent novel scaffolds and are thus promising starting points for development of new pharmacological tools for studying TAAR1 biology.

Key words: TAAR1 ligands, homology model of TAAR1, in silico screening

Hall D:
Molecular clocks and chronopharmacology

(Organized by Turkish Pharmacological Society)
Organizers and chairs:
Francis Levi (Warwick University)
Alper Okyar (İstanbul University)
09:00-12:00

09:00 Discovery of the small molecules that regulates circadian rhythm
İbrahim Halil Kavaklı
Koç University, TURKEY

09:30 Modelling the link between circadian clock and cancer
Nuri Öztürk
Gebze Technical University, TURKEY

10:00 Systems chronopharmacology of anticancer drugs from cells to patients
Francis Lévi
Warwick University, UK

10:30 Coffee break

11:00 Circadian control of drug metabolism, detoxification and pharmacokinetics
Alper Okyar
İstanbul University, TURKEY

11:30 Elevated talk (C031): Relevance of everolimus dosing time for toxicity and circadian clock effects in mice
Xiao Mei Li
INSERM, Université Paris-Sud, FRANCE
(Authors: Xiao Mei Li, Thinh Doan, Narin Ozturk, Alper Okyar, Barbel Finkenstadt Rand, Sylvie Giacchetti, Francis Lévi)

11:45 Elevated talk (C032): The circadian timing system as a toxicity target of the anticancer mTOR inhibitor everolimus in mice
Narin Öztürk
İstanbul University, TURKEY
(Authors: Narin Ozturk, Dilek Ozturk, Zeliha Pala Kara, Engin Kaptan, Serap Sancar Bas, Suzan Cinar, Gunnur Deniz, Xiao Mei Li, Sylvie Giachetti, Francis Levi, Alper Okyar)

12:00 End of symposium

Discovery of the small molecules that regulates circadian rhythm

İ. Halil Kavaklı

Authors: Şeref Gül[1], Tuba Korkmaz[2], Ibrahim Barış[3], Nuri Öztürk[2], Metin Türkay [4], İ. Halil Kavaklı[1,3]

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Circadian rhythm controls the behavioral, biochemical and physical activities of the organisms in which they are found from cyanobacteria to human. Clock mechanism is controlled by two interconnected transcription/translation feedback loops (TTFL) at the molecular level. Four integral clock proteins drive the clock. These are two activators (BMAL1 and CLOCK) and two repressors (PER and CRY). BMAL1-CLOCK forms the dimer and binds to E-box (positive arm of the TTFL) in the promoter region of genes (~40% of all genes) including *Cry* and *Per*. PER and CRY proteins accumulate in the cytoplasm and translocate to nucleus to inhibit BMAL1-CLOCK driven transactivation (negative arm of the TTFL).

During the last decade it has been shown that overexpression or knockout of CRY causes significant consequences in different diseases such as type-II diabetes and cancer. Recent studies showed that *Cry* knockout in *p53* knockout (cancer) mice caused 50% extended life span as a result of increase in the susceptibility of cells to apoptosis. In addition other studies revealed that overexpression of CRY decreases the blood glucose level by inhibiting the accumulation of cAMP in response to G protein-coupled receptor (GPCR) activation; and improves insulin sensitivity in insulin resistant *db/db* mice. These findings suggest that molecules that modulate the stability of the CRY have potential to be utilized in for the treatment type-II diabetes and cancer.

In this talk I will summaries the recent works from my group about the molecules that regulates the stability of the mammalian CRY.

This work is supported by TUBITAK 114Z622

Modelling the link between circadian clock and cancer

Nuri Öztürk
Authors: Tuba Korkmaz, Gözde Özçelik, Handan Emişoğlu, Nuri Öztürk
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Many physiological events are regulated in a daily rhythmic manner by the circadian clock. The circadian clock controls 10-20% of the transcripts, and its deregulation is associated with diseases such as cancer, metabolic abnormalities and neurological disorders. It was previously suggested that the disruption of the circadian clock increases the risk of cancer, however experimental findings and especially those involving studies with animal models do not always support this generalization. This suggests that a comprehensive mechanistic study would help to understand the link between circadian clock and cancer.

Our previous experience indicated that not only the way in which the circadian clock is disrupted, but also genetic background affects the relationship between the circadian clock and carcinogenesis. Based on this experience and considering the genetic heterogeneity in cancers, we wanted to screen the effect of the circadian clock on carcinogenesis in a systematic way. We were especially interested in the responses to chemotherapeutic drugs, which might be affected by the status of the circadian clock and other events involved in metastasis. For this purpose, we generated a panel of (normal and tumorigenic epithelial) cell lines with mutations in single or multiple genes by CRISPR method. We then used these cell lines to analyze multiple events associated with carcinogenesis.

The availability of easy and fast genome editing methods made us able to inspect the relationship between multiple genes or pathways in a systematic and convenient manner. These new approaches will be beneficial for designing new in vivo strategies for better management of cancer in affected individuals.

Funding: This study was supported by Turkish Scientific and Technical Research Council-TUBİTAK (Project Number: 114S446).

Systems chronopharmacology of anticancer drugs from cells to patients

Francis Lévi

Authors: Francis Lévi, Annabelle Ballesta, Sandrine Dulong, Xiao Mei Li, Sandra Komarcynski, Monique Maurice, Pasquale Innominato

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Cancer Chronotherapy and Post-operative Liver, INSERM UMRS 935, Campus CNRS, Villejuif, France

Both pharmacokinetics and pharmacodynamics of anticancer drugs vary largely according to dosing time in cell cultures, mice and patients. Such chronopharmacology results from the rhythmic control of drug metabolism and cellular proliferation by the Circadian Timing System (CTS), a coordinated network of 15-genes molecular clocks which reside in all mammalian cells. The CTS determines up to 5-fold predicted changes in experimental treatment tolerability and/or efficacy, as shown for nearly 50 anticancer cytostatics and targeted agents. Clinical benefit was shown for 8 drugs including 5-fluorouracil, oxaliplatin, and irinotecan in cancer patients. However, sex and circadian robustness critically determined the optimal chronotherapeutic schedule, in meta-analyses of international clinical studies (Giacchetti et al. Ann Oncol 2012; Lévi et al. Chronobiology Int 2014). The reciprocal circadian regulation of clock genes Rev-erbα and Bmal1 shapes chronopharmacology and sets up optimal timing in individual cells, mice or patients, as shown for the topoisomerase-I inhibitor irinotecan (Li et al. Cancer Res 2013). Irinotecan-induced apoptosis was four-fold as high following dosing near Bmal1 transcription peak as compared to trough in CaCo2 cells. Bmal1 silencing suppressed irinotecan chronopharmacology including circadian bioactivation, detoxification, and Top1-DNA complex formation (Dulong et al. Mol Cancer Ther 2015). The combination of experimental methods and mathematical models within a systems chronopharmacology approach encompassed both genetically-based and sex-related CTS variabilities in experimental models (Lévi at al. Annu Rev Pharm Toxicol 2010; Dallmann et al. Trends in Mol Med 2016). Currently, the monitoring of multidimensional CTS biomarkers such as rest-activity and temperature (Roche et al. Chronobiology Int 2014; Ortiz-Tudela et al. Int J Cancer 2013, BMC Cancer 2016) jointly with automatic chronotherapy delivery at home aim at optimizing chronotherapy in individual cancer patients. The integration of circadian rhythms into advanced ICT devices and services further address intrapatient CTS variability in chronopharmacology.

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Circadian control of drug metabolism, detoxification and pharmacokinetics

Alper Okyar
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Circadian rhythms (~24h period) have been shown for most biological variables in living organisms and are generated by molecular clocks involving clock genes. The molecular clocks are coordinated by the suprachiasmatic nuclei along the ~24h and orchestrates the circadian timing system (CTS). The CTS generates daily rhythms in cellular and organism physiology and adjusts them to environmental cycles. Twenty-four-hour rhythms have long been known to moderate xenobiotic absorption, distribution, metabolism and excretion. These key processes determine the shape and levels of cellular exposure to drugs and toxicants, i.e., pharmacokinetics and toxicokinetics. Phase I oxidation, reduction and hydrolysis reactions, Phase II conjugation reactions and Phase III xenobiotic transport ultimately increase xenobiotic water solubility and facilitate excretion mainly via urine, bile and the intestine. Phase III transport in liver, kidney and intestine is mainly accomplished by ATP-Binding Cassette (ABC) transporters, in particular P-glycoprotein (P-gp), is the most outstanding one among ABC transporters, as it confers the strongest resistance (MDR) to the antineoplastics which are narrow therapeutic index and severe adverse effects to the host. Indeed, circadian timing modifies the toxic effects of 40 anticancer medications in rodents and in patients. The mechanisms of drug detoxification involve the CYP metabolism and the cellular efflux of drugs and/or their metabolites via transporters. This detoxification rhythm can importantly contribute to host tolerability for some anticancer drugs such as irinotecan, docetaxel, doxorubicin and everolimus. However, we should consider also that several mechanisms jointly account for the chronopharmacology of the anticancer agents.

Elevated talk (C031): Relevance of everolimus dosing time for toxicity and circadian clock effects in mice

Xiao Mei Li[1], Thinh Doan[2], Narin Ozturk[3], Alper Okyar[3], Barbel Finkenstadt Rand[2], Sylvie Giacchetti[1], Francis Lévi[4]

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The molecular circadian clock regulates mTOR (mammalian target of rapamycin) activity, the main target of everolimus. Its use in human cancers is hampered by adverse events. To determine the relevance of everolimus timing for body weight loss, and circadian clock effects. In experiment 1, everolimus (5-10 mg/kg/day x 14 days po) was administered at Zeitgeber times (ZT)1, ZT7, ZT13, or ZT19 to 32 C57BL/6 mice, with body weight loss and lethal toxicity as endpoints. In experiment 2, liver molecular clock alteration on everolimus was investigated in freely moving *Per2*^{luc} male C57BL/6 mice singly housed in Real Time-Biolumicorder (RT-BIO) units. Everolimus (5 mg/kg/day x 6d) was administered at ZT1 or ZT13. D-luciferin was dissolved into drinking water. Mouse liver *Per2::luc* expression and rest-activity were monitored every minute with the photomultiplier tube and infrared sensor respectively. Time series of moving 3-day window with 6-h shifts were analyzed according to the Spectrum Resampling method. In Experiment 1, lethal toxicity of 10 mg/kg/d varied from none at ZT13 to 50% at ZT1 or ZT7. Maximum body weight loss of the non-lethal 5 mg/kg/d ranged from -5% at ZT13 to -12% at ZT1 (ANOVA $p<0.001$). Similarly, mean body weight loss was twice as large following everolimus at ZT1 compared to ZT13 in Experiment 2 ($p<0.001$). Both rest-activity and *Per2::luc* circadian rhythms displayed stable 24-h periodic rhythms before everolimus and in both vehicle-treated controls. Everolimus ablated circadian rhythms in rest-activity and liver *Per2::luc* in both mice treated at ZT1. In contrast *Per2::luc* remained rhythmic in both mice dosed at ZT13. Everolimus toxicity was least following dosing at ZT13, i. e. near the onset of the activity span in male mice. This timing avoided circadian disruption in the liver molecular clocks. Morning administration of everolimus could minimize metabolic alterations and fatigue in humans.

Keywords: circadian clocks, everolimus toxicity, optimal dosing time, rest-activity, *Per2::luc*

Elevated talk (C032): The circadian timing system as a toxicity target of the anticancer mTOR inhibitor everolimus in mice

Narin Ozturk[1], Dilek Ozturk[2], Zeliha Pala Kara[3], Engin Kaptan[4], Serap Sancar Bas[4], Suzan Cinar[5], Gunnur Deniz[5], Xiao Mei Li[6], Sylvie Giachetti[6], Francis Levi[7], Alper Okyar[3]

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The circadian timing system (CTS) determines optimal timing and waveform of drug tolerability, yet treatment itself can alter this system. The aim of this study was to investigate dose and dosing-time dependent effects of anticancer drug everolimus on body temperature and locomotor activity rhythms as physiological biomarkers of the CTS in mice and to investigate the chronotoxicity of everolimus. C57BL/6J male and female mice were implanted with telemetry transmitters and exposed to different oral doses of everolimus (5-10 mg/kg in males; 15-20 mg/kg in females) at four different Zeitgeber times (ZT; ZT1, ZT7, ZT13 or ZT19) for 14 days. Most severe alterations on rhythm parameters were found at ZT1. Based on the telemetry study findings, everolimus was administered to male (5 mg/kg) and female mice (15 mg/kg) orally at ZT1-most toxic time- and ZT13-best tolerated time- in subacute toxicity study for 28 days. Body weight loss, hematological and histological toxicity were investigated. Based on the general toxicity findings and effects of everolimus on the circadian physiology outputs, tolerability of the drug was best at ZT13 in both sexes. Body weight loss in mice dosed at ZT1 was greater than ZT13 (ANOVA, $p<0.05$ in both sexes and all dose groups). Observed cortical atrophy in thymus upon everolimus administration was more evident in male mice at ZT1 than ZT13 (ANOVA, $p<0.05$). Total leukocytes ($p<0.01$, ANOVA), T helper (+CD4) and B (+CD19) cells were decreased significantly ($p<0.01$ for both lymphocytes, ANOVA) at ZT1 as compared to control whereas no significant differences observed at ZT13 in males. Delivery of everolimus near its time of least toxicity produced least alterations in circadian physiological outputs, a finding suggests that the extent of circadian disruption contributes to the toxicity of everolimus. These findings support the concept of everolimus chronotherapy for increasing the tolerability of this drug.

Keywords: everolimus, chronotoxicity, circadian rhythm, cancer chronotherapy, circadian biomarkers

Workshop Wednesday, 29th June 2016, Hall E: ORPHEUS platform: “Best Practices for High Research Standards and PhD Students training in Responsible Research”

(Organized by ORPHEUS: ORganisation of PhD Education in Biomedicine and Health Sciences in the EUropean System)
Organizers and chairs:
Zdravko Lacković (University of Zagreb, CROATIA)
09:00-12:00

09:00	Best practices for high research standards and PhD students training in responsible research Zdravko Lacković University of Zagreb, CROATIA
09:30	Institutional procedures as a tool to promote responsible research environment Hakan Sedat Orer Koç University, TURKEY
10:00	ORPHEUS tools for assisting institutions in implementing best practices for responsible research training Michael John Mulvany Aarhus University, DENMARK
10:30	Coffee break
11:00	Impact of ORPHEUS on PhD training: The experience of a Turkish university Gül Güner Akdoğan İzmir University of Economics, TURKEY
11:30	Discussion
12:00	End of symposium

Best practices for high research standards and PhD students training in responsible research

Zdravko Lacković
University of Zagreb, CROATIA

The PhD programs leading to the PhD degree should provide candidates with competences to become a scientist able to conduct "significant, responsible, independent and original research". This workshop will show how ORPHEUS (Organisation for PhD Education in Biomedicine and Health Sciences in the European System) is providing tools to assist institutions in developing best practices to achieve high scientific standards of PhD programs. These programs should include appropriate training in the international and local rules concerning research ethics, including medical ethics and bioethics. Institutional organization and rules of managing suspected research misconduct, as well as training PhD candidates in responsible research should allow students to distinguish between scientific misconduct, questionable research practices and poor science, and institution how to deal with.
This workshop will describe:

- New forms of scientific misconduct associated with new information and communication technologies. Best practices for PhD students training in responsible research
- Institutional procedures as a tool to promote a responsible research environment. Ethics committee reviews, laboratory book keeping rules and mentor-mentee agreements could be instrumental to better the formation of PhD candidates and to promote good research environment inside the institutions
- Tools for assisting institutions in implementing best practices for responsible research training. Experiences of International Evaluation of PhD Programs (ORPHEUS Labeling) and New Initiatives
- How it is to be evaluated by Orpheus: Experience of Dokuz Eylul University, Izmir

The workshop will be two-way, and all participants of EPHAR 2016 are invited to present their experience and suggestions and thus actively participate in shaping the future of PhD in health sciences in Europe.

Institutional procedures as a tool to promote responsible research environment

Hakan S. Orer
Koç University, Medical Faculty, TURKEY

In a globalized and competitive world, producing independent researchers with high ethical values becomes a critical task. The pace of scientific discovery in health sciences is such that the emerging global and bioethical challenges need to be addressed properly. The issue of dual use research of concern will be a central point of this discussion. In this context, doctorate students should be accommodated in an institutional environment where they can acquire a mature ethical posture at the onset of their careers. Throughout their formation years, PhD candidates encounter with many institutional procedures where ethical conduct and research integrity play an integral part. Ethics committee applications, exams and data acquisition processes are real-life situations and institutional environment, by and large, determines the future conduct of the aspiring researchers apart from lectures and workshops. Another important avenue is the thesis supervision. In a growing number of graduate schools, there are genuine efforts to establish formal training programs for both supervisors and students. Such institutional procedures may serve as means to reinforce proper scientific and ethical conduct, had these been organized in concert with the graduate programs. The role of institutional review boards (ethics committees) in the making of PhD thesis and administrative policies to implement best practice guidelines are key elements of this endeavor. Ethics committee reviews, laboratory book keeping rules and mentor-mentee agreements could be instrumental to better the formation of PhD candidates and to promote good research environment inside the institutions.

ORPHEUS tools for assisting institutions in implementing best practices for responsible research training

Michael J. Mulvany
Department of Biomedicine, Aarhus University, Aarhus, DENMARK

Doctoral training is a key element in ensuring that research is performed in responsible fashion. There is therefore currently considerable focus on ensuring that all PhD candidates receive training in the principles of ethical conduct in addition to training in academic excellence and transversal skills. To assist this process, ORPHEUS (Organisation for PhD Education in Biomedicine and Health Sciences in the European System) has – building on Bologna and Salzburg principles – over many years developed a number of tools. These are based on the publication "*Best Practices for PhD Training",* published by ORPHEUS and AMSE (Association of Medical Schools in Europe), and a self-evaluation questionnaire. The "*Best Practices"* document (previously known as the "*ORPHEUS Standards"* document) provides a number of recommendations, and the purpose of the questionnaire is to provide a framework for institutions to discuss these recommendations and to reflect on their PhD programmes. The effectiveness of the process is enhanced by relevant stakeholders (dean, head of graduate school, head of graduate school administration, research programme directors, supervisors, PhD candidates) being involved in completing the questionnaire.

Other tools that are offered by ORPHEUS to ORPHEUS members are first the possibility to submit the completed questionnaire to the ORPHEUS Labelling Board which then appoints experienced facilitators to provide feedback and advice for possible improvements in the training being offered. Institutions completing this stage are qualify for an ORPHEUS Evaluation Certificate. Secondly, if the institution wishes, a site-visit is arranged for facilitators to meet with local stakeholders and thus provide more detailed feedback. Lastly, for institutions complying with the recommendations of the *Best Practices* document an ORPHEUS label is awarded. So far, seven ORPHEUS labels have been awarded.

With these tools, ORPHEUS is building a community of institutions abiding by the same principles of PhD training, in particular regarding responsible research conduct.

Impact of ORPHEUS on PhD training: The experience of a Turkish university

Gül Güner Akdoğan
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ORPHEUS has the mission of promoting PhD training not only in Europe, but throughout the World. The main pillars of PhD training within the main focus of ORPHEUS are: the mindset, organizational structure, and practice. Dokuz Eylül University (Izmir) has benefited to a large extent from being an institutional member of ORPHEUS. Organising the 6th ORPHEUS Conference in Izmir (2011), it has had the opportunity to share its “best practices” with the 250 participants from all over Europe. Having been selected as a “pilot candidate” institution for the ORPHEUS labelling procedure, it has had outstanding input from ORPHEUS to reform the PhD training programmes to achieve the “best-practices” of ORPHEUS. Obtaining the approval of the related “Ethics Committee” for any research project and organising a “mandatory” ethics course is highly recommended by ORPHEUS. At the outset, Dokuz Eylul met the first requirement, but not the second. Accordingly, the “elective” ethics course was made “mandatory”- which upgraded the ethical considerations of the PhD candidates. The other main outcomes are: requirement of three papers before the thesis defence, two of which should have been accepted in a SCI-extended cited journal, and one, prepared as a manuscript for a peer-reviewed journal (ORPHEUS requires “equivalent of three research papers”), an enriched list of transferable courses (with two new inaugurations: “Personal Development” and “Teaching skills”), an independent thesis defence jury two of whom are from outside the university, with the supervisor not having the right to vote. All these significant reforms have only been possible with the support of ORPHEUS. In fact, these positive outcomes have paved the way for the award of “ORPHEUS Label” to Dokuz Eylül University Graduate School of Health Sciences. ORPHEUS team is also supportive in helping safeguard the ORPHEUS best-practices.

*The author was a faculty member of Dokuz Eylül University School of Medicine (1986-2016) and Chair of the ORPHEUS labelling Board of Dokuz Eylül Graduate School of Health Sciences (2014- 30 March 2016).

Plenary Lecture, Hall A (13:30-14:30)
Poly(ADP-ribose) polymerase: pathomechanisms and therapeutic opportunities

Csaba Szabo

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The goal of this lecture is to overview the pathophysiological roles of poly(ADP-ribose) polymerase (PARP) in various diseases, and to emphasize the current and potential clinical translation of pharmacological inhibitors of poly(ADP-ribose) polymerase (PARP) for the therapy of various diseases. The first section of the talk will summarize the available preclinical and clinical data with PARP inhibitors in various forms of cancer. In this context, the role of PARP in single-strand DNA break repair is relevant, leading to replication-associated lesions that cannot be repaired if homologous recombination (HRR) repair is defective, and the synthetic lethality of PARP inhibitors in HRR-defective cancer. HRR defects are classically associated with BRCA1 and 2 mutations associated with familial breast and ovarian cancer, but there may be many other causes of HRR defects. Part of the therapeutic action of some PARP inhibitors involve trapping of PARP in the DNA replication forks. Multiple lines of preclinical data demonstrate that PARP inhibition increases cytotoxicity and tumor growth delay in combination with temozolomide, topoisomerase inhibitors and ionizing radiation. PARP inhibitors are currently viewed as the drugs of choice for BRCA mutant breast and ovarian cancers. The second part of the lecture will summarize the role of PARP in selected non-oncologic indications. In a number of severe, acute diseases (such as stroke, neurotrauma, circulatory shock and acute myocardial infarction) the clinical translatability of PARP inhibition is supported by multiple lines of preclinical data, as well as observational data demonstrating PARP activation in human tissue samples. In these disease indications, PARP overactivation due to oxidative and nitrative stress drives cell necrosis and pro-inflammatory gene expression, which contributes to disease pathology. Emerging data indicate that not only nuclear PARP, but also mitochondrially localized PARP plays a role in the pathogenesis of oxidative cell injury. Multiple lines of preclinical data indicate the efficacy of PARP inhibitors to preserve viable tissue and to down-regulate inflammatory responses. This includes emerging data with the clinically approved PARP inhibitor, olaparib, in various forms of critical illness and inflammatory diseases. These data raise the possibility of the therapeutic repurposing of currently approved oncological PARP inhibitors to be used in various non-oncologic indications.

Oral Presentation Sessions (15:00-16:30)

Oral Presentation Session 11, Hall A

Pain and Inflammation; Gasotransmitters (C093-C098)

Chairs: Nuran Ogulener (Çukurova University, Turkey)

15:00 C093: Ondansetron shortens the duration of the thermal antinociceptive effects of bupivacaine in the rat paw
Aydin Mermer, Ali Özgül Saltali, Ipek Duman, Bülent Hanedan, Sengal Taylan Bagci, Yasin Tire, Seza Apiliogullari

15:15 C094: The effects of vascular cytocrome P450 Inhibitors on mesenteric blood flow, vascular hyporeactivity, organ damage and survival in an experimental septic shock model
Selda Ertaç Serdar, Kemal Kösemehmetoğlu, Alper Bektaş İskit

15:30 C095: Immunomodulatory effect of minocycline in mouse colitis
Jose Garrido Mesa, Alba Rodriguez Nogales, Francesca Algieri, Teresa Vezza, Natividad Garrido Mesa, Deseada Camuesco, Maria Pilar Utrilla, Maria Elena Rodriguez Cabezas, Federico Garcia, Natalia Chueca, Julio Galvez

15:45 C096: Blocking of urotensin receptors as new target for treatment of carrageenan induced inflammation in rats
Elif Cadirci, Zekai Halici, Muhammed Yayla, Erdem Toktay, Yasin Bayir, Emre Karakus, Atilla Topcu, Basak Buyuk, Abdulmecit Albayrak

16:00 C097: Do penile hemodynamics change in the presence of hydrogen sulfide (H2S) donor in the metabolic syndrome-induced erectile dysfunction?
Ezgi Dayar, Erkan Kara, Gunay Yetik Anacak, Nil Hocaoglu, Ozan Bozkurt, Sedef Gidener, Nergis Durmus

16:15 C098: The effects of cyclooxygenase, nitric oxide, phosphodiesterase IV and Rho-kinase inhibitors on hydrogen sulfide-induced relaxant response in mouse corpus cavernosum
Fatma Aydinoglu, Nuran Ogulener

C093: Ondansetron shortens the duration of the thermal antinociceptive effects of bupivacaine in the rat paw

Aydin Mermer[1], Ali Özgül Saltali[2], Ipek Duman[3], Bülent Hanedan[1], Sengal Taylan Bagci[4], Yasin Tire[5], Seza Apiliogullari[6]

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In clinical practice there is no approved antagonist for local anesthetics. There is conflicting evidence that ondansetron, a $5HT_3$ receptor antagonist causes reduction in sensory block durations and analgesic activity of local anaesthetics after local or systemic administration. We designed this experimental behavioural study to assess the effectiveness of locally and systemic injected ondansetron on the duration of the thermal antinociceptive effects of bupivacaine in the rat paw.

After Institutional approval 28 Sprague-Dawley rats weighing 400-450g were randomized. Thermal antinociception was determined as achieve cut-off latency by the paw withdrawal latency (PWL) measurements. Group 1 (control group, n=7) received left intraplantar injection of plain 50µL bupivacaine 250µg. Group 2 (n=7) received left intraplantar injection of plain 50µL bupivacaine 250µg + left intraplantar injection of 50µL ondansetron 100µg, Group 3 (n=7) received left intraplantar injection of plain 50µL bupivacaine 250µg + left intraplantar injection of 100µL ondansetron 200µg. Group 4 (n=7) received left intraplantar injection of plain 50µL bupivacaine 250µg + intraperitoneal 100µL ondansetron 200µg. The data are reported in terms of duration of PWL. Statistical comparisons were made using Kruskal-Wallis H test followed by Man Whitney U (with Bonferroni correction, $\alpha = 0.008$) test. $p<0.05$=significant.

Ondansetron significantly reduced the duration of thermal antinociceptive effect of bupivacaine both when applied with the same area of local anesthetic and systemically administered. Local administration is more effective than intraperitoneal injection at the same ondansetron dose.

Systemic and locally injected ondansetron shortens the duration of the thermal antinociceptive effects of bupivacaine in the rat paw. Local administration is more effective.

Keywords: antinociception, bupivacaine, ondansetron

Table 1. Paw withdrawal latency.

	PLW duration (min) (mean±SD)
Group 1 (n=7) bupivacaine 250µg	95.4±9.3
Group 2 (n=7) bupivacaine 250µg+ local ondansetron 100µg	45.4±17.1*
Group 3 (n=7) bupivacaine 250µg+ local ondansetron 200µg	31.4±1.8*
Group 4 (n=7) bupivacaine 250µg+ intraperitoneal ondansetron 200µg	67.7±8.2*¥

**p=0.002 compared to group 1, ¥p=0.002 compared to group 3*

C094: The effects of vascular cytocrome P450 Inhibitors on mesenteric blood flow, vascular hyporeactivity, organ damage and survival in an experimental septic shock model

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The effects of the vascular cytochrome P450 (CYP) epoxygenase and omega(ω)hydroxylase which catabolize the arachidonic acid into the vascular tone and inflammation regulators, epoxeicosatrienoic acids (EET's) and 20hydroxyeicosatetraenoic acid (20-HETE) respectively, on the hemodynamic changes, organ damage and survival in an experimental sepsis model have not been clearly investigated yet. For this purpose, male Wistar albino rats were divided into 2 groups as LPS (lipopolysaccharide induced experimental septic shock model, 2 mg.kg-1, i.p) and control (%0.09 NaCl, 2 ml.kg-1, i.p.) groups. 4 hours later, under chloralhydrate (400 mg kg-1, i.p.) anesthesia, the effects of non-selective CYP inhibitor, miconazole, 3 mg.kg-1; CYP epoxygenase inhibitor, PPOH, 5 mg.kg-1; CYP omega(ω)-hydroxylase inhibitor, HET0016, 5 mg.kg-1 and their vehicules (0.5 ml.kg-1 i.v.; n=6), on blood pressure, mesenteric blood flow and mesenteric vasoconstrictor response to phenylephrine (1-1000 µg.kg-1 i.v.) were evaluated. At the end of the experiment, lung, liver, kidney and spleen tissues were taken for the oxidative and histopathological damage examination. Furthermore, male Swiss albino mice were administered lethal dose of LPS (80 mg.kg-1, i.p.) and observed for 48 hours survival. We observed that LPS-treated rats displayed about 30% reduction in blood pressure, mesenteric blood flow and phenylephrineinduced vasoconstrictor response and none of the inihibitors affected on these parameters. CYP ω-hydroxylase inhibitor reversed the oxidative and histopathological damage in tissues and augmented the survival rate from 30% to 50%. Contrarily, all of the CYP epoxygenase inhibitor administered mice died in the first day. These findings show that while CYP epoxygenase inhibitor augments the organ damage and mortality, CYP ω-hydroxylase inhibitor reverses the organ damage and partially attenuates the mortality in this sepsis model.

Keywords: 20HETE, CYP epoxygenase, CYP ω-hydroxylase, EET, septic shock

C095: Immunomodulatory effect of minocycline in mouse colitis

Jose Garrido Mesa[1], Alba Rodriguez Nogales[1], Francesca Algieri[1], Teresa Vezza[1], Natividad Garrido Mesa[1], Deseada Camuesco[1], Maria Pilar Utrilla[1], Maria Elena Rodriguez Cabezas[1], Federico Garcia[2], Natalia Chueca[2], Julio Galvez[1]
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Minocycline exerts immunomodulatory effects that could be beneficial in inflammatory bowel disease (IBD). The mechanism underlying it is not completely understood and different actions on distinct immune populations might be implicated. The aim of the study was to evaluate the impact of minocycline on the different immune populations involved in DSS-mice colitis.

Male C57BL/6J mice were assigned into non-colitic and DSS-colitic groups. Colitis was induced by dextran sodium sulfate (DSS) in the drinking water (3%) for 5 days. Once the colitis process was established, colitic mice were divided in two groups: DSS-control (without treatment) and MNC (receiving minocycline 50 mg/kg/day). After five days of treatment, all mice were sacrificed. The inflammatory status was evaluated by a disease activity index (DAI), qPCR of inflammatory markers and multiparametric flow-cytometry of lamina propria leukocytes.

According to the DAI values, minocycline treatment improved the recovery of colitic mice, ameliorating some of the inflammatory markers. The characterization of immune populations involved in DSS-induced intestinal inflammation showed that minocycline affected to several subsets, reducing the recruitment of B cells but increasing the numbers of eosinophils, T cells and antigen presenting cells, macrophages and dendritic cells. On the T cell compartment, the effect of minocycline stands out by an increase in T regulatory cells and further analysis of macrophages and DC subsets showed that the treatment improved the ratio between mature macrophages over recently arrived inflammatory monocytes and between tolerogenic DCs over inflammatory DCs.

Minocycline is able to modify the expression of different inflammatory markers, which influence the recruitment and functions of immune populations present in the intestinal lamina propria. These observations sugest that minocycline could induce a shift towards regulatory and Th2 response, which helps to control intestinal inflammation. These immunomodulatory properties could be of great interest to face inflammatory disorders such as IBD.

Keywords: minocycline, flow cytometry, IBD, inflammation, mouse colitis

C096: Blocking of urotensin receptors as new target for treatment of carrageenan induced inflammation in rats

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This study investigated possible role of urotensin-II (U-II) and its receptor expression in inflammation by using UTR agonist and antagonist in carrageenan induced acute inflammation. Rats were divided into 5 groups as (1) Healthy control, (2) Carrageenan control, (3) Carrageenan +Indomethacin 20 mg/kg, orally, (4) (4) Carrageenan +AC7954 (U-II receptor agonist, intraperitoneally) 30 mg/kg and (5) Carrageenan +SB657510 (UTR antagonist, intraperitoneally) 30 mg/kg. 1 hour after drug administration, carrageenan was injected. UTR expression increased in carrageenan induced paw tissue. At the 3rd hour after carrageenan injection, agonist produced no effect while antagonist 63% anti-inflammatory effect respectively. Antagonist administration prevented the decrease in antioxidant system and also capable to decrease TNF-alpha and IL-6 mRNA expressions. This study showed the role of urotensin-II receptors in the physiopathogenesis of acute inflammatory response that underlying many diseases accompanied by inflammation.

Acknowledgements: This study was supported by Turkish Academy of Sciences (TUBA) The Young Scientists Award Programme (GEBIP) with project number "EC/TUBAGEBIP20135"

Keywords: urotensin-II, inflammation, urantadine, rats, TNF-alpha

Table 1. Effects of urotensin 2 receptor agonist (AGO), antagonist (ANTA) and indomethacin (IND) on carrageenan (CAR)-induced paw oedema (third hour) in rats *(mean values with their standard deviations).* Columns in with same letter (i.e. a or b) is statistically insignificant. Columns with different letters are statistically significant (p<0.05).

Groups	Paw Volume-before carrageenan	Paw Volume-3 hours After carrageenan	Distinction	Anti-inflammatory effect
CAR	0,85±0,07	1,85±0,15	1,00±0,12 a	-
CAR+IND	0,93±0.05	1,25±0,07	0,32±0.05 b	%68
CAR+AGO	0,90±0.05	1,95±0.05	1,05±0,03 a	-
CAR+ANTA	0,87±0,06	1,24±0.05	0,37±0.05 b	%63

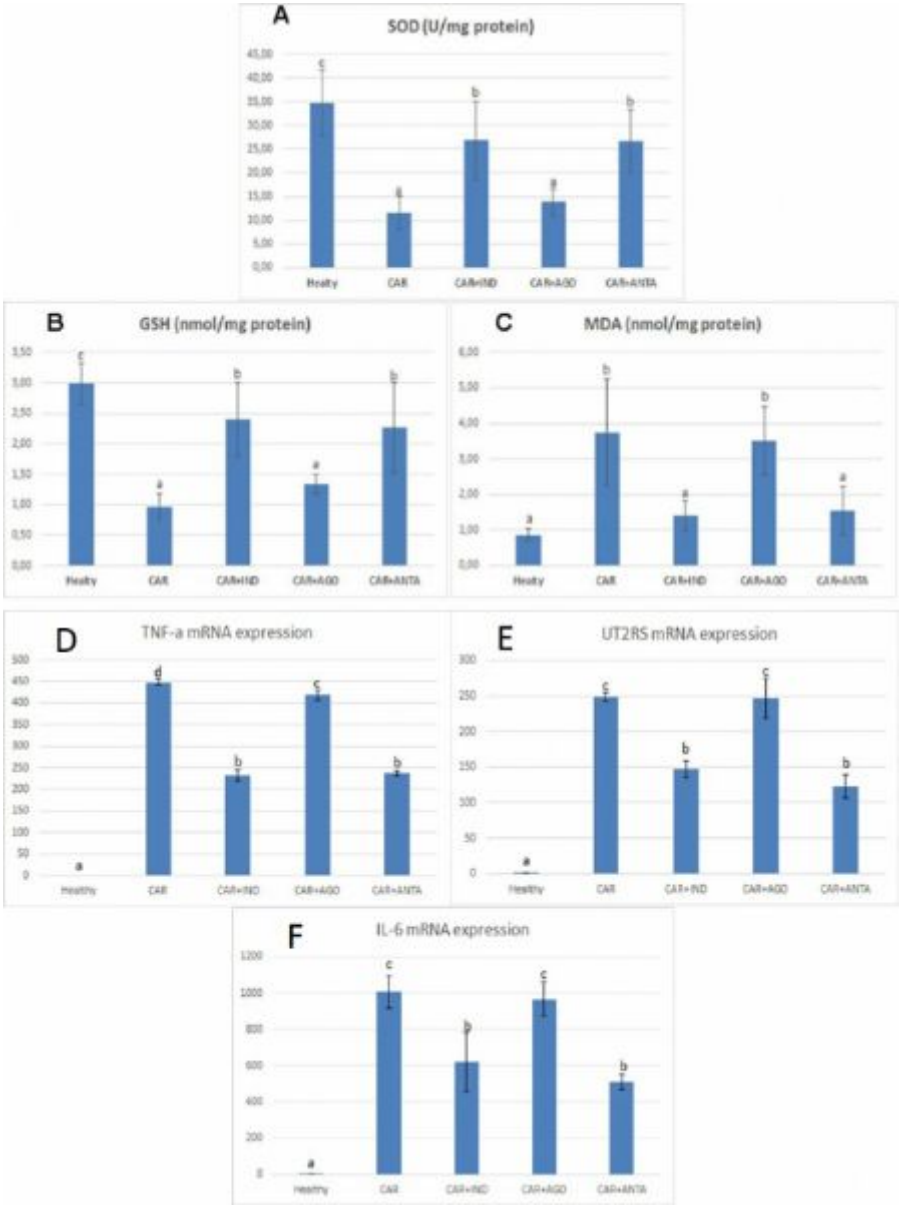

Figure 1. A: Effects of Urotensin 2 agonist and antagonist on changes in the activity of SOD enzyme in carrageenan-injected rat paws (third hour). B: Effects of Urotensin 2 agonist and antagonist on changes in the activity of GSH in carrageenan-injected rat paws (third hour). C: Effects of Urotensin 2 agonist and antagonist on changes in the MDA level in carrageenan -injected rat paws (third hour). D: Effects of Urotensin 2 agonist and antagonist on changes in the expression of TNF-a mRNA level in carrageenan-injected rat paws (third hour). E: Effects of Urotensin 2 agonist and antagonist on changes in the expression of UTR mRNA level in carrageenan-injected rat paws (third hour). F: Effects of Urotensin 2 agonist and antagonist on changes in the expression of IL-6 mRNA level in carrageenan-injected rat paws (third hour). CAR: carrageenan, IND: indomethacin, AGO: agonist, ANTA: antagonist.

C097: Do penile hemodynamics change in the presence of hydrogen sulfide (H_2S) donor in the metabolic syndrome-induced erectile dysfunction?

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Erectile dysfunction (ED), a general marker for metabolic syndrome (MetS), occurs as a result of impairment in nitric oxide pathway. Hydrogen sulfide (H_2S), an other gasotransmitter, has been revealed to involving in hypertension, insulin secretion, regulation of vascular tone especially in erectile physiology. The aim of this study is to investigate sodium hydrosulfide (NaHS, H_2S donor) on penile hemodynamics and H_2S levels in the penile tissues in MetS rat model.

Wistar rats (weight 190-230g) were randomly divided into 2 groups as; control and MetS, which were fed with standard diet (SD) and 60% high fructose diet (HFD) for 10 weeks, respectively. MetS model was evaluated with biochemical analyses

[serum triglyceride (TG), uric acid, blood glucose, insulin levels], waist circumference/tibial length ratio and HOMA index. Penile hemodynamic parameters were evaluated by the measurement of intracavernous pressure (ICP)/mean arterial pressure (MAP) ratio and area under curve (AUC) during cavernous nerve (CN) stimulation in the presence and absence of intracavernous injection of NaHS (100mg/50µl) and its control %0.9NaCl 50µl) in Mets and control groups. H_2S levels were measured in penile tissues by methylene blue assay.

TG and insulin levels, HOMA index, weight, waist circumference/tibia percent and blood pressure were increased significantly in MetS group when compared to control group ($p<0.01$, $p<0,0001$, $p<0,0001$, $p<0,0001$, $p<0,0001$, $p<0.01$ respectively). HFD attenuated ICP/MAP and AUC in MetS group ($p<0,0001$, $p<0.01$ respectively). Intracavernous administration of NaHS caused a significant increase in ICP/MAP ratio in the MetS group ($p<0,1$) but not in control group (Figure 1). H_2S levels were decreased significantly in the penil tissues of the MetS group ($p<0,001$).

Decreased H_2S levels in penile tissue and recovery of penile hemodynamics by NaHS in MetS group reveals the significant role of H_2S in the MetS-dependent ED that could be a new therapeutic target.

Keywords: hydrogen sulphide, metabolic syndrome, erectile dysfunction

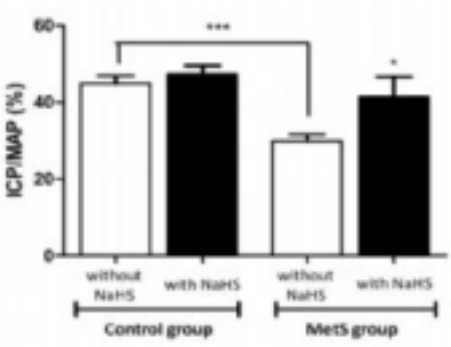

Figure 1. Evaluation of ICP/MAP in the presence and absence of NaHS in control and MetS groups. *Percentage of intracavernous pressure /mean arterial pressure ratio (ICP/MAP). Statistical analysis was performed by Student's t-test (Graphpad, San Diego, CA). P<0.05 was considered as statistically significant. ICP/MAP (without NaHS), control vs metS group *** p<0.0001; ICP/MAP (with NaHS), control vs metS group * p<0.05.*

Table 1. Biochemical parameters, weight, waist circumference (WC)/ tibia length (TL), HOMA index and blood pressure in control and metS groups at the end of 10th weeks.

	TG (mg/dl)	**UA (mg/dl)**	**Glucose (mg/dl)**	**Insulin (mU/L)**	**Weight (g)**	**WC/TL cm**	**HOMA index**	**Blood Pressure (mmHg)**
Control (n=21)	44,4±2,2	1,9±0,2	316,1±12,5	0,1±0,02	339±5,5	3,7±0,03	0,09±0,02	83,29±1,62
MetS (n=21)	58,1±3,8**	2,2±0,3	351,7±19,0	3,9±0,4***	447,2±9,7***	4,6±0,08****	3,42±0,3***	92,52±2,06**

*Statistical analysis was performed by Student's t-test (Graphpad, San Diego, CA). P<0.05 was considered as statistically significant. Control vs MetS group, TG **p<0.01, Insulin ***p<0,0001, weight ***p<0,0001, WC/TL ***p<0,0001, HOMA index ***p<0,0001, Blood pressure **p<0.01.*

C098: The effects of cyclooxygenase, nitric oxide, phosphodiesterase IV and Rho-kinase inhibitors on hydrogen sulfide-induced relaxant response in mouse corpus cavernosum

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Recent studies have been demonstrated that hydrogen sulfide (H_2S) synthesizes endogenously from L-cysteine substrate and causes the relaxant effect in corpus cavernosum (CC) tissues. Also, it has been suggested that L-cysteine/H_2S pathway may regulate penile erection and the mechanism of H_2S regulating penile erection may provide a new therapeutic approach in erectile dysfunction. Therefore, the aim of this study was to evaluate the possible role of cyclooxygenase (COX), nitric oxide (NO) phosphodiesterase IV (PDEIV) and Rho-kinase in relaxant responses induced by exogenous H_2S in the mouse CC.

In the present study, the relaxant responses to sodium hydrogen sulfide (NaHS; exogenous H_2S; 10^{-6}–10^{-3} M) were obtained in isolated mouse CC tissues which were pre-contracted by phenylephrine ($5x10^{-6}$ M). The effects of COX enzyme inhibitor indomethacin (10^{-6} M), NO synthase inhibitor N-nitro-L-arginine (L-NA: 10^{-4} M) and combination of indomethacin (10^{-6} M) plus L-NA (10^{-4} M) on the relaxant response to exogenous H_2S were investigated. The effect of theophylline ($5x10^{-4}$ M), a non-selective PDEIV inhibitor, was studied on the relaxant response of exogenous H_2S. Also, the influence of fasudil ($3x10^{-6}$ M) and Y-27632 ($3x10^{-6}$ M), specific Rho-kinase inhibitors, on the relaxant response to exogenous H_2S were examined. Also, we evaluated effect of L-cysteine (10^{-3}, 10^{-4} M) as an endogenous H_2S on the relaxant response to exogenous H_2S.

Exogenous H_2S-induced relaxations were significantly reduced by indomethacin. However, L-NA increased the relaxations to exogenous H_2S. The combination of indomethacin and L-NA significantly augmented the exogenous H_2S-induced relaxant response. Furthermore, theophylline, fasudil and Y-27632 were markedly reduced relaxant responses to exogenous H_2S-induced relaxations. Also, exogenous H_2S-induced relaxations significantly reduced in the presence of L-cysteine.

These results suggest that COX, NO, PDEIV and Rho-kinase pathways may involve the relaxant response induced by exogenous H_2S in mouse CC tissues.

Keywords: corpus cavernosum, cyclooxygenase, hydrogen sulfide, mouse, rho-kinase

Oral Presentation Session 12, Hall B

Cancer Chemotherapy; Respiratory Pharmacology; Genitourinary and Reproductive Pharmacology (C099-C104)

Chairs: Zeliha Yazıcı (İstanbul University, Turkey)
Ahmet Altun (Cumhuriyet University, Turkey)

15:00 C099: Protective effect of hypericum triquetrifolium turra. on cyclophosphamide–induced myelotoxicity and hemotoxicity in rat
Songül Çetik, Adnan Ayhancı, Cumali Keskin

15:15 C100: The cytotoxic effects of estrogen targeted nano-silver molecules on platinum resistant ovarian cancer cells line (OVCAR-3)
Ahmet Altun, Nevcihan Gursoy, Marija Sevcenko, Ahmet Turan Demir

15:30 C101: Expressional alterations of CYP1A2, CYP2J3 and CYP3A1 in rat liver and heart upon doxorubicin and ciprofloxacin treatment
Andleeb Shahzadi, Esra Guzel, Omer Faruk Karatas, Ikbal Sonmez, Mustafa Ozen, Zeliha Yazici

15:45 C102: Pharmacokinetics of chronomodulated capecitabine as a part of first line XELOX chemotherapy in metastatic colorectal cancer patients
Zeliha Pala Kara, Kezban Nur Pilanci, Sezer Saglam, Alper Okyar

16:00 C103: Protective effect of hypericum perforatum extract on cigarette smoke induced lung inflammation
Halil Mahir Kaplan, Ergin Şingirik, Figen Doran

16:15 C104: Chronic unpredictable mild stress impairs neurogenic and endothelium-dependent relaxation of rabbit corpus cavernosum smooth muscle: improvement with chronic administration of resveratrol
Semil Selcen Gocmez, Tugce Demirtas Sahin, Tijen Utkan

C099: Protective effect of hypericum triquetrifolium turra. on cyclophosphamide–induced myelotoxicity and hemotoxicity in rat

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Possible protective effects of Hypericum triquetrifolium Turra. (HT) against Cyclophosphamide (CP)-induced hemotoxicity and myelotoxicity were examined in this study. The toxic effects of the CP on erythrocyte, leukocyte, platelets and bone marrow cells were compared. Albino rats (Wistar, 3-4 months old, male, weight 220±20 g, healthy) were randomly divided in 9 groups, each including seven animals: Group 1 (control) treated with saline; groups 2 treated with 150 mg/kg CP, respectively; group 3, 4 and 5 treated with 25, 50 and 100 mg/kg HT; groups 6, 7 and 8 treated with 25, 50 or 100 mg/kg HT+CP, group 9 treated with 0,5ml - %0,2 DMSO. The results were analyzed by One Way Analysis of Variance (ANOVA) and Kruskal-Wallis One Way Analysis of Variance on Ranks Test. When compared with the control group, in rats given 150 mg/kg CP, the number of erythrocyte, leukocyte, platelets and bone marrow core cells were decreased. There was a dose-dependent effect on CP-induced myelotoxicity and hemotoxicity. By the administration of 25, 50 and 100 mg/kg HT, there was an important decrease with respect to CP toxicity. It has been shown that dose of 25, 50 or 100 mg/kg HT+CP significantly increased number of cells when compared with CP. Based on these findings, it could be proposed that HT is a strong candidate in preventing the CP-induced hemotoxicity and myelotoxicity. This property of HT may suggest that it's related with antiinflammatory and cytoprotective effects.

Keywords: cyclophosphamide, myelotoxicity, hemotoxicity, hypericum triquetrifolium

C100: The cytotoxic effects of estrogen targeted nano-silver molecules on platinum resistant ovarian cancer cells line (OVCAR-3)

Ahmet Altun[1], Nevcihan Gursoy[2], Marija Sevcenko[3], Ahmet Turan Demir[2]
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Resistance to chemotherapy commonly compromises the treatment of many advanced cancers. Although platinum derivate cancer drugs are able to provide strong cytotoxicity and high survival rates, resistance to the platinum derivate makes it more dangerous and alternative approaches are needed for successful treatment. The main purpose of the present study was to investigate anticancer effects of estrogen-AgNP complex on platinum resistant ovarian cancer.

OVCAR-3 platinum resistant cell line purchased from American Type Cell Collection (ATCC) was used to test to test our hypothesis. OVCAR-3 cells were grown in RPMI- 1640 medium. All cell cultures were supplemented with 1% (w/v) penicillin/ streptomycin, 10% (v/v) FBS. Cells were grown at 37˚C in a 5% CO2 humidified atmosphere. The surface morphology of the estrogen molecules was observed by a Transmission electron microscope (FEI Tecnai G2 Spirit Bio (TWIN) TEM) operating at the accelerating voltage of 120 kV. Molecule size was verified by Atomic Force Microscope (AFM) (Park System XE-100E). XTT test has been used to evaluate viability in cytotoxicity experiments.

AFM analyses showed successful synthesis of AgNPs which has a round shape complexes with 20-174 nm in diameter. TEM images clearly show that silver nanoparticles were homogeneously distributed on estrogen molecules. While, estrogen alone did not cause any effect on proliferation, estrogen-AgNP complex caused concentration dependent strong cytotoxic effect. It has been observed that estrogen-AgNP complex caused statistically significantly low cytotoxic effect on healthy L929 fibroblast cells ($p<0.05$) which may indicate the specificity of the treatment for estrogen receptor positive cells.

The present study suggests that we accomplished to the synthesis of silver nanoparticles on estrogen molecules and estrogen molecules which have high expression levels in ovarian cancer can be targeted by estrogen-AgNP complex. Estrogen-AgNP complex seems to be an alternative in the treatment of platinum resistant advance ovarian cancer.

Keywords: platinum resistant ovarian cancer (OVCAR-3), nano silver molecules, cytotoxicity, Atomic Force Microscope (AFM), transmission electron microscope (TEM)

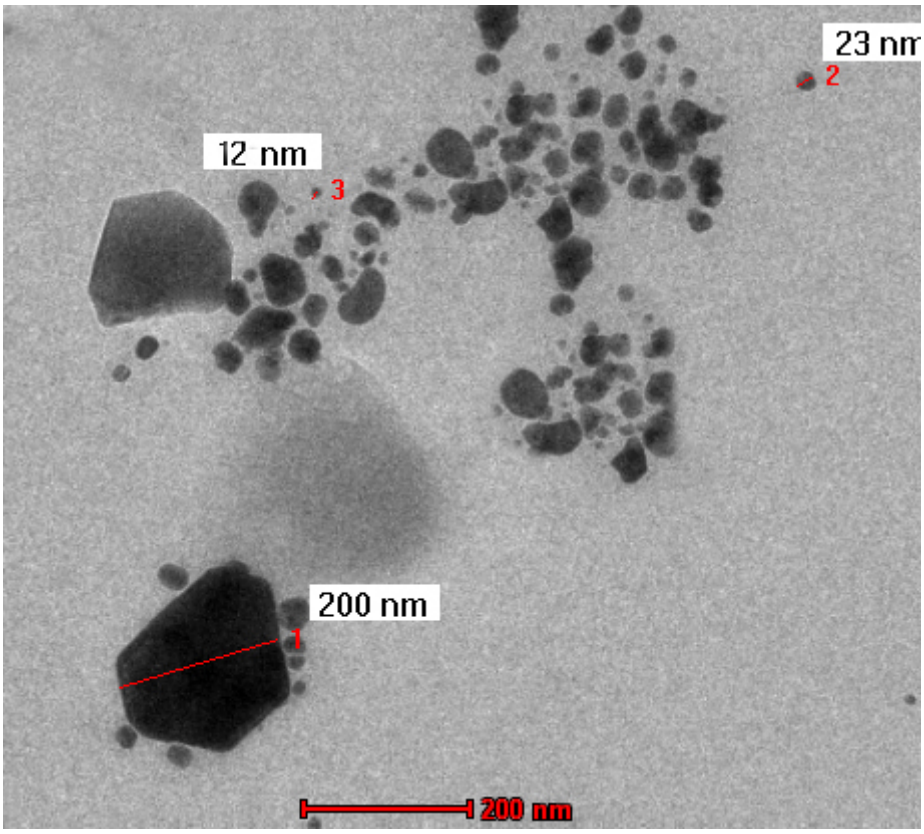

Figure 1. TEM image of estrogen+AgNP. TEM images clearly show that silver nanoparticles were homogeneously distributed on estrogen molecules.

C101: Expressional alterations of CYP1A2, CYP2J3 and CYP3A1 in rat liver and heart upon doxorubicin and ciprofloxacin treatment

Andleeb Shahzadi[1], Esra Guzel[2], Omer Faruk Karatas[3], Ikbal Sonmez[1], Mustafa Ozen[4], Zeliha Yazici[1]

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The present study was designed to investigate doxorubicin and ciprofloxacin induced cardio-toxicity in relation to CYP1A2, CYP2J3 and CYP3A1 mRNA expression.

Adult male Sprague Dawley rats followed chronic 3-week and acute single dose DOX and/or CIP administration. The DOX and cardiac troponin-I (cTnI) plasma levels were measured by using enzyme-linked Immuno Sorbent Assay (ELISA) technique. Cardiac CYP1A2, CYP2J3 and CYP3A1 gene expressions were determined by real-time polymerase chain reaction (qPCR).

Cardiac CYP1A2 gene expression after acute administration did not show any significant difference. CYP1A2 mRNA was increased 50% ($p<0.05$) after chronic administration of DOX. Acute high dose DOX showed 69% ($p<0.005$) induction while chronic high dose DOX increased the CYP2J3 mRNA expression by 83%, addition of CIP down regulated it to 99% ($p<0.01$). CYP3A1 cardiac mRNA level was induced to 65% ($p<0.05$) with acute high dose DOX administration although, chronic administration showed non-significant induction of cardiac CYP3A1 mRNA expression. Acute and chronic group showed a strong positive correlation with CYP3A1 gene expression and cTnI ($r = 0.818$ $p <0.05$).

We found an induction of CYP2J3 CYP1A2 and CYP3A1 gene expression with DOX and CIP down regulate these expressions. CYP3A1 gene showed a strong correlation with cardiac toxicity.

Keywords: CYP, cardiotoxicity, ciprofloxacin, doxorubicin, drug-drug interaction

C102: Pharmacokinetics of chronomodulated capecitabine as a part of first line XELOX chemotherapy in metastatic colorectal cancer patients

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Chronomodulated therapy is based on the administration of chemotherapy according to circadian rhythms to improve tolerability and effectiveness of drugs in cancer patients. Capecitabine is an oral, tumor-selective, fluoropyrimidine carbamate molecule, a precursor of 5-Fluorouracil for the treatment of colorectal, gastric and breast cancers. The aim of this study was to evaluate the pharmacokinetics of chronomodulated capecitabine administered in the morning (08:00 am) and noon (12:00 pm) according to a specific time schedule (Brunch Regimen: Breakfast and Lunch) as a part of the first line XELOX chemotherapy in patients with metastatic colorectal cancer (n= 5). Plasma concentration of capecitabine was measured by HPLC method. Capecitabine was rapidly absorbed and the mean time to reach Cmax was 1 h at both administration times. However, C_{max} was two-fold higher in the morning (2.88µg/ml ±0.45) as compared to noon time plasma level (1.45µg/ml ±0.58; p=0.31, t-test). The calculated mean AUC levels from 0 to 4 hours were 62% higher when the drug administered at 08.00 am than the one at 12:00 pm (3.70 µg/ml vs 2.30 µg.h/ml; p=0.06, t-test). Elimination half-life was shorter following administration at 12:00 p.m. than the one at 08:00 a.m. (p=0.42, t-test). This study has reported the first pharmacokinetic results with the brunch regimen chronomodulated administration of capecitabine in cancer patients. Our findings highlight the further studies to show the correlation between tolerability and pharmacokinetics of capecitabine in choronomodulated regimen versus traditional regimen.

Keywords: capecitabine, cancer chronotherapy, pharmacokinetics, brunch regimen

C103: Protective effect of Hypericum perforatum extract on cigarette smoke induced lung inflammation

Halil Mahir Kaplan[1], Ergin Şingirik[1], Figen Doran[2]
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Hypericum perforatum is a plant, which blooms between july and september at farms, borders of roads and woods, top of hills and grasslands, and whose anti-inflammatory effects are shown in various studies. It is shown that cigarette smoke causes increase in inflammation mediators in the lung. Due to this reason, we planned a study to examine the protective effects of Hypericum perforatum aganist smoke exposure induced lung damage in mice. For this purpose, mice were exposed to smoke during 2 mounth and 70 mg/kg Hypericum perforatum extract per a day and cyclooxygenase-2 (COX-2), cytosolic phospholipase A2 (cPLA2) and inducible nitric oxide synthase (iNOS) enzymes in their lungs are analyzed by using ELISA method. Smoke exposure increased the expression of COX-2, cPLA2 and iNOS enzymes. Hypericum perforatum administration to mice that are exposured to smoke previously decreased the rate of increase of the cPLA2, COX-2 and iNOS. In conclusion, our study shows that smoke exposure causes inflammation mediated lung damage and the use of Hypericum perforatum extract can be helpful against this toxic effect.

Keywords: cigarette smoke, lung, Hypericum perforatum, cyclooxygenase-2 (COX-2), cytosolic phospholipase A2 (cPLA2), inducible nitric oxide synthase (iNOS)

C104: Chronic unpredictable mild stress impairs neurogenic and endothelium-dependent relaxation of rabbit corpus cavernosum smooth muscle: Improvement with chronic administration of resveratrol

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Accumulating epidemiological and experimental evidence has indicated that psychological stress may significant contributions to the development of erectile dysfunction (ED). Resveratrol has antiinflammatory, antioxidant, vasorelaxant and cardioprotective effects which may have protective and restorative potentials in stress-induced ED. This study investigated the effects of resveratrol on chronic unpredictable mild stress (CUMS)-induced impairment of neurogenic and endothelium-dependent relaxation of rabbit corpus cavernosum smooth muscle.

Male New Zealand adult rabbits were randomly divided into three groups (n=6): animals not exposed to CUMS (control), animals exposed to CUMS, and animals treated with resveratrol while exposed to CUMS (CUMS+resveratrol). Resveratrol was administrated intraperitoneally, 20 mg/kg/day for 12 weeks to the animals being exposed to the CUMS battery. CUMS was induced by a set of defined adverse conditions applied in a shuffled order for 4, 8, 12 weeks. Rabbits were sacrificed 24 h after the end of the CUMS protocol and the reactivity of the corpus cavernosum tissue from all groups was studied in organ chambers. Significance was tested by one-way analyses of variance (ANOVA) with a posthoc Tukey's test.

Carbachol-induced endothelium-dependent and electrical field stimulation (EFS)-induced neurogenic relaxations were significantly reduced in CUMS group compared to the control group. The impaired relaxations of strips were markedly improved by treatment of resveratrol. There was no significant difference in the relaxant response to sodium nitroprusside and contractile respose to KCl among the groups.

These data indicate that chronic stress may impair both neurogenic and endothelium-dependent relaxation of corpus cavernosum smooth muscle, and may contribute to the etiology of ED. Chronic treatment of resveratrol prevents impairment of functional responses to the penile erectile tissue of rabbits, suggesting the potential for new treatment approaches to treatment of ED during psychological stress.

Keywords: chronic stress, corpus cavernosum, resveratrol, rabbit

Oral Presentation Session 13, Hall C

Gastrointestinal Pharmacology; Rational Drug Use (C105-C110)

Chairs: Atila Karaalp (Marmara University, Turkey)
Elif Çadırcı (Atatürk University, Turkey)

15:00 C105: Proton pump inhibitor use for a twelve-month period is not associated with changes in serum magnesium levels: a prospective open label comparative study
Elton Bahtiri, Hilmi Islami, Rexhep Hoxha, Hasime Qorraj Bytyqi, Shaip Krasniqi, Kujtim Thaçi, Valon Krasniqi, Liridon Haziri, Malbora Musa

15:15 C106: The role of TLR4 in gut-brain cross talk in a murine model for Parkinson's disease
Paula Perez Pardo, Hemraj B Dodiya, Johan Garssen, Ali Keshavarzian, Aletta D Kraneveld

15:30 C107: The effect of urantide, an urotensine receptor antagonist, on the healing of indomethacin-induced gastric ulcer in rats
Zekai Halici, Elif Cadirci, Busra Diyarbakir, Muhammed Yayla, Damla Cetin, Zerrin Kutlu, Emin Sengul

15:45 C108: Potentially inappropriate drug use in elderly patients admitted to the intensive care unit of a university hospital
Medine Gulcebi, Songul Ozkula, Nesrin Duman, Gozde Ayhan, Zehra Turgan, Rezzan Gulhan, Atila Karaalp, Filiz Onat, Zafer Goren

16:00 C109: Self-Medication practice among preclinical medical students and law students of Marmara University
Akif Köse, Asibe Türkkan, Melike Ülkü Aydın, Özlem Alhan, Yasin Yıldız, Mikail Özdemir, Seyhan Hıdıroğlu, Rezzan Gülhan Aker

16:15 C110: Assessment of antibiotic prescribing performance of dentists in Turkey
Mehtap Aydın, Cenker Z Koyuncuoglu, Ipek Kirmizi, Esma Kadi, Ali Alkan, Ahmet Akici

16:30 C119: Investigation of the effects of oleuropein rich diet on rat enteric bacterial flora
Aslı Kiraz, Tuncer Şimşek, Satı Zeynep Tekin, Sait Elmas, Murat Tekin, Hasan Şahin, Hatice Betül Altınışık, Çiğdem Uysal Pala*

* Transferred from Hall E.

C105: Proton pump inhibitor use for a twelve-month period is not associated with changes in serum magnesium levels: A prospective open label comparative study

Elton Bahtiri[1], Hilmi Islami[1], Rexhep Hoxha[1], Hasime Qorraj Bytyqi[1], Shaip Krasniqi[1], Kujtim Thaçi[2], Valon Krasniqi[1], Liridon Haziri[1], Malbora Musa[1]

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Proton pump inhibitors (PPIs) are a widely used class of drugs because of a generally acceptable safety profile. Among recently raised safety issues of long-term use of PPIs is increased risk of developing hypomagnesaemia. As there have been very few prospective studies measuring serum magnesium levels before and after PPI therapy, we aimed to prospectively assess the potential association between one year PPI therapy and the risk of hypomagnesaemia as well as the incidence of new-onset hypomagnesaemia during the study. In addition, the association of PPI therapy with the risk of hypocalcaemia was assessed.

The study included 250 patients who had normal serum magnesium and total calcium levels and had been initiated a long-term PPI treatment. Serum magnesium, total calcium and parathormone (PTH) levels were measured at baseline and after one year.

Of the 250 study participants, 209 completed 12 months of treatment and were included in the statistical analysis. The Wilcoxon signed rank test showed stable serum Mg levels all study groups and subgroups, with no statistically significant differences between measurements at two different time points. However, there were statistically significant differences in serum total calcium and PTH levels in PPI users.

This study demonstrated stable serum magnesium levels after 12 months of PPIs use in the general population thus failing to show an association between PPIs use and risk of hypomagnesaemia. However, our study did demonstrate significant reduction of serum total calcium levels among PPIs users compared to nonusers thus serum calcium and PTH level measurements should be considered prior to and periodically after initiating long-term PPI therapy.

Keywords: magnesium, calcium, parathormone (PTH), proton pump inhibitors

Table 1. One year changes in biochemical parameters according to PPI use.

Groups	Variables	Before treatment	After treatment	Wilcoxon test (Z-score)	Wilcoxon test (p-value)
PPI users (n=167)	Parathormone	38.26±15.23 (36.39)	44.77±18.51 (41.94)	-4.617	<0.001
	Total Ca	2.46±0.16 (2.45)	2.37±0.18 (2.40)	-4.650	<0.001
	Mg	0.83±0.09 (0.80)	0.82±0.09 (0.83)	-0.902	0.367
PPI non-users (n=42)	Parathormone	47.28±22.15 (43.46)	43.60±16.11 (39.67)	-0.925	0.355
	Total Ca	2.44±0.19 (2.41)	2.39±0.15(2.36)	-1.351	0.177
	Mg	0.82±0.09 (0.80)	0.82±0.08 (0.81)	-0.525	0.599

All variables are presented as Mean±SD (Median)

C106: The role of TLR4 in gut-brain cross talk in a murine model for Parkinson's disease

Paula Perez Pardo[1], Hemraj B Dodiya[3], Johan Garssen[2], Ali Keshavarzian[3], Aletta D Kraneveld[1]
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Parkinson's disease (PD) clinical picture is usually dominated by motor impairments. However, non-motor symptoms, such as gastrointestinal dysfunctions, often precede the motor symptoms by many years and their occurrence in otherwise healthy people is associated with an increased risk of developing PD. Toll-like receptors (TLRs) are expressed by innate immune cells in the gut and TLR4 is involved in intestinal permeability regulation. It has been previously shown that abnormal intestinal permeability correlates with α-synuclein accumulations in the enteric nervous system (ENS).The gut might be an early site for PD in response to an environmental toxin or pathogen.

We aimed to investigate whether intestinal TLR4 dysregulation plays a role in PD pathology.

Rotenone exposure in rodents is a frequently used model for studying PD since it is able to reproduce many pathological features found in PD patients. TLR4 deficient and wild type C57BL/6J mice received 10mg/kg of rotenone orally once a day for 28 days. Control animals received vehicle. Readout parameters included motor function, intestinal transit time and histological examination of brain and gut to assess PD-like pathology.

Oral administration of rotenone induces motor deficits, delayed intestinal transit time and α-synuclein accumulation in the ENS in mice. Moreover, we observed inflammation in the gut in rotenone-treated mice characterized by a reduction in colon length and an increase in the number of T-cells. TLR4 deficient mice were partly protected against rotenone-induced motor deficits, delayed intestinal transit time and gut inflammation.

Oral administration of rotenone caused: 1. PD like motor deficits; 2. α-synuclein pathology in ENS and 3. intestinal motor dysfunction and inflammation. TLR4 is partly involved in the motor dysfunction and intestinal phenotype found after rotenone administration. Our results support the hypothesis that gut/brain cross talk possibly via TLR4 plays a central role in α-synuclein-induced PD pathology.

Keywords: Parkinson's disease, TLR4, gut-brain axis, alpha-synuclein

C107: The effect of urantide, an urotensine receptor antagonist, on the healing of indomethacin-induced gastric ulcer in rats

Zekai Halici[1], Elif Cadirci[1], Busra Diyarbakir[1], Muhammed Yayla[2], Damla Cetin[2], Zerrin Kutlu[3], Emin Sengul[4]

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The aim of this study was to investigate the role of urotensin and its receptors in indomethacin induced gastric ulcer formation by using urotensin receptor antagonist urantide.

36 male albino wistar rats were used in this study. Indomethacin induced gastric ulcer model was performed. 24 hour fasted rats were administered 0,3-0,6 and 1,2 mg/kg doses of urantide and 20 mg/kg dose of famotidine as standard antiulser agent. 25 mg/kg dose of indomethacin was administered to induce gastric ulcer. Six hours after indomethacin administration rats were sacrificed, stomach tissues were collected for macroscopic, biochemical and molecular analyses. Besides acid secretory effects of urantide and endogenous Human urotensin-II was compared on an isolated stomach perfusion model.

Urantide administration decreased ulcer formation in gastric tissue at 0.3 mg/kg dose and this effect was similar with standard drug famotidine. Urantide decreased gastric malondialdehide (MDA) levels when compared to indomethacin group. Indomethacin administration decreased gastric superoxide dismutase (SOD) activity and 0.3 mg/kg urantide increased this activity as well as famotidine. Indomethacin increased gastric inducible nitric oxide synthase (iNOS) mRNA expression while urantide (0.3 mg/kg) and famotidine decreased this activity significantly. When we evaluated gastric urotensin receptor expression we saw that in damaged tissues receptor expression decreased and both famotidine and low dose urantide restored this parameter. In gastric pH both human urotensin-II and urantide exerted variable effects, namely 10^{-7} M HU-II dose did not changed gastric pH while 10-6 M dose significantly increased. Also 10^{-7} dose of urantide increased gastric pH while 10^{-6} M dose caused no change.

This study showed that urotensin and its receptor can contribute in gastric ulcer formation and blocking urotensin receptors by urantide can have potential antiulcer effects at low doses.

This work is supported by the Ataturk University Medical Research Council, Grant No: 2014/151.

Keywords: indomethacin, rat, ulcer, urantide, urotensin-II

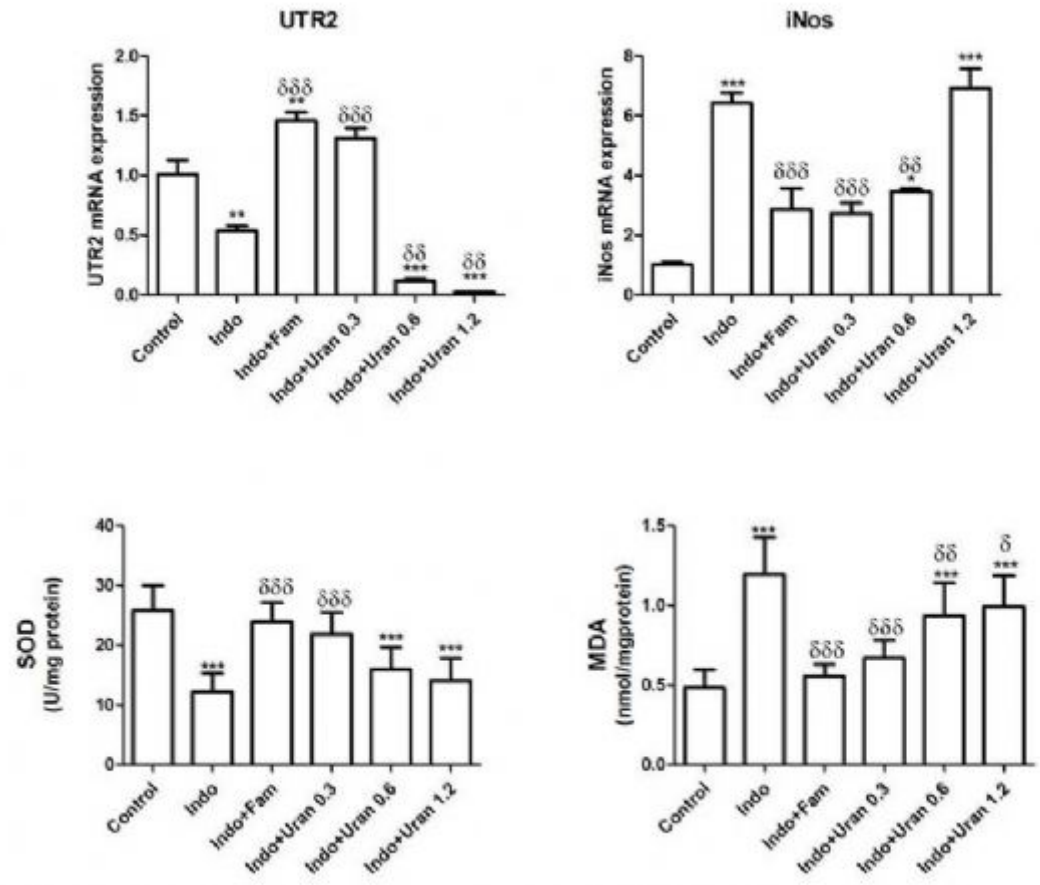

Figure 1. Stomach tissue superoxide dismutase (SOD) activity malondialdehyde (MDA) level and mRNA expression levels of Urotensin receptor (UTR2) and inducible nitric oxide synthetize (iNOS) in experimental groups (Indo: indomethacin, Fam: famotidine and Uran: Urantide). *p<0.05, **p<0.01, ***p<0.001 when compared to control and $^{\delta}$<0.05, $^{\delta\delta}$<p<0.01,$^{\delta\delta\delta}$p<0.001 when compared to indomethacin group according to one-way ANOVA test Tukey option.

C108: Potentially inappropriate drug use in elderly patients admitted to the intensive care unit of a university hospital

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Polypharmacy and drug-drug interactions (DDIs) play critical roles in the production of adverse drug reactions. Potentially inappropriate drugs (PIDs), identified in Beers Criteria, are associated with poor outcomes in elderly patients. We aimed to assess the use of PIDs according to Beers Criteria and clinically relevant DDIs in elderly patients admitted to intensive care unit (ICU) of a university hospital.

This study was performed in the patients aged 65 or older in surgical ICU at Marmara University Hospital who were consulted to Medical Pharmacology Department from March, 2014 to December, 2015. The PIDs in the medication lists of the patients were examined according to Beers Criteria which was recently updated by American Geriatrics Society. Clinically relevant potential DDIs (D and X risk rating category) were evaluated using databases such as the Micromedex Health Care Series Volume 148 and Lexi-Interact™ Online "interactions checker".

There were 145 different active ingredients among the drug lists of postoperative elderly patients (n=129) admitted to ICU for therapy of mostly hypertension, diabetes, heart failure, gastrointestinal bleeding, asthma, sepsis or cancer. Of these medications 14% (n=20) were detected to be PID according to Beers Criteria. Of the patients with at least one PID (n=89), 63% (n=56) had potential DDIs which are considered to have clinical importance. The most common PIDs were metoclopramide (25%, n=32), tramadol (19%, n=24) and insulin (14%, n=18) which can result with extrapyramidal effects, epilepsy seizures and hypoglycemia in the elderly patients, respectively. Thirty-four % of patients on metoclopramide therapy, 21% of patients on tramadol therapy and 18% of patients on insulin therapy had potential DDIs.

In conclusion, prediction of inappropriate drug use in elderly patients will provide not only better prescribing and drug safety but also reduce clinically relevant DDIs in this age group.

Keywords: polypharmacy, pharmacovigilance, drug safety

C109: Self-Medication practice among preclinical medical students and law students of Marmara University

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Self-medication (SM), defined as the 'use of a drug without medical prescription in order to treat a self-diagnosed disease or symptom', becomes an important issue in health system. We aimed to evaluate and compare medical and law students' attitude about SM and determine the correlation of SM and anxiety.

A questionnaire based survey was applied to Marmara University Medical (undergraduate phase 1) (n=180) and Law Faculty students (n=153) in Haydarpaşa campus for this cross-sectional study. The survey included two parts: 1- Sociodemographic features and the attitudes about self-medication; 2-SHAI (short health anxiety inventory). The data were analyzed in SPSS 18.

Fifty-five % (n:99) of medical students were women and 64.1% (n:98) of law students were men. 81.1% of medical students and 78.4% of law students used unprescribed drugs previously (p:0.54). The most frequent reason of SM was headache in medical students, and flu/common cold in law students. 71% (n:128) of medical students and 57.2% (n:87) of law students preferred only prescribed drugs in their medical problems. The use of antibiotics without a prescription was significantly lower in medical students (18.5%) compared to law students (%30) (p:0.02). Similarly, herbal substance use in medical school students was lower than law students (p:0.007). Students who had high illness likelihood factor (p:0.001) and high body vigilance factor (p:0.01) practiced more SM in the last 30 days. Students with chronic illness had high illness likelihood factor (p:0.001) and high body vigilance factor (p:0.005).

Medical students with higher health anxiety have increased use of SM. However, law students use more antibiotics without any prescription and herbal substances than medical students. This can be related to a lower knowledge level of law students compared to medical students about the side effects of drugs and harm of wrong treatment.

Keywords: self-medication, health anxiety inventory, nonprescription drugs

C110: Assessment of antibiotic prescribing performance of dentists in Turkey

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Irrational use of antibiotics is a serious problem in all over the world. Antibiotic utilization in Turkey is quite high compared to European Union and surrounding countries. These drugs are also commonly prescribed in dentistry. Population based dental antibiotic prescribing data have not been studied extensively from Turkey in the literature. The aim of this study was to determine the utilization of antibiotics written by dentists in Turkey.

The database contained antibacterial for systemic use that prescribed by the dentists from Turkish Medicines and Medical Devices Agency's Prescription Information System (PIS) were used retrospectively from the beginning of 2013 to the ending of August 2015. Since this study involves extensive coverage of collected data, a part of study findings that related general antibiotic utilizations has been presented in this abstract.

Total numbers of prescriptions written by dentists from the PIS were 15.702.854 in study period and 82.4% of them (n=12.944.522) included systemic antibiotics (anatomical therapeutic chemical classification subgroup, J01). Beta-lactam antibiotics, penicillins (J01C) were the most commonly used agents (71.3%) in the ATC-3 subgroup. Based on the ATC-5 classification analysis, amoxicillin-clavulanic acid (J01CR02) was found as the most frequently prescribed antibiotic (57.6%) and followed by spiramisin (J01FA02), (10.7%) and amoxicillin (J01CA04), (9.4%).

According to the basic principal of rational use of medicine, antibiotics should be used in limited indications. The present data showed that like other physicians' over-prescribing performance, dentists also prescribe antibiotics excessively in Turkey. Additionally, they preferred antibiotics mostly with broad-spectrum and used with inappropriate indications as irrational. These findings reveal that new educational strategies, practical guidelines on rational use of antibiotic and surveillance programs may become important to improve the dentists' prescribing performance.

Keywords: antibiotic, prescribing, dentists

Oral Presentation Session 14, Hall D

Drug Discovery, Development and Evaluation; Drugs for Infectious Diseases (C111-C116)

Chairs: Hakan Parlakpınar (İnonü University, Turkey)
Atilla Akdemir (Bezmialem Vakif University, Turkey)

15:00 C111: Effects of perineural administration of phenytoin in combination with levobupivacaine in a rat sciatic nevre block
Ahmet Selim Ozkan, Sedat Akbas, Mehmet Akif Durak, Mehmet Ali Erdogan, Hakan Parlakpınar, Nigar Vardı, Onurhal Ozhan, Ali Ozer

15:15 C112: 1H-indole-2,3-dione 3-[N-(4-sulfamoylphenyl)thiosemicarbazone] derivatives as putative anticancer and antifungal agents
Atilla Akdemir, Nilgün Karalı, Claudiu T. Supuran

15:30 C113: In vitro investigation of therapeutic effects of Myrtus communis L. in Cancer Cell Lines
Ayşe Gürel, Hacer Esra Gürses Cila, Ömer Faruk Hatipoğlu, Esra Gündüz

15:45 C114: Protective effects of coenzyme Q10 on survival, vascular and tissue injury in sepsis
Erdem Kamil Ozer, Mustafa Tugrul Goktas, Ibrahim Kilinc

16:00 C115: Has infliximab protective effects on survival, vascular and tissue injury in sepsis?
Erdem Kamil Ozer, Mustafa Tugrul Goktas, Ibrahim Kilinc

16:15 C116: Protective effects of celecoxibe on survival, vascular and tissue injury in sepsis
Erdem Kamil Ozer, Mustafa Tugrul Goktas, Aysun Toker

16:30 C118: Adverse drug reactions to anti-TB Drugs: Pharmacogenomics Perspective for identification of host genetic markers
Kamal Kishor, Roshan Kumar Sahu*

* Transferred from Hall E.

C111: Effects of perineural administration of phenytoin in combination with levobupivacaine in a rat sciatic nevre block

Ahmet Selim Ozkan[1], Sedat Akbas[1], Mehmet Akif Durak[2], Mehmet Ali Erdogan[1], Hakan Parlakpınar[3], Nigar Vardı[4], Onurhal Ozhan[3], Ali Ozer[5]
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Peripheral nerve blocks are often used to provide anesthesia and post-operative analgesia. The adjuvant agents are added to local anesthetics to extend the duration of block in management post-operative pain. Extension of the block time increases patient comfort and reduces the consumption of drugs have serious side effects. Phenytoin stabilizes and inactivates sodium channels similar to the local anesthetics. In our study, we investigated the effect of perineural phenytoin on duration of peripheral nerve blocks in combination with levobupivacaine in rats.

After the approval obtained from Inonu University Medical Faculty of Animal Experiments Local Ethical Committee, 32 male Sprague-Dawley (250-350 g) rats were randomized and divided into 4 groups as Group 1 sham, n=8; Group 2 perineural levobupivacaine (0.2 mL of 0.5% levobupivacaine), n=8; Group 3 perineural phenytoin (0.2 mL, 62.5 mg/kg), n=8; Group 4 perineural phenytoin+levobupivacaine, n=8. After 6-8 mg/kg ketamine anesthesia, 0.2 ml of local anesthetic was injected the bilateral sciatic nerve. Sensory block was evaluated with paw clamping test and withdrawal response of the paw test (hot-plate).The measurements were maintained every 30 minutes during 120 minutes or until the disappearance of full block.

At 30, 60 and 90 minutes, the latency times of the rats in Group 2 and Group 4 were longer than those Group 1 in the paw withdrawal response and this difference disappeared at 120 minutes (p=0.001). Compared with rats in sham group, those in levobupivacaine+phenytoin group showed significantly higher latency times at 30 minutes in the hot plate test (p=0.018). This difference disappeared at 60 minutes.

Our results showed that the duration of sensory blocks was prolonged by phenytoin and levobupivacaine mixture. The present results can be used as guidance for future work.
Keywords: phenytoin, levubupivacaine, sciatic nerve block, rat

C112: 1H-indole-2,3-dione 3-[N-(4-sulfamoylphenyl)thiosemicarbazone] derivatives as putative anticancer and antifungal agents

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Carbonic anhydrases (CAs, EC 4.2.1.1) are important enzymes that perform a very simple but crucial chemical reaction, i.e., the reversible hydroxylation of carbon dioxide. They provide bicarbonate ions to various metabolic and biosynthetic processes and they regulate physiological pH values. The CA enzymes are not an uniform enzyme family. They consist of a wide variety of structurally different enzymes that all perform the same task, i.e., α-CAs (humans and other mammals), β-CAs (most prokaryotes and fungi), γ-CAs (methanogens), δ-CAs (diatoms), ζ-CAs (some chemolitotrophs) and the recently identified η-CAs (plasmodium).

Selective carbonic anhydrase inhibitors (CAIs) that target human CAs (hCAs) isoforms are pharmacological agents in clinical use. The antiglaucoma drug dorzolamide is an inhibitor of hCA II. In addition, selective inhibitors of hCA IX and XII are putative targets in cancer treatment. Some CAs are important in the physiology and virulence of several pathogens and selective CAIs that target these enzymes have putative roles as antimicrobial agents.

1H-indole-2,3-dione (isatin) is a synthetically versatile molecule which has led to an array of derivatives displaying a broad spectrum of pharmacological actions, including anticancer, antiviral and antimicrobial activities. We synthesized several isatin-containing analogs and tested them on pharmacologically interesting CA enzyms, including the tumor-associated hCA IX and XII and the fungal CgNce103 and CaNce103 (from Candida spp.). Several analogs displayed KI values in the lower nanomolar range for hCA IX, hCA XII, CgNce103 or CaNce103, but not for the off-targets hCA I and II. Subsequently, molecular modelling studies were performed to rationalize the obtained selective and potent CA inhibition values. These findings will guide our future ligand-optimization projects to obtain more selective and potent CAIs against various pharmacologically interesting CAs.

Keywords: isatins, carbonic anhydrase, docking, enzyme inhibition, candida

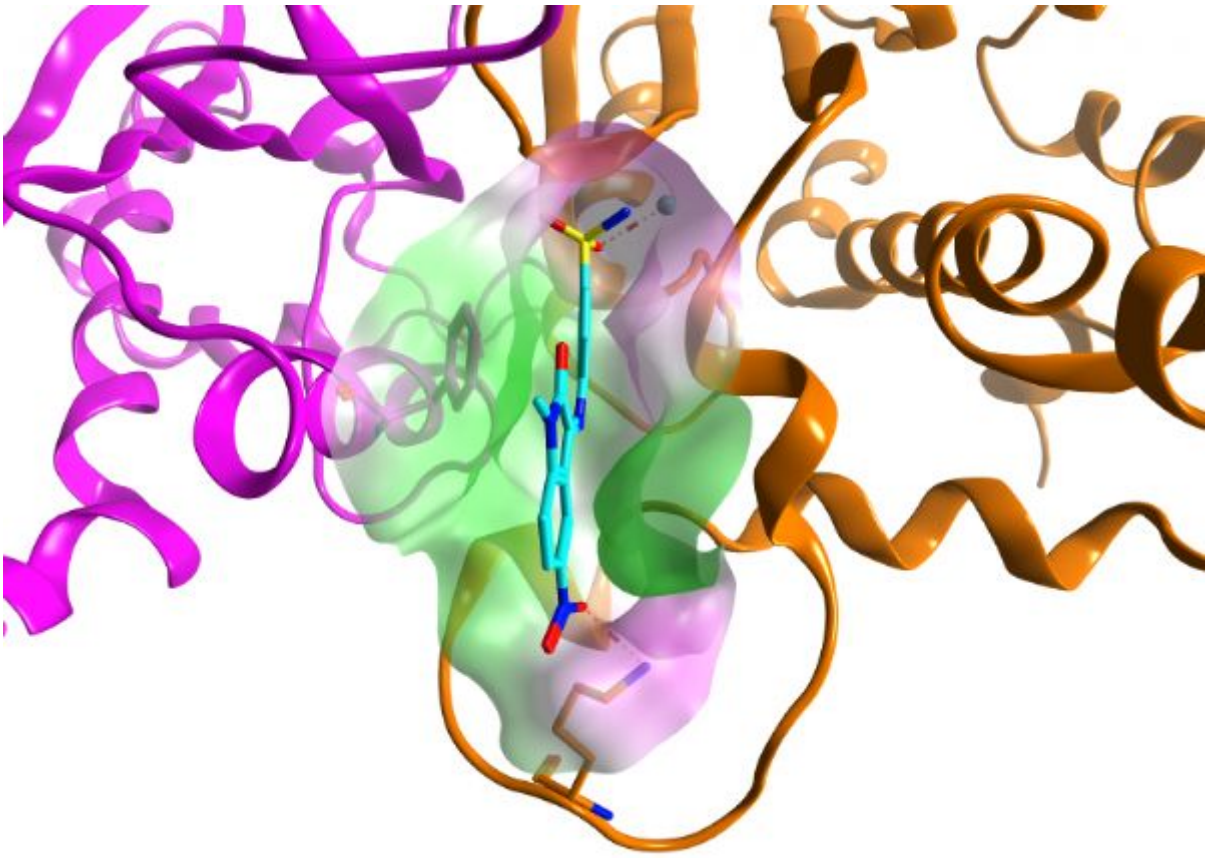

Figure 1. Candida carbonic anhydrase in complex with isatin analogs.

C113: In vitro investigation of therapeutic effects of Myrtus communis L. in cancer cell lines

Ayşe Gürel[1], Hacer Esra Gürses Cila[2], Ömer Faruk Hatipoğlu[2], Esra Gündüz[2]
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Myrtus communis L. (MC) grown in Mediterranean countries likewise in Turkey has been reported that induces apoptosis in different cancer cell lines via the mitochondrial pathway. We aimed to determine both the effect of MC extract on breast cancer, head and neck cancer, colon cancer and glioblastoma cancer cell lines and the cytotoxicity of MC on control cells, skin fibroblasts.

MC extract were isolated from myrtle leaves. The compounds were dissolved in dimethyl sulfoxide (DMSO) and medium. IC50 values for breast cancer cell line (MCF-7) is 113 µg/ml, glioblastoma cell line (YKG-1) is 172 µg/ml, colon cancer cell line (HT-29) is 133 µg/ml, primer head and neck cancer cell line (UT-SCC-74A) is 185 µg/ml, metastatic head and neck cancer cell line (UT-SCC-74B) is 143 µg/ml and lung fibroblast normal cell line (IMR-90) 2 mg/ml. These doses were determined by using the xCELLigence® RTCA DP instrument. To figure out the whether the apoptotic pathway is induced or not, BAX, BCL-2, Caspase-3, Caspase-9 and PARP proteins were analyzed through western blot analysis. Also, BAX and BCL-2 genes expression level were determined by using qRT-PCR. Cell growth and migration abilities were established using scratch assays.

As a result of in vitro experiments, MC extract caused cell death on cancer cell lines, and it had a little cytotoxic effect on fibroblasts. Cell death observed, indeed occurred through apoptosis by an increase in expression of pro-apoptotic genes and decrease in expression of anti-apoptotic genes.

In this study, it was found that MC extract causes apoptotic cell death in cancer cell lines.

This project is supported by TUBITAK, Project No:113S920.
Keywords: apoptosis, cancer, *Myrtus communis* L

C114: Protective effects of coenzyme Q10 on survival, vascular and tissue injury in sepsis

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CoQ10 is endogenously synthesized in the mitochondria and has a crucial role for ATP production. It has powerful antioxidant and anti-inflammatory properties which is reduced during sepsis because of mitochondrial dysfunction. We investigated effects of CoQ10 on survival, vascular and tissue injury in a polymicrobial sepsis model.

Wistar albino rats (n=56) were divided into four groups; sham (SH), cecal ligation and puncture (septic rats, CL), SH+CoQ10 (SHQ), CL+CoQ10 (CLQ). For 15 days rats were injected with CoQ10 (10 mg/kg/day, i.p.) in SHQ and CLQ groups or its solvent (olive oil, 1 ml/kg/day, i.p.) in SH and CL groups. At 16th day animals were underwent CL and SH operations. After 20 hours, the operations mesenteric arterial blood flow (MBF) and phenylephrine responses of isolated aortic rings were measured. Tissue damages were assessed biochemically.

Phenylephrine responses of aortic muscles and MBF decreased in CL groups that were prevented by CoQ10 pretreatment. Survival rate decreased in CL groups ($p<0,001$) which was partially ameliorated by CoQ10. Serum biochemical parameters AST, ALT, LDH, BUN, Cr and TNF-alpha, IL-1β, IL-6 levels were increased in septic rats. The increase in biochemical parameters except for AST were reversed by CoQ10, which partially restored AST levels. Malondialdehyde (MDA) levels in liver, lung, spleen and kidneys were increased and GSH levels decreased in septic rats. The increase in lung, spleen and kidney MDA levels and the decrease in liver, spleen GSH levels were prevented by CoQ10. The increase in liver MDA was partially restored by CoQ10.

CoQ10 has preventive effects on sepsis mortality, vascular dysfunction and tissue injury possibly due to its antioxidant and anti-inflammatory properties.

Keywords: coenzyme Q10, sepsis, protective effects

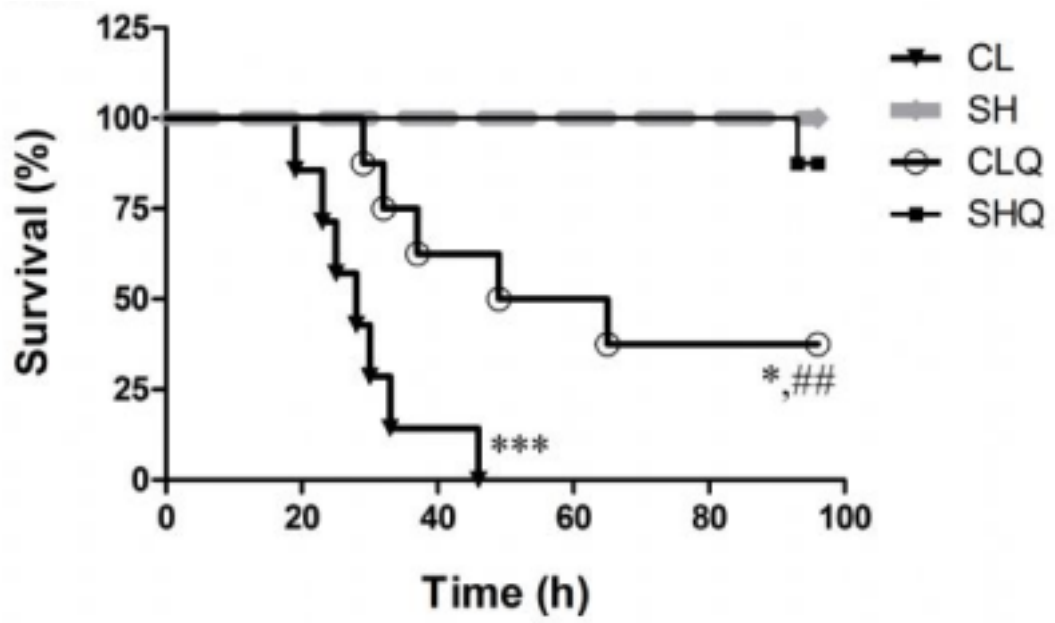

Figure 1. Survival rates obtained from rats challenged with CL or SH operations and were pretreated either with CoQ10 or saline for 96 h. n=7-8 for the groups. Survival curves were compared by the Gehan Breslow Wilcoxon test. *Denotes significant difference between SH vs. other groups. #Denotes significant difference between CL vs. CLQ groups. (*, p<0.05; ##, p<0.01; ***, p<0,001).

Table 1. -log EC50 (an indicator of potency) and Emax (an indicator of efficacy) values of phenylephrine for aortic muscle contractions obtained from dose-response curve. *For statistical analysis, one-way ANOVA with post hoc Newman-Keuls multiple comparison post test were done. *Denotes significant difference between SH vs. other groups. #Denotes significant difference between CL vs. CLQ groups. (*,#, p<0.05; **, p<0.01; ###, p<0,001).*

	-log EC_{50}	**E_{max} (g)**
SH	7,22±0,16	1,49±0,09
CL	6,82±0,18**	0,92±0,11**
SHQ	7,67±0,17*	1,48±0,14
CLQ	7,55±0,14*,###	1,36±0,11#

C115: Has infliximab protective effects on survival, vascular and tissue injury in sepsis?

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[2]Department of Pharmacology, Faculty of Medicine, Yildirim Beyazit University, Ankara, Turkey.
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TNF-alpha plays a role in the pathophysiology of inflammatory disease such as septic shock, rheumatoid arthritis, psoriasis. TNF-alpha increases over the first stages of septic shock and leads to organ dysfunction by triggering the release of proinflammatory mediator and cytokines. So, inhibition of TNF-alpha by infliximab (INF) may be protective in septic shock. In this study, we investigated the effects of INF on survival, vascular and tissue injury in a polymicrobial sepsis model.

Wistar albino rats (n=53) were divided into four groups; sham (SH), cecal ligation and puncture (septic group, CL), SH+INF (SHINF), CL+INF (CLINF). Before 24 h the SH or CL operations animals in these groups were given INF (7 mg/kg/day, i.p.) or its solvent (nonpyrogenic sterile saline). After 20 h the operations, mesenteric arterial blood flow (MBF) and responses of isolated aortic rings to phenylephrine were measured. Organ damages were examined biochemically.

INF prevented the decrease MBF in septic rats. Phenylephrine responses of aortic rings decreased in septic rats that were ameliorated by INF treatment. All rats in the CL group died at 43 h after undergoing CL operation. Pretreatment with INF increased the survival rate by 57% at the end of 96 h. Serum TNF-alpha, IL-1β, IL-6 levels and levels of biochemical parameters (AST, ALT, LDH, BUN, Cr) increased in CL group and these increases were prevented by INF. In septic tissues GSH reduced and malondialdehyde (MDA) increased. The increase in liver, lung, spleen and kidney MDA levels and the decrease in liver, spleen and kidney GSH levels were prevented by INF.

INF has protective effects on sepsis mortality, tissue injury and vascular dysfunction biochemically possibly due to its antioxidant and anti-inflammatory properties.

Keywords: infliximab, sepsis, protective effects

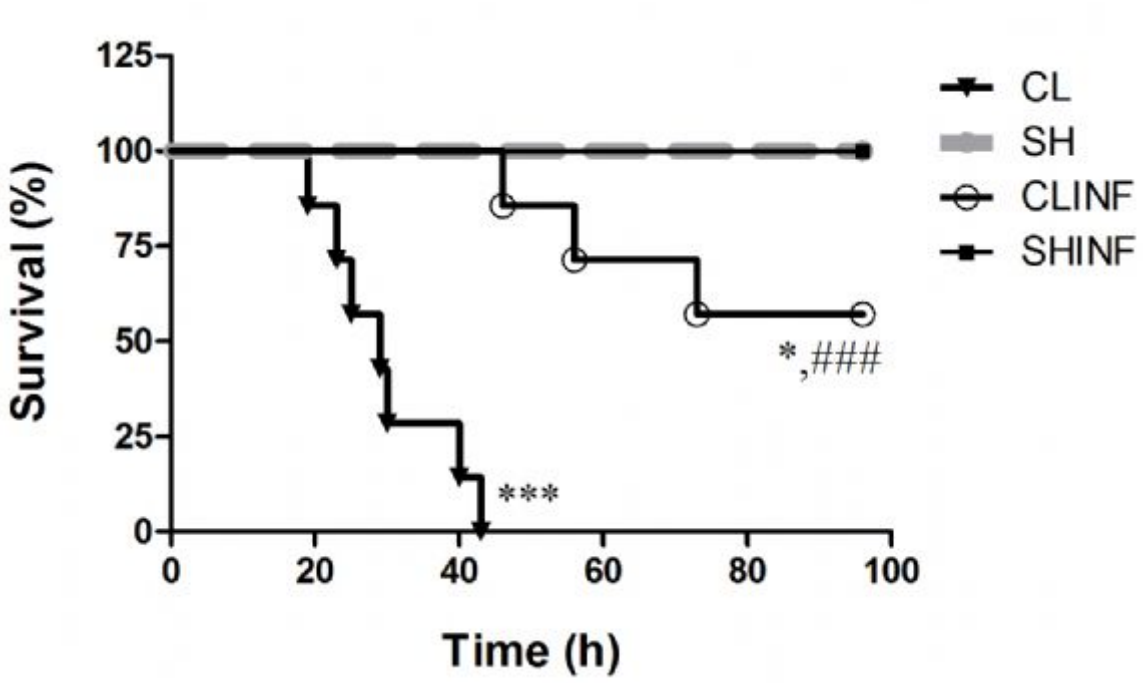

Figure 1. Survival rates obtained from rats challenged with caecal ligation and puncture (CL) and Sham (SH) operations for 96 hours. The animals in SHINF and CLINF groups were also given or infliximab (INF; 7 mg/kg, i.p.) or its solvent (saline 1 ml/kg/day, i.p.) 24 h before the CL or SH operations. n=7-8 for the groups. Survival curves were compared by the Gehan Breslow Wilcoxon test. *Denotes significant difference between SH vs. other groups. #Denotes significant difference between CL vs. CLINF groups. (*, $p<0.05$; ###,***, $p<0.001$).

C116: Protective effects of celecoxibe on survival, vascular and tissue injury in sepsis

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Constitutively COX-1 expression and basal COX-2 expression lead to production low level prostanoids which have protective effects for the body, but high level prostanoids produced by COX-2 overexpression lead to hyperinflammation and have detrimental effects for the body. Low doses of celecoxib (CCX), a specific COX-2 inhibitor, may be protective in sepsis rather than fully inhibit COX-2. We investigated effects of CCX on survival, vascular and tissue injury in a polymicrobial sepsis model.

Wistar albino rats (n=51) were divided into four groups; sham (SH), cecal ligation and puncture (septic group, CL), SH+CCX (SHCCX), CL+CCX (CLCCX). At 2 h after the SH or CL operations animals in these groups were given CCX (0,5 mg/kg/day, o.g) or its solvent (nonpyrogenic sterile saline). After 20 h, mesenteric arterial blood flow (MBF) and responses of isolated aortic rings to phenylephrine were measured. Organ damages were examined biochemically.

CCX prevented the decrease MBF in septic rats. Phenylephrine responses of aortic rings decreased in septic rats that were partially prevented by CCX treatment. Survival rate was %0 at 49th h in septic group which was increased to 42,8% by CCX at the end of 96th h. Serum TNF-alpha, IL-1β, IL-6 levels and levels of biochemical parameters (AST, ALT, LDH, BUN, Cr) increased in CL group and these increases were prevented by CCX. In septic tissues GSH reduced and MDA increased. The increase in liver, lung, spleen and kidney malondialdehyde (MDA) levels and the decrease in liver, spleen GSH levels were prevented by CCX.

CCX has protective effects on sepsis mortality, tissue injury and vascular dysfunction biochemically and histopathologically possibly due to its antioxidant and anti-inflammatory properties.

Keywords: celecoxib, sepsis, tissue injury

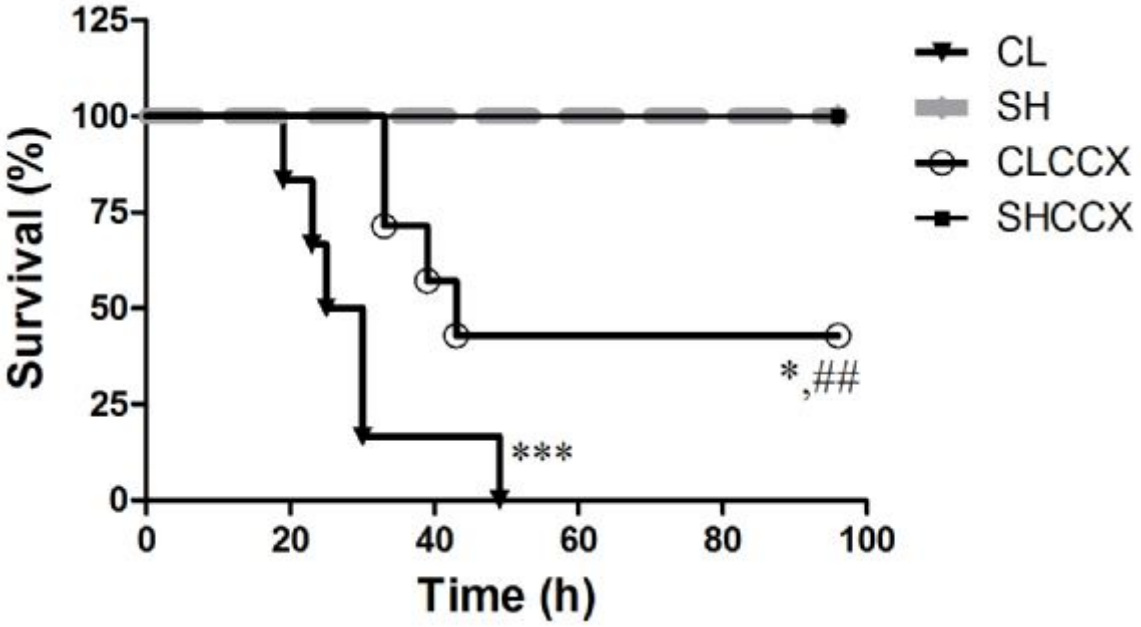

Figure 1. Survival rates obtained from rats challenged with caecal ligation and puncture (CL) and Sham (SH) procedures for 96 h. n=6-7 for the groups. Survival curves were compared by the Gehan Breslow Wilcoxon test. *Denotes significant difference between SH vs. other groups. #Denotes significant difference between CL vs. CLCCX groups. (*, $p<0.05$; ##, $p<0.01$; ***, $p<0.001$).

Oral Presentation Session 15, Hall E

Miscellaneous (C118-C120)

This session is cancelled.

15:15 C118: Adverse drug reactions to anti-TB Drugs: Pharmacogenomics Perspective for identification of host genetic markers
Kamal Kishor, Roshan Kumar Sahu
Transferred to Hall D, 16:30

15:30 C119: Investigation of the effects of oleuropein rich diet on rat enteric bacterial flora
Aslı Kiraz, Tuncer Şimşek, Satı Zeynep Tekin, Sait Elmas, Murat Tekin, Hasan Şahin, Hatice Betül Altınışık, Çiğdem Uysal Pala
Transferred to Hall D, 16:30

15:45 C120: Marjoram inhibits norepinephrine-induced malignant phenotype of triple negative breast cancer cells
Reem Alolabi, Alaaeldin Saleh, Ali Hussein Eid
No registration, abstract will be deleted.

C118: Adverse drug reactions to anti-TB Drugs: Pharmacogenomics Perspective for identification of host genetic markers

Kamal Kishor[1], Roshan Kumar Sahu[2]
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Adverse drug reactions (ADRs) are associated with clinical morbidity and, in severe cases, even mortality. Globally billions of dollars are spent on managing these ADRs for common and uncommon diseases. The developing world suffers from a high burden of tuberculosis, which requires 6-8 months of multi-drug treatment. In spite of most cases being treatable the problem persists mainly due to a high attrition rate associated with ADR mediated complications. Due to these reasons drug resistant strains have emerged and are now a serious challenge to TB eradication. To effectively deliver the available treatment regimen and ensure patient compliance it is important to manage ADRs more efficiently. Recent studies have demonstrated that drug outcomes are patient-specific and can, therefore be predicted. A few of these drugs, including a few administered for TB, have shown excellent correlation with response rates and development of ADRs. In present work, we profile information available in public domain for existing anti-TB drugs to understand the genesis of ADRs and patient response. Additionally, human genome variation databases have been used to correlate the frequency of these markers and their genomic variants in different populations. Nowadays our group is involved in gene and its adverse effect of TB drug, Linezolid

Keywords: linezolid, adverse drug reaction, anti-TB drugs

C119: Investigation of the effects of oleuropein rich diet on rat enteric bacterial flora

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Bacterial changes in intestinal flora are associated with metabolic and immunological problems. Biofenols such as oleuropein with antimicrobial activities regulate intestinal flora by reducing pathogenic bacteria. We aimed to investigate how intestinal flora is affected in rats fed predominantly with oleuropein.

Twenty adult, male Wistar albino rats were randomly divided into two groups. Group O (n=10) received olive leaf extract 20 mg/kg/day by intragastric gavage in addition to standard rat chow and water for 30 days. Group C (n=10) were fed with standard rat chow and water for 30 days. After 30 days, laparotomy was performed in all rats and one gram of ceacal contents were collected. Sterile physiological saline solution of 9 mL was added to all ceacal contents and consecutive 10-fold serial dilutions were prepared with a final concentration of 10^{-8}. The diluted samples were inoculated on Plate Count agar and Violet Red Bile Glucose agar to determine the total number of enteric bacteria. After incubation at 37 °C for 48 hours, isolated bacterial counts from the plates were recorded as CFU/g (colony forming unit/gram).

Total aerobic bacterial counts of Group O were significantly lower than Group C in all plates inoculated with ceacal samples in every dilution ($p<0.005$) (Table 1).

Oleuropein reduced the bacterial count isolated by conventional aerobic culture methods we used, however other bacteria that could not be isolated with this method were not counted and this is a limitation of our study. It is known that patients in intensive care units are prone to develop bacterial translocation (BT). Mortality rates will decrease by starting enteral nutrition earlier in critically ill patients. Addition of oleuropein on enteral nutrition solutions may be helpful in recovery of critically ill patients predisposing to BT by reducing enteric bacterial count.

Keywords: enteric bacteria, intensive care, oleuropein, rat

Table 1. Total aerobic bacterial counts in two groups (CFU/g)

	Group O (n=10)	**Group C (n=10)**
Dilution of caecal samples	PCA/VRBG Mean (min-maks)	PCA/VRBG Mean (min-maks)
10^{-1}	62.1x104 (55x104-69x104)*/32.5x104 (26x104-38x104)*	78.4x106 (72x106-86x106)/50.9x106 (46x106-55x106)
10^{-2}	59.1x104 (51x104-74x104) */28.5x104 (21x104-35x104)*	72.8x106 (66x106-79x106)/45.8x106 (42x106-49x106)
10^{-3}	53.1x104 (45x104-64x104) */24.8x104 (19x104-29x104)*	66.8x106 (62x106-74x106)/40.6x106 (37x106-44x106)
10^{-4}	46.8x104 (40x104-57x104) */21x104 (17x104-24x104)*	60.8x106 (54x106-68x106)/34.6x106 (30x106-39x106)
10^{-5}	37.6x104 (22x104-52x104) */16.4x104 (13x104-19x104)*	54.4x106 (49x106-61x106)/29.1x106 (24x106-34x106)
10^{-6}	31.4x104 (17x104-46x104) */11.9x104 (8x104-15x104)*	48.1x106 (43x106-56x106)/22.7x106 (19x106-27x106)
10^{-7}	26x104 (12x104-42x104)*/ 8.7x104 (5x104-12x104)*	40.8x106 (34x106-49x106)/17.3x106 (13x106-22x106)
10^{-8}	16.7x104 (3x104-31x104) */3.8x104(1x104-8x104)*	33.8x106 (29x106-41x106)/11.1x106 (6x106-17x106)

*PCA: Plate Count Agar, VRBG: Violet Red Bile Glucose Agar CFU/g: colony forming unit/gram *: with Mann-Whitney U test p<0.005*

C120: Marjoram inhibits norepinephrine-induced malignant phenotype of triple negative breast cancer cells

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Breast cancer is a major contributor to cancer-related mortality both in Qatar and the world. Among other factors, stress appears to be associated with increased breast cancer risk. Norepinephrine (NE), a stress hormone, is a potent physiologic inducer of malignant phenotype of breast adenocarcinoma cells. Triple negative breast cancer (TNBC), in particular, is relatively resistant to various treatment regimens and is hence associated with poor prognosis. This mandates that alternative approaches like herbal medicine be sought. Indeed, a large number of anticancer phytochemicals have already been identified. We recently showed that Origanum majorana (marjoram) extract (OME) inhibits the malignant phenotype of TNBC cells. Here, we provide evidence that OME also abolishes the NE-induced proliferation, migration, adhesion and invasion of MDA-MB-231 cells. Moreover, pretreatment with OME appears to inhibit both basal and NE-induced production of MMP-9, IL6, VEGF, Nitric Oxide and ERK1/2 phosphorylation. Importantly, NE-stimulated adhesion of MDA-MB-231 to fibronectin via beta1 integrins was abrogated when cells were pre-treated with OME. Furthermore, these effects of OME appear to be mediated, at least in part, by beta 2-adreneroceptors. Collectively, our results show that Origanum majorana, by blocking β2-adrenoceptors, diminished the NE-promoted malignant phenotype of human breast cancer cells. These findings suggest that marjoram could be beneficial in the treatment of stress-induced breast cancer malignancy. However, further studies are warranted to better determine the in vivo efficacy of this “medicinal” herb.

Keywords: breast cancer, norepinephrine, stress, marjoram, malignancy

Thursday, 30th June 2016

Main Sessions (09:00-12:00)

Hall A:
Targeting inflammation in disease

(Organized by Spanish Society of Pharmacology)
Organizers and chairs:
Maria-Jesus Sanz (University of Valencia)
Juan Tamargo (Complutense University of Madrid)
09:00-12:00

09:00 Inflammation plays a role in the pathophysiology and represents a therapeutic target in atrial fibrillation
Juan Tamargo
Complutense University of Madrid, SPAIN

09:30 The microbiota as a pharmacological target for the treatment of intestinal inflammatory diseases
Julio Gálvez
University of Granada, SPAIN

10:00 Elevated talk (C033): Correlation of IL-1 beta levels and acute phase proteins in patients' sera from a phase 2 familial mediterranean fever trial with canakinumab
Serhan Sevgi
Novartis Pharma Medical Department, TURKEY
(Authors: Serhan Sevgi, Huri Ozdogan, Ozgur Kasapcopur, Burak Erer, Serdal Ugurlu, Soner Turgay, Ahmet Gul)

10:15 Elevated talk (C034): Protective effects of pentoxifylline and its potentiation with low dose nitric oxide (NO) modulators in complete Freund's adjuvant-induced rheumatoid arthritis in rats
Rishi Pal
King George's Medical University, INDIA
(Authors: Rishi Pal, Prafulla Chandra Tiwari, Rajendra Nath, Kamlesh Kumar Pant)

10:30 Coffee break

11:00 Is there a room for antiinflammatory drugs in neuropsychiatry?
Juan-Carlos Leza
Complutense University of Madrid, SPAIN

11:30 Therapeutic potential of blood glutamate grabbing in stroke: a proof of concept
Maria-Isabel Loza
University of Santiago de Compostela, SPAIN

12:00 End of symposium

Inflammation plays a role in the pathophysiology and represents a therapeutic target in atrial fibrillation

Juan Tamargo
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Atrial fibrillation (AF) is the most prevalent cardiac arrhythmia and a major cause of hospitalization, morbidity, and mortality. AF is a slowly progressing, chronic disease that produces electrical, structural and contractile changes (atrial remodelling) that create the arrhythmogenic substrate contributing to its self-perpetuation. Unfortunately, drugs available for treating AF and its complications are limited. There is experimental and clinical evidence that inflammation plays a key role in AF-induced atrial remodeling. Atrial biopsies of patients with AF show inflammatory infiltrates, myocyte necrosis, and fibrosis and the incidence of AF increases in inflammatory conditions (ie. pericarditis, myocarditis and cardiac surgery). Serum levels of inflammatory biomarkers and expression of pro-inflammatory markers (hs-CRP, TNFα, MPO, interleukins-1β, 6, 8, 10 and 18, TGF-β1) are elevated in cardiac tissues of AF patients and their presence has been correlated with the presence or recurrence of AF after successful electrical cardioversion or catheter ablation. They are also related with outcomes (stroke, vascular events, and mortality) of AF independently of other cardiovascular risk factors. Conversely, inflammatory biomarker levels decrease after cardioversion. Inflammation has been associated with electrophysiological (slow heterogeneous conduction and dispersion of atrial refractoriness, abnormal calcium handling, increased automaticity and triggered activity), structural (fibrosis, apoptosis) and contractile (negative inotropism, altered response of β-adrenergic signalling) remodelling. Additionally, inflammation is linked to various pathological processes, i.e. fibrosis, apoptosis and oxidative stresss that participate in the atrial arrhythmogenic substrate. Furthermore, inflammation may induce endothelial dysfunction and platelet activation in patients with AF, thus linking inflammation and thrombogenesis. All this evidence supported the hypothesis that pharmacological interventions with anti-inflammatory effects might be efficacious in the prevention of AF. Unfortunately, and in contrast to the promising findings in experimental models, the clinical results of drugs with antiinflammatory and antioxidant properties (statins, angiotensin-converting enzyme inhibitors, or glucocorticoids) have been quite disappointing. Therefore, we need a better understanding of the role of inflammation in the pathophysiological mechanisms involved in atrial remodelling and AF maintenance. This would allow us to identify new therapeutic targets and the design new mechanism-based therapeutic strategies aimed at reducing the AF burden.

The microbiota as a pharmacological target for the treatment of intestinal inflammatory diseases

Julio Gálvez
CIBER-EHD, Department of Pharmacology, ibs.GRANADA, CIBM, University of Granada, Granada, SPAIN.

Gut microbiota constitutes a complex ecosystem that establishes a commensal relationship with the host through the intestinal epithelium, thus promoting homeostatic functions such as immunomodulation and maintenance of the barrier function. A dysregulation of the microbiota composition, known as dysbiosis, may disturb the interaction bacteria-host, and trigger an inappropriate immune response, which, in turn, might ultimately lead to different inflammatory conditions like inflammatory bowel disease (IBD). Supporting this, those strategies targeted to modify and restore the unbalance intestinal microbiota could ameliorate the inflammatory response. Among these strategies, the administration of probiotics may play a prominent role in the management of IBD. Several mechanisms have been proposed: enhancement of the epithelial barrier function, inhibition of pathogen adhesion and modulation of the immune system. Other approach may be the administration of antibiotics, since, in fact, broad-spectrum antibiotics have been already used empirically in human IBD. Lately, different studies have reported the ability of some antibiotics, like minocycline, doxycycline or rifaximin, to modulate both the innate and the adaptative immune responses. Therefore, the use of a single compound with both immunomodulatory and antimicrobial activities could be interesting in the pharmacological treatment of IBD. Unfortunately, several studies have reported that discontinuation of antibiotic therapy results in a high relapse rate, suggesting a need for long-term therapy which in turn could increase the risk of drug side effects. Therefore, it is interesting the development of combined approaches that would both restore the gut dysbiosis and reinstate the altered immune response that characterises the inflamed intestine in the long term. Then, the combination of antibiotics and probiotics could be useful in the treatment of IBD, with the rationale of opening a microbial niche with the antibiotics that the probiotics can then occupy, and thus preserving the microbiota composition and preventing IBD progression.

Elevated talk (C033): Correlation of IL-1 beta levels and acute phase proteins in patients' sera from a phase 2 familial mediterranean fever trial with canakinumab

Serhan Sevgi[1], Huri Ozdogan[2], Ozgur Kasapcopur[2], Burak Erer[3], Serdal Ugurlu[2], Soner Turgay[1], Ahmet Gul[3]
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Familial Mediterranean Fever (FMF)-related MEFV mutations are associated with increased IL-1β production. Therefore, inhibition of IL-1β activity may decrease both frequency and severity of acute attacks in patients with FMF. Our Phase 2 clinical trial in 9 patients confirmed the role of IL-1β by showing the efficacy of canakinumab, a monoclonal anti-IL-1β antibody in these patients. The objective of this study is to investigate the correlation of serum IL-1β with serum amyloid A (SAA) and C-Reactive Protein (CRP) acute phase proteins for identification of better biomarkers in monitoring colchicine resistant (cr) FMF patients.

In this Phase 2 trial, FMF patients with ≥1 attack/month in the preceding 3-months despite the highest tolerated colchicine dose were eligible to enter a 30-day run-in period. Those with an attack in the run-in period advanced to a 3-month treatment period to receive canakinumab 150mg sc every 4-weeks. IL-1β, canakinumab, SAA and CRP levels measured and data collected for regression and variant analysis.

Thirteen patients enrolled in the run-in and 9 (range 12-34 yrs) entered treatment periods. Median baseline elevated CRP (58mg/L) and SAA (162mg/L) levels normalized (CRP, 2.5mg/L; SAA, 5.8mg/L) by Day 8 and remained low throughout the study. The correlation analysis for serum IL-1β by Pearson method revealed a high score (>0,5) for CRP and SAA in 5 of 9 patients and in 8 of 9 patients, respectively. The correlation between canakinumab levels and IL-1β was also high (R-Sq= 86,7%) and the regression equation is; Total IL-1β= 8,35+1,13 Canakinumab levels.

Correlation of serum IL-1β levels with CRP, and especially SAA levels supports further the value of these acute phase proteins in the monitoring of crFMF patients, and suggest preference of SAA as a better biomarker when possible in the management of these patients with anti-IL-1 treatments.

Keywords: FMF, IL-1, Canakinumab, CRP, SAA

Elevated talk (C034): Protective effects of pentoxifylline and its potentiation with low dose nitric oxide (NO) modulators in complete Freund's adjuvant-induced rheumatoid arthritis in rats

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Rheumatoid arthritis is an inflammatory autoimmune disorder of joints. Pentoxifylline, a nonspecific phosphodiesterase inhibitor and TNF-alpha antagonist may have potential role in reducing inflammation. Nitric oxide (NO) plays a significant role in regulation of inflammatory mechanisms involved in immunological disorders. Therefore, present study was designed to evaluate protective effects of pentoxifylline and its interaction with low dose of NO modulators in adjuvant-induced RA in rats. Wistar rats (200-300 g, n=6 per group) were used in the study. On day '0' experimental arthritis was induced by injecting 0.2 ml of complete Freund's adjuvant (CFA) in sub-planter region of right hind paw in 0.1 ml of squalene intradermally injected animals. Pentoxifylline treatment alone and in combination with NO modulators, L-arginine and L-NAME were given (i.p) from day '14' to '28'. On 28th day, blood and joint synovial fluid were collected for pro-inflammatory (TNF-alpha) and anti inflammatory (IL-10) cytokine estimations. Paws were excised for histopathology. Data obtained was analyzed by using two way ANOVA followed by post hoc test. A p value of <0.05 was considered as significant. CFA inoculation significantly increases a) arthritic index b) ankle diameter c) paw volume d) histopathology score e) serum & joint synovial TNF-alpha levels while body weight and serum IL-10 levels was significantly decreased ($P<0.001$). These CFA-induced arthritic changes were significantly reversed by pentoxifylline 5, 10 and 20 mg/kg in a dose dependant manner. Low dose of L-arginine (100 mg/kg) and/or L-NAME (10 mg/kg) significantly potentiated protective effects of pentoxifylline in all parameters ($p<0.01$) corroborated with histopathological findings. These results are suggestive of involvement of NO in anti-arthritic effects of pentoxifylline on these adjuvant-induced arthritic changes which are mediated through pro-inflammatory/anti-inflammatory cytokine networks. Further research needs to understand the molecular mechanisms involved and their regulation by NO in rheumatoid arthritis. So that new pharmacotherapy designed for this non-curable disease.

Keywords: rheumatoid arthritis, pentoxifylline, NO-modulators, cytokines, arthritic index

Is there a room for antiinflammatory drugs in neuropsychiatry?

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There is a renewed interest in immune/inflammatory changes and their associated oxidative/nitrosative consequences as key pathophysiological mechanisms in several mental diseases. Both brain cell components and peripheral immune cells have been implicated in brain and systemic inflammation and in the resulting oxidative/nitrosative stress in depression and schizophrenia, and several molecules related with the control of inflammation correlate with symptoms in neuropsychiatric diseases. Furthermore, down-regulation of endogenous antioxidant and anti-inflammatory mechanisms has been identified in biological samples from patients, although the degree and progression of the inflammatory process and the nature of its self-regulatory mechanisms vary from early onset to full blown diseases. Diverse pharmacological strategies have been studied to deal with these processes (mainly treatments with anti-inflammatory or antioxidant drugs as add-on to antidepressants or antipsychotics). These augmentation strategies, although in general effective and safe, obtain only partial symptomatic relief in the majority of studies. While the focus has traditionally been on antagonizing the pro-inflammatory pathways, little effort has been made to investigate the anti-inflammatory side of the balance; including stimulation of intra and intercellular anti-inflammatory pathways (i.e. anti-inflammatory prostaglandins and transcription factors). Future studies should include strategies combining neurotransmitter based therapies with the control of inflammatory pathways in mental diseases.

Therapeutic potential of blood glutamate grabbing in stroke: a proof of concept

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After ischemic stroke, there is a rapid elevation of glutamate into the extracellular space following by brain edema and inflammation with result of neuronal death, increase in infarct volume and bad prognosis. We observed that plasma glutamate levels correlate with brain edema after ischemic stroke in human patients. Therefore, blood glutamate grabbing has becoming a novel translational strategy validated by the Clinical Neurosciences Research Laboratory with successful protective effects during acute phase of experimental ischemic stroke.

With the aim of identifying low molecular weight drugs acting as glutamate grabbers, the Biofarma group (Innopharma pharmacogenomics USC platform) developed a miniaturized *in vitro* assay for measuring Glutamate-Oxaloacetate-Transaminase (GOT) activity, as well as, an off-target screening measuring succinate-dehydrogenase activity. Innopharma chemical library was screened finding 6 hits that reduced glutamate levels over a 30%, being riboflavin the safer glutamate grabbing agent *in vitro.* Moreover, combination of riboflavin with low concentrations of oxaloacetate, showed synergistic effects and higher efficiency in decreasing blood glutamate concentration. *In vivo* analysis showed that 1mg/kg of riboflavin in ischemic animal models induced a significant lowering of blood glutamate levels and a reduction of infarct volume at 7 days after ischemia.

A clinical proof of concept was carried out to clinically evaluate the novel mechanism in stroke (ClinicalTrials.gov Identifier: NCT02446977). Acute (i.v.) administration of riboflavin to 25 ischemic patients in the first 4 hours after ischemic stroke led to a significant reduction of glutamate plasmatic levels after 6 hours of drug administration. These results validate the translation of both *in vitro* and *in vivo* results to decreasing human blood levels of glutamate. We are currently carrying out the preclinical studies of the synergistic combination in order to initiate a human clinical trial for this new mechanism.

Hall B:
European Registered Pharmacologist Project

(Organized by EPHAR and EACPT)
Organizers and chairs:
Thomas Griesbacher (Medizinische Universität Graz, AUSTRIA)
09:00-10:30
Speakers from the relevant societies to be announced
10:30 End of symposium
10:30 Coffee break

Certification of medical specialists in pharmacology and toxicology within the EPHAR/EACPT European Certified Pharmacologists Programme in Austria

Thomas Griesbacher

Authors: Thomas Griesbacher[1], Georg Wietzorrek[2]

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The European Certified Pharmacologists (EuCP) Programme was launched in 2014 by the Federation of European Pharmacological Societies with the intention to identify experts in the field of pharmacology whose competency profile, in addition to their personal specialised scientific expertise, covers expert knowledge in all major fields of the discipline. National certification schemes must meet all requirements of the EuCP Programme including a clear catalogue of criteria with respect to knowledge, practical awareness and skills, as well as general rules including rules for final assessment of candidates.

The Austrian Pharmacological Society (APHAR) has set up a EuCP certification program based on the diploma for Medical Specialists in Pharmacology and Toxicology of the Austrian Medical Chamber. The training requirements for medical pharmacologists have recently been reformed following a general training structure common to all medical specialties: within a 6-year period, trainees begin with a 9-month period of basic clinical training followed by a 36-month period of basic training and a 27-month period of specialised training. While basic training in the specialty has to be covered by all training institutions, the period of specialised training is organised in several facultative modules. A formal exam has to be taken at the end of training. For certification as EuCP, applicants have to submit their diploma as medical pharmacologists along with further documents that support their credentials, such as proof of current activity in pharmacology, publications showing the practical expertise of the applicant, etc. All applications are evaluated by an independent panel of pharmacologists to ensure that all criteria of the EuCP Programme are met.

The APHAR EuCP scheme has been approved by the EuCP Committee in November 2015 and its regulations are available on the EuCP website (www.eucp-certification.org). Further, separate regulations shall be set up by APHAR for clinical and non-medically qualified pharmacologists.

EJP Educational Seminar, Hall B (11:00-12:00)

How to write great papers: from title to references, from submission to publication

Jaap Van Harten

Elsevier BV, The NETHERLANDS

Good research deserves to be published, to be widely read, and to be recognized by your fellow researchers and community. The current research (and funding) climate makes it even absolutely necessary that you are successful in being able to be published: “Publish or Perish”. This then raises the question, how can you achieve that goal?
“Success” essentially depends on three components: 1) the ability to determine the best possible publication strategy for your research findings, 2) the best possible way to write your article, and 3) the most effective interaction with editors. Key to success in this context is your ability to put yourself in the position of readers, reviewers and editors.

Key considerations in journal selection are a realistic assessment of the quality of the research and the audience you intend to reach. As an example of the latter: publication in a clinically oriented journal may require more background information on the chemical/pharmaceutical aspects of the research, but more detailed clinical results, whereas the opposite is the case when you publish the same research in a pharmaceutical journal.

The art of manuscript writing is not just applying one “golden tip”. It is essentially “telling your story” to your readers in an engaging way, and avoiding common mistakes and deficiencies including poor language. Avoidable mistakes can lead to unnecessary rejection of your manuscript.

Finally, it is your open, non-defensive attitude towards the editors and the reviewer comments, that will not only increase the likelihood of getting your manuscript accepted for publication, it is also likely that your published paper has improved thanks to their comments.

By consistently applying these principles you are likely to become a more successful author.

Hall C:
New insights in neuropsychiatric disorders: models & molecules with therapeutic potential

(Organized by Turkish Pharmacological Society)
Organizers and chairs:
Ece Genç (Yeditepe University)
Feyza Aricioğlu (Marmara University)
09:00-12:00

09:00 Current and future pharmacology of post-traumatic stress disorder
M. Zafer Gören
Marmara University, TURKEY

09:30 Convulsions triggered by food intake in antimuscarinic-treated fasted animals: a model for eating seizures?
Nurhan Enginar
İstanbul University, TURKEY

10:00 Novel targets focusing on neuroinflammation and neurogenesis in schizophrenia
Feyza Aricioğlu
Marmara University, TURKEY

10:30 Coffee break

11:00 Can valproic acid have a therapeutic potential in Parkinson's disease?
Ece Genç
Yeditepe University, TURKEY

11:30 Elevated talk (C035): Neuroprotective effects of epicatechin against homocyctein-induced oxidative stresss in hippocampal area in rat
Ramin Ataee
Mazandaran University of Medical Sciences, IRAN
(Authors: Ramin Ataee, Yaghub Shayesteh, Amin Ataie, Esmaeil Akbari, Fatemeh Shaki)

11:45 Elevated talk (C036): The effect of the hyperpolarization-activated cyclic nucleotide-gated (HCN) channel blocker ZD72688 on spike-and-wave discharges in animal models of absence epilepsy
Nihan Çarçak Yilmaz
İstanbul University, TURKEY
(Authors: Nihan Çarçak Yılmaz, Francois David, Filiz Onat, Vincenzo Crunelli)

12:00 End of symposium

Current and future pharmacology of post-traumatic stress disorder

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Post-traumatic stress disorder (PTSD) is a mental disorder characterized by re-experiencing the trauma when confronted with the clues related to the trauma. Although the etiology of the disease is known exactly, the interactions in the neurobilogical systems have not been fully understood yet. The 5th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5; APA 2013) defines PTSD as comprising four distinct symptom clusters:
(1) intrusion symptoms in which the traumatic event is re-experienced;
(2) avoidance of trauma-related thoughts, feelings or reminders;
(3) negative alterations in cognition or mood (e.g., dissociative amnesia, distorted blame of self or others, pervasive negative trauma-related emotions such as fear, horror or shame);
(4) alterations in arousal or reactivity, which may include irritability, insomnia, hypervigilance and exaggerated startle. In the DSM-5, there is a new symptom cluster, the negative alterations in cognition or mood such as aggressive or risky behavior. These symptoms must persist for longer than 1 month and cause significant distress or functional impairment.

Neuroanatomical studies showed that the noradrenaline and the noradrenergic system in the central nervous system constitute a skeleton for the brain regions involved in the disease. The majority of noradrenergic neurons in the central nervous system are found in the locus ceruleus (LC) of the brainstem. The LC receives from and sends noradrenergic fibers to the limbic system, the forebrain and the thalamus (Gören and Cabadak, 2015). Studies have proven that there is an increased noradrenergic tonus in PTSD (Terzioğlu et al, 2013). The neurochemical findings suggest that there is excessive adrenergic activation after a traumatic event and this may enhance the memory consolidation of the event and increase the probability of a re-experience the trauma with a reminder (Gören and Cabadak, 2015). Pharmacologically, it was also demonstrated that administration of the [illegible]2 receptor antagonist yohimbine could induce flashbacks and increase autonomic responses in patients with PTSD. Adrenergic receptor blockers seem to be promising (Sofuoğlu et al, 2014). Corticotropin releasing factor is also involved in feed-forward circuit between amygdala and hypothalamus where low cortisol levels may produce unopposed autonomic and neuroendocrine responses (Berridge and Waterhouse 2003).
Neurochemical studies have also revealed that the hippocampal cholinergic system is also involved in the integration of anxiety and memory and we also demonstrated participation of muscarinic receptors in a predator scent test performed with cat litter (Aykaç et al, 2012). Valproic acid (valproate/divalproex sodium) and other anticonvulsants were also found to be effective in PTSD, especially as an adjunct in exposure-based psychotherapies (Yoshiike and Kuriyama, 2015). We also observed that D-cycloserine is helpful in extinction training sessions in predator scent test in rats (Sarıdoğan et al, 2015). DCS is a partial agonist for the glycine binding site on NMDA type glutamate receptors and it is helpful in the extinction of avoidance responses and risk assessment behaviors.

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Convulsions triggered by food intake in antimuscarinic-treated fasted animals: a model for eating seizures?

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Mice and rats treated with scopolamine, atropine or biperiden after fasting for two days or less develop partial or secondarily generalized tonic-clonic convulsions soon after refeeding (1). All of the complicated acts occurring during eating (e.g. chewing and swallowing movements, smelling and tasting) and stimulation of the amygdala by repetitive oral and masticator movements are suggested to be triggering and underlying factors because convulsions develop only after solid food intake, but not slurry or fluid feeding (2). Food deprivation itself, but not its hypoglycemic consequence, seems to play a critical role in the occurrence of convulsions.

Fasting for 48 h produces changes in the kinetics of [^{3}H]glutamate binding in the brain, implying that neuroadaptive changes occur during food deprivation. Chlorpromazine, haloperidol, clonidine, tizanidine and MK-801 provide effective treatments, but most of the major antiepileptic drugs are ineffective. Interestingly, eating-induced seizures in patients have similar triggering factors and manifestations (3,4). Attacks mostly occur during meal, and more precisely at the onset of meals when the patient begins to masticate food. The type of seizure is usually simple or complex partial, with or without secondary generalization (3). The precise underlying mechanisms are unknown. Sight or smell of food, chewing, swallowing or the complete act of eating a meal or conditioned reflex is suggested to act as triggering factors. Genetic susceptibility has been reported for some patients (5). In most cases, response to antiepileptic drugs is poor (6). With respect to the phenomenological similarities, the validity of convulsions in fasted animals as a model of eating seizures needs to be discussed.

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Novel targets focusing on neuroinflammation and neurogenesis in schizophrenia

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Schizophrenia is a serious mental illness that affects approximately 1% of the population worldwide, with positive, negative and cognitive dysfunctions and significant impairment in psychosocial functioning. Interactions between genetic susceptibility and environmental stressors at the early stages of life, subsequently neurodegeneration process is important in the development of schizophrenia. Current approaches suggest that cytokines induced neuroinflammation might have a role in the development of several psychiatric disorders, including schizophrenia. Uncontrolled microglia activation, increase in pro-inflammatory cytokines, and neurotransmitter dysfunctions can induce schizophrenia. Microglial activation induced by pro-inflammatory cytokines in central nervous system is responsible for the initiation and proceeding of inflammatory process and consequently developing neurodegeneration. In this context, new approaches regarding with elucidating the complex pathogenesis of the disease are gradually increasing. Among these approaches studies performed in recent years refer that abnormal inflammatory responses might play a role in the pathophysiology of schizophrenia. Obtaining promising results by adding anti-inflammatory agents such as monocycline, which is an antibiotic belonging to tetracycline group, and acetyl salicylic acid, which is a non-selective COX inhibitor, celecoxib, which is a selective COX-2 inhibitor as adjuvant to the standard therapy with antipsychotics, is supportive to this approach. In this regard, elevated pro-inflammatory cytokine levels in the plasma and cerebrospinal fluids of schizophrenic patients have been reported in the studies performed. It is thought that immune activation which occurs by various mechanisms in the fetus brain during the development period could lead to schizophrenia in the future periods of life by contributing neuro-developmental disorders comprising abnormal neurogenesis and neurodegeneration. Today, peripheral immune changes in various neuropsychiatric diseases are known to modulate the brain functions and behaviors. Truly elucidating the role of inflammatory mechanisms, which are increasingly reported in schizophrenia, is promising to comprehend the pathophysiology of the disease and establish effective therapeutic approaches.

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Can valproic acid have a therapeutic potential in Parkinson's disease?

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Parkinson's Disease (PD) is the second most common neurodegenerative disorder worldwide effecting 1% of the population over 60 years of age. Dopaminergic neurons in substantia nigra pars compacta progressively die and unfortunately very few symptoms become manifest before a high percentage of neurons die. The current therapeutic regimens such as combination of levodopa with aromatic amino acid decarboxylase inhibitors, amantadine, anticholinergics, entecapone/talcapone, selegiline, dopaminergic receptor agonists all work for a limited period of time. Gene therapy, fetal substantia nigra tissue implantation have all been tried, however, the results have been inconclusive. Antiapoptotic drugs, glutamate antagonists and antiinflammatory drugs were used for their antioxidant effects and deep brain stimulation has also been applied as functional neurosurgery,

Although the pathogenesis of Parkinson's disease is not fully understood, mechanisms related to free radical stress, mitochondrial dysfunction and apoptosis are the major factors in the degeneration of dopaminergic neurons (1). Since it is very difficult to develop new drugs, the ones that are already in the market might be tried to help patients. Anticonvulsant drug valproic acid has been known to have neuroprotective effects (2).

In an animal model of Parkinson's disease developed in rats stereotaxic injection of 6-OHDA (8 µg / 2 µL) or saline (2 µL) to the right substantia nigra pars compacta was done. The following coordinates of substantia nigra pars compacta was used: (AP) = –4.8 mm, (ML) = –1.8 mm and (DV) = –8.2 mm (3). Only the rats showing pronounced rotational behaviour (more than 5 contralateral turns) were included in the study after apomorphine (0.5 mg/kg sc) test. The effects of valproic acid were compared with levodopa. We were able to demonstrate the effects of valproic acid on oxidative stresss parameters (3). Similar effects were observed when motor function and apoptotic parameters were analyzed.

Valproic acid's potential as an adjunct therapeutic agent might be discussed.

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Elevated talk (C035): Neuroprotective effects of epicatechin against homocyctein-induced oxidative stresss in hippocampal area in rat

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Alzheimer disease is a neurodegenerative disease with complicated pathophysiology. Oxidative stress is an important event which related to the pathogenesis of Alzheimer. Epicathechin is a flavanol compound found in some herbs as green tea which its' antioxidant property has been known in some disease as cancer, diabetes and some neurodegenerative diseases but informations about this compound are few. So, in this study we evaluated the neuroprotective effects of epicatechin during homocysteine-caused hippocampal toxicity in vivo.

Epicathechin (50 mg/kg) was gavaged once daily for a period of 10 days beginning 5 days prior to homocysteine (0.5 µmol/µl) intra hippocampal injection in rats. Biochemical and behavioral studies, including passive avoidance learning and locomotor activity tests were studied 24 h after the last treatment. Also oxidative stresss markers as MDA, ROS, GSH and protein carbonyl were assayed.

Homocysteine induced lipid peroxidation, increased MDA, protein carbonyl and decreased GSH content. Moreover, homocysteine diminished passive avoidance learning and locomotor activity, however, Epicathechin treatment decreased MDA, protein carbonyl, ROS levels significantly as well as improved learning and memory.

Our results showed that oxidative damage of the hippocampus by homocysteine was diminished during administration of Epicatechin.

Keywords: epicathechin, homocysteine, oxidative stresss, lipid peroxidation, passive avoidance learning

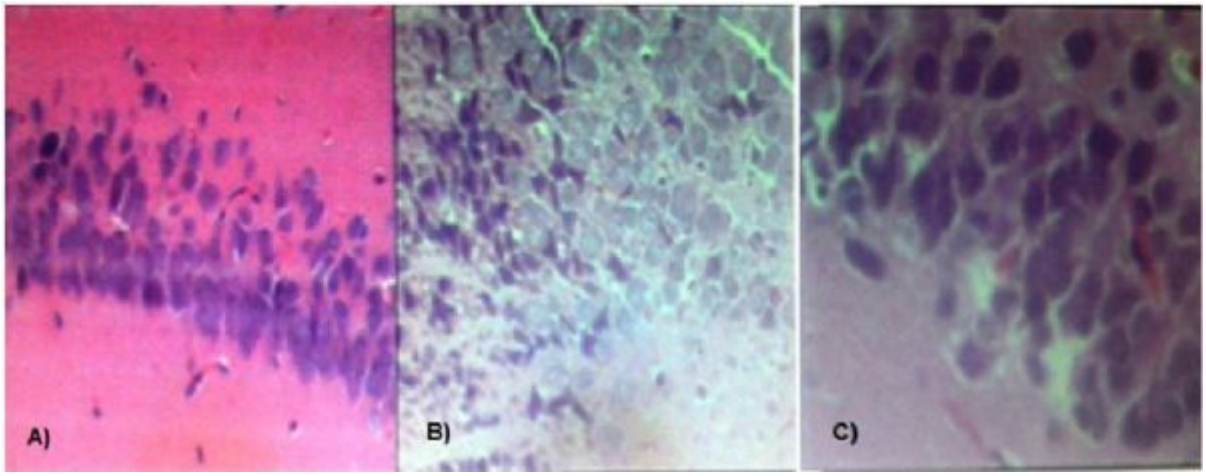

Figure 1. Rats' hippocampal sections stained with Hematoxylin and Eosin and observed optically (X10 & X40). (A) Control group: Normal stratified layers of cells showing smooth and nuclear borders, (B) Hcy group: Necrotic pyramidale cells characterized by piknotic hyperchromatic nuclei and irregular nuclear borders (C) Ec +Hcy group, signs of repair characterized by layers of granular cells with distinct cell borders.

Elevated talk (C036): The effect of the hyperpolarization-activated cyclic nucleotide-gated (HCN) channel blocker ZD72688 on spike-and-wave discharges in animal models of absence epilepsy

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Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels and the corresponding pacemaker current I_h has been shown to be a key player in thalamocortical dynamics linked to spike-and-wave discharges (SWDs) which are typical for childhood absence epilepsy. The pacemaker properties of I_h current has been extensively investigated *in-vitro*, however there are no *in-vivo* studies addressing the role of thalamic I_h in animal models of absence epilepsy. Here, we investigated the effect of thalamic I_h current by pharmacological block through ZD72688, a selective blocker of HCN channels, in genetic (Genetic Absence Epilepsy Rats from Strasbourg/GAERS) and pharmacological γ-hydroxybutyrate (GHB) models of absence epilepsy. Male GAERS and Wistar rats were implanted with microdialysis guide cannula bilaterally into the ventrobasal (VB) thalamic nucleus and epidural EEG electrodes were placed over the frontal and parietal cortex. Microdialysis probes were inserted into each VB nucleus and an artificial cerebrospinal fluid solution (aCSF) alone or mixed with ZD7288 at various concentrations from 10µM to 1mM was passed through the membrane in freely moving animals. Thalamic I_h blocker ZD72688 significantly reduced the total time spent in seizure and the duration of SWDs when compared to aCSF ($p<0.001$) in GAERS. In GHB model, the total time spent in seizure and the duration were also significantly reduced within the first 20 min (the peak of GHB action on seizure) of ZD72688 treatment ($p<0.01$). The reported decrease in total time and duration of SWDs in both genetic and pharmacological animal models of absence epilepsy suggest that thalamic I_h currents may be considered as a specific thalamic therapeutic target against absence epilepsy.
Keywords: GAERS, GHB, I_h current, reverse microdialysis, ventrobasal thalamic nucleus (VB)

Hall D:
Biased signaling - far beyond arrestin

(Organized by Dutch Pharmacological Society)
Organizers and chairs:
Martina Schmidt (University of Groningen, The NETHERLANDS)
This section will be performed via Skype connection
09:00-10:30

09:00 PKA and Epac as biased signaling paradigms in cAMP
Martina Schmidt
University of Groningen, The NETHERLANDS

09:30 Differential G protein activation by a single GPCR as a signaling bias
Martin Lohse
University of Würzburg, GERMANY

10:00 Elusive equilibrium: The influence of kinetics on observed signaling bias
Steven Carlton
The University of Nottingham, UK

10:30 End of symposium

Local workshop, Hall E:
Rational Drug Use

11:00-12:00

12:00 End of congress

Paralel meetings

Monday, 27th June 2016		
14:00-15:00	Meeting of the EPHAR EC Executive	Meeting room
Tuesday, 28th June 2016		
12:30-13:30	EUCPT Committee Meeting	Meeting room
17:00-18:30	EPHAR Council Meeting	Hall C
Wednesday, 29th June 2016		
15:00-16:00	Meeting of the new EPHAR EC Executive	Meeting room

Poster Sessions

Poster Session Day 1, Monday, 27th June 2016, Hall Poster hall 15:00-16:30

Receptors and Cell Signalling

P001: Effect of cronical delta9-tetrahidrocannabinol treatment on rho/rho-kinase signalization pathway in brain

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Delta9-tetrahidrocannabinol (Δ9-THC) shows their effect by activating cannabinoid receptors which is on some tisues and neurons. Some studies showed that cannabinoid systems involved in neuron and have role on cell proliferation and devolepment. Furthermore, it is interesting that cannabinoid system and rho/rho-kinase signalization pathway which have important role on cell development and proliferation may have role on neuron proliferation and development together. Thus, we aimed to investigate rhoA protein and rho-kinase enzyme expressions and activities in the brain of cronical Δ9-THC treated mice. In our study, mice treated with Δ9-THC for one month period and three times per day. At the end of this period, rhoA protein and rho-kinase enzyme expressions and activities were analyzed by ELISA method in their brain. Administration of Δ9-THC decreased the expression of rhoA. Administration of Δ9-THC did not effect expression of rho kinase while increased its activity.

Keywords: delta9-tetrahidrocannabinol, rhoA, rho-kinase, brain, mice

P002: Characterization of simultaneous 5-HT and glutamate release in response to optogenetic stimulation of median raphe afferents

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Serotonergic neurotransmission has been implicated extensively in behavioural, affective and cognitive functions in the brain. Previous studies regard the raphe as an area composed of functionally distinct subpopulations of 5-HT and non-5-HT neurons and necessity to examine these characteristics of the release of

[3H]5-HT in terms of its dependence of nerve impulse.

Here, we used an optogenetic approach to study the release of [3H]5-HT and [3H]Glu from median raphe (MR) nuclei and hippocampal slices in vitro. To measure the extracellular neurochemical changes in MR we combined optogenetic method with in vivo microdialysis and determine the effects of optical stimulation on mice behaviour. We have also applied for drugs such as TTX, CNQX and DL-AP5, and modified Krebs solution, to investigate the nature of [3H]5-HT release and interaction between serotonin and glutamate system in MR in vitro.

We found that optical stimulation significantly increased [3H]5-HT and [3H]Glu release in vitro and the extra cellular levels of 5-HT and Glu in vivo. Behaviourally, the optogenetic stimulation of neurons in MR increased locomotor activity of mice.

These results indicate that acute increase in 5-HT release from MR depends on glutamate-mediated effect and it exerts stimulant effect of locomotor behaviour under control condition.

Keywords: serotonin, release, optical stimulation, median raphe nucleus

P003: Mechanical forces activate histamine H_1 receptors

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G-protein coupled receptors play a major role in the transduction of extracellular signals into the cell and they are not only sensors for small molecules and hormones, but they can even perceive mechanical forces. It is known that agonist stimulation induces active receptor conformations allowing for G-protein activation. In this study we aimed to analyze whether mechanical stimulation might cause distinct active receptor conformations. For this, we analyzed the $G_{q/11}$-protein-coupled histamine H_1 receptor that showed a high degree of mechanosensitivity in previous studies. This receptor regulates the physiological process like sleep-wake regulation. To find out whether agonist and mechanical receptor stimulations would lead to different active receptor conformations, we performed intramolecular dynamic FRET measurements, which allow monitoring of conformational changes of the receptor. In case of agonist stimulation transmembrane domain 6 is supposed to be more involved and in the other case membrane stretch might induce movement of transmembrane domain 7. Therefore, Cerulean, a stable cyan fluorescent protein was attached to the C-terminus of the H_1 receptor and a small tetracysteine-binding motif was inserted at five different positions, four in the third intracellular loop of the receptor and one at the beginning of the C-terminus. The binding motifs allow labeling with the small fluorescent arsenical hairpin binder FlAsH, a yellow fluorescent dye. Agonist stimulation with histamine and mechanical stimulation with hypotonic bath solution were performed using a focal pressurized perfusion system. When compared with agonist stimulation, mechanical stimulation with hypotonic bath solution resulted in a significantly larger decrease of the FRET signals. As a more direct mechanical stimulus shear stress was applied. Notably, FRET signals were decreased in dependency of the strength of shear stress. Altogether, our results suggest that histamine H_1 receptors are mechanosensitive and adopt distinct active receptor conformations upon mechanical stimulation.

Keywords: mechanosensation, histamine H_1 receptor, intramolecular FRET, receptor conformation, shear stress

P004: Effect of cigarette smoking on rho/rho-kinase signalization pathway in lungs

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Cigarette smoke exposure has side effects lungs, cardiovascular systems and some tissue and organs and most affected organs is lungs. Rho/rho-kinase signalization pathway has important role one bronchial smooth muscle contractions and cancer development of lung fibroblast. Mechanism of molecular of some side effects due to cigarette smoke exposure has not yet been clearly identified. In the present study, we aimed to research effect of cigarette smoke exposure on rho/rho-kinase signalization pathway in lungs. For this propose mice (male age: 8 weeks) separated into two groups as controls smoke exposure. The cigarette smoke application continued 7 days in a week during two months. In the end of two months, mice were killed by cervical dislocation and their lungs were isolated. Then, rhoA and rho-kinase enzymes expressions and rho kinase enzyme activity have been determined. Cigarette smoke exposure upregulated rhoA and rho-Kinase enzyme expressions and elevated rho-kinase enzyme activity. As a result, cigarette smoke elevated activity of rho/rho-kinase signalization pathway.

Keywords: cigarette smoke, rhoA, rho-kinase, lung, mice

P005: The effects of selective MC_3 agonist PG990 and PG992 on TNF-alpha induced chondrocyte cell death and pro-inflammatory cytokine release

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Osteoarthritis (OA) is a degenerative joint disease partially mediated by the catabolic cytokine TNF-alpha, leading to progressive and permanent degeneration of cartilage. A role for melanocortin peptides exerting anti-inflammatory and chondroprotective effects have been shown, although the receptor subtype involved is unclear. This study aims to assess the chondroprotective and anti-inflammatory effects of the selective melanocortin-3 (MC_3) agonists PG990, PG992 and partially selective agonist D [Trp^8]-γ-MSH on TNF-alpha induced cell death, pro-inflammatory inflammatory cytokine and matrix metalloproteinase release.
Human C-20/A4 chondrocytic cell-line were cultured in 6 well plates ($1x10^6$ cells/well) and harvested to determine MC expression by RT-PCR. In separate experiments, C-20/A4 cells were treated with D [Trp^8]-γ-MSH (10µg/ml), PG990 or PG992 (0.1–30.0µg/ml) for 30 mins prior to TNF-alpha (60pg/ml) stimulation. Cell viability was determined by MTT, whilst, IL-6, IL-8 and MMP-1 release were detected by ELISA in cell free supernatants. Data expressed as Mean ± SD of n=4-8 determinations in triplicate. #$p \leq 0.05$ vs. control, and *$p \leq 0.05$, **$p \leq 0.01$ vs. TNF-alpha.

RT-PCR showed MC_1 and MC_3 expression on C-20/A4 cells. TNF-alpha stimulation caused a maximal cell death of 19.8% at 6h, D [Trp^8]-γ-MSH inhibited cell death by 49%, whilst, PG990 (10µg/ml) and PG992 (30µg/ml) caused a 182% and 58% reduction, respectively. TNF-alpha caused significant increase in IL-6, IL-8 and MMP-1 release. PG990 inhibited both IL-6 and IL-8 release at 6h, whilst, MMP-1 was reduced by PG992 but not by PG990.

Selective MC_3 agonists (PG990 and PG992) exhibited chondroprotective and modulation of inflammatory and tissue destructive pathways following TNF-alpha activation of chondrocytes. This data highlights a preferential role for MC_3 in modulating the inflammatory response in OA.
Keywords: arthritis, cytokines, immunopharmacology, melanocortins

P006: Pterygial tissue Rho/Rho-kinase gene expressions

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Pterygium is a proliferative wing-shaped fibrovascular tissue overgrowth arising from bulbar conjunctiva and growing onto the cornea. Pterygium is associated with chronic UV exposure and is characterized by proliferation, inflammatory infiltrates, fibrosis, angiogenesis and extracellular matrix breakdown. The pathogenesis of pterygium formation is not completely understood. The aim of this study was to investigate the possible contribution of Rho/Rho-kinase gene expressions in pterygial tissues.

A total of 30 patients with primary, and 15 patients with recurrent pterygium were included to this study. Primary and recurrent pterygia and uninvolved superior temporal bulbar conjunctiva specimens were obtained from patients who underwent pterygium excision and conjunctiva autograft surgery. mRNA from tissue samples was extracted, and real time polymerase chain reaction on the BioMark HD dynamic array system (Fluidigm, South San Francisco, Calif., USA) was performed for the Rho/Rho-kinase gene expressions. Gene expressions were calculated with QIAGEN online GeneGlobe program (http://www.qiagen.com/geneglobe) and Student's t-test was used for statistical comparisons.

Gene expression analysis showed that ROCK1, ROCK2, RHOC, RHOD, and RND3 (RHOE) mRNA contents in primary pterygial tissues were markedly elevated when compared to the control tissues ($p<0.05$). However, there were no changes in RHOA and RHOB gene expressions. On the other hand, marked augmentations in ROCK2, RHOB, RHOC, RHOD, and RND3 (RHOE), but not in ROCK1 and RHOA, gene expressions were observed in recurrent pterygium ($p<0.05$).

To the best of our knowledge, these results are the first to demonstrate the contribution Rho/ROCK gene expressions in pterygium. Our data showed that these genes may involve to the pathology of pterygium development.

This study is supported by a project (SBAG 114S562) from the TUBITAK, Ankara, Turkey.

Keywords: gene expression, pterygium, rho proteins, rho-kinase

P007: Epac1 links prostaglandin E2 to β-catenin transcriptional activity during epithelial-to-mesenchymal transition in A549 cells

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Epithelial-to- mesenchymal transition (EMT) is a major hallmark during carcinogenesis in which the cells lose their epithelial phenotype, including a loss of cell-cell junctions, and acquire a motile, mesenchymal phenotype underlying dissemination. In epithelial cells, β-catenin is localized at cell-cell junctions where it links the actin cytoskeleton to E-cadherin. When cell-cell junctions are lost, β-catenin can translocate to the nucleus where it activates transcriptional programs involved in survival and migration. Recent research has indicated that prostaglandin E_2 (PGE_2) can activate β-catenin transcriptional programs through the cyclic AMP effector protein kinase A (PKA). Here, we aimed to identify if the cyclic-AMP effector exchange protein directly activated by cyclic AMP (Epac) plays a role in β-catenin activation by PGE2 in cells undergoing EMT.

In the non-small cell lung carcinoma cell line A549, PGE_2 induced downregulation of E-cadherin and promotes migration, indicative of EMT. Interestingly, we observed increased expression of the Epac subtype Epac1 following PGE_2 treatment. In addition, PGE_2 induced β-catenin translocation from the cell membrane to the nucleus and activated β-catenin transcriptional programs, including upregulation of the EMT regulator ZEB1. Importantly, pharmacological inhibition of Epac1 with the specific inhibitor CE3F4 or Epac1 siRNA abolished β-catenin nuclear translocation, expression of β-catenin target genes and cell migration. Inhibition of Epac2 had no effect. Recently, a nuclear pore localization sequence was identified in the catalytic domain of Epac1 which is required for Epac1 localization in the nucleus. We found that expression of an Epac1 mutant with a deletion of this domain abolished β-catenin target gene transcription. Additionally, we observed that β-catenin and Epac1 co-immunoprecipitated in Epac1 overexpressing cells. The adaptor protein Ezrin is known to bind to E-cadherin, β-catenin and Epac1, thus possibly bringing all molecular components together. We found that downregulation of Ezrin expression resulted in attenuation of β-catenin transcriptional activity, suggesting a possible role for Ezrin in sequestering Epac1 and β-catenin together.

Taken together our data indicate a novel role for Epac1 in PGE_2-induced EMT via activation of β-catenin transcriptional programs.

Keywords: Epac, A549 cells, epithelial to mesenchymal transition

Ion Channels

P008: Effects of Capsaicin on human alpha7-nicotinic acetylcholine receptors

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Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is an active component of chili pepper. The burning and painful sensations associated with capsaicin result from its chemical interaction with sensory neurons. Capsaicin has been shown to bind to a receptor called the vanilloid receptor subtype 1 and also interacts with several voltage-dependent channels. However, the effect of capsaicin on nicotinic acetylcholine receptors (nAChR), a prototype for the ligand-gated ion channels, is currently unknown. In this study, using two-electrode voltage clamp technique, ACh (100 µM)-induced currents were recorded in *Xenopus* oocytes expressing the cloned α7-subunit of human nAChRs and the effect of capsaicin was tested on the function of this receptor. Bath application of 10 µM capsaicin inhibited 100 µM ACh-induced currents to 48 ± 4 of controls (n=6). The effect of capsaicin reached steady-state levels within 15 min and it was concentration-dependent with respective IC_{50} and slope values of 11.7 µM and 1.2. In the presence of capsaicin, although the potency of ACh was not altered, maximal ACh-induced responses were significantly suppressed indicating that capsaicin acts in a non-competitive manner. In conclusion, the results indicate that capsaicin inhibits the function of human α7- nAChRs.

Keywords: capsaicin, nicotinic acetylcholine receptors

P009: The decrease in αENaC surface abundance in cultured alveolar epithelial cells in hypoxia depends on HIF-2α

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Epithelial Na Channels (ENaC) play a central role in maintaining alveolar fluid balance because Na-transport drives the reabsorption of excess fluid. Hypoxia decreases alveolar fluid reabsorption. As cellular adaptation to hypoxia is regulated by hypoxia-inducible transcription factors (HIF), we tested whether HIFs are also involved in decreasing ENaC activity in hypoxic alveolar epithelium.

HIF-1α and HIF-2α expression was suppressed in cultured rat primary alveolar epithelial cells (AEC) with shRNAs introduced by adenoviral infection. Cells were exposed to normoxia and hypoxia (1.5% O2) for 24h. ENaC activity was measured in Ussing chambers, mRNA by RT qPCR, protein abundance of ENaC by surface biotinylation and mTOR targets by western blot.

Hypoxia (1.5% O2, 24 h) decreased amiloride-sensitive transepithelial Na transport, decreased the mRNA expression of αβγENaC, and reduced the amount of αβγENaC protein in the plasma membrane as well as intracellular αβENaC. Silencing HIF-2α, but not HIF-1α, partially prevented the hypoxia-induced decrease in ENaC activity. HIF-2α silencing fully prevented the decrease in αENaC inserted into the apical plasma membrane and of intracellular αENaC, and did not prevent the hypoxia-induced internalization of β- and γENaC. Decreased mRNA expression of αβγENaC in hypoxic AEC was not affected by HIF silencing. Hypoxia decreased active S6 kinase and 4E-BP1 and increased the active form of Nedd4-2 independent of HIFs.

These results indicate that the decrease in ENaC in hypoxic AEC is due to decreased transcription and translation as well as HIF-2α dependent degradation of αENaC, resulting in reduced ENaC membrane abundance and decreased ENaC dependent Na reabsorption.

Keywords: ENaC, hypoxia, HIF-2α, lung

P010: Bioinformatical investigations of Androctons crassicauda scorpion toxins: Presence of GG4 domains

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Scorpions are living fossils and they present on earth more than 400 million years. The primary weapons of scorpions are their deadly venoms. The toxins of the venoms are effective on almost all living animals including humans.

Scorpion venom toxins act many systems especially on cardiac and nervous systems. Main action of their toxins are on ion channels. Due to the envenomations they exhibit serious medical problems but they also give chance for new drug candidates.

The aim of this study was to investigate possible motifs on toxins of Androctonus crassicauda scorpion venoms.

The protein sequences were downloaded from uniprot site (www.uniprot.org). Androctonus crassicauda toxin sequences were extracted using slackware linux operating system.

bio3d and protr packages of the R programming language (cran.r-project.org) and clustalo were used for alignment of the toxin sequences.

Presence of the GG4 motif was seen on SCX8 toxin. Since GG4 motif is one of the important motifs for mammalian proteins, its presence on the toxin is suggested to involve in its mode of action. This is the first report for the presence of GG4 motifs on Androctonus and the other scorpions which requires further investigations

Keywords: venom, toxin, scorpion, motif

Cardiovascular Pharmacology

P011: The role of IκB-α/NF-κB pathway activation in hypotension and inflammation reversed by BAY 61-3606, a selective Syk Inhibitor, in rat models of septic and non-septic shock

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Septic shock is a systemic inflammatory response with abnormal microcirculatory function and severe hypotension. Non-septic shock, triggered by zymosan, leads to systemic inflammation and multiple organ dysfunction. Spleen tyrosine kinase (Syk) is reported to be involved pathogenesis of various inflammatory diseases. In this study, we aimed to investigate the contribution of IκB-α/NF-κB pathway activation to the effect of Syk inhibition on hypotension and inflammation regarding the production of vasodilator and proinflammatory mediators in septic and non-septic shock.

Male Wistar rats received saline (4 ml/kg, i.p.), LPS (10 mg/kg, i.p.) or zymosan (500 mg/kg, i.p.) at time 0. Saline, LPS or zymosan-treated rats were given BAY61-3606 (3 mg/kg, i.p.) 1 h after injections. Mean arterial pressure (MAP) and heart rate (HR) were measured by using tail-cuff device. Rats were sacrified 4 h after LPS or zymosan challenges. Blood, kidney, heart, thoracic aorta, and superior mesenteric artery were collected for the measurement of COX-2 and iNOS activities in addition to IL-8 and TNF-alpha levels. Expressions and/or phosphorylations of IκB-α, NF-κB p65, and β-actin were measured in cytosolic and/or nuclear fractions of these tissues.

LPS and zymosan decreased MAP and increased HR. LPS and zymosan caused an increase in both expressions and/or phosphorylations of NF-κB p65 and IκB-α in cytosolic and/or nuclear fractions of the tissues as well as a decrease in IκB-α expression. COX and iNOS activities, TNF-alpha and IL-8 levels were increased in sera and/or tissues with increased activity of IκB-α/NF-κB p65 pathway and NF-κB p65 translocation.

These results demonstrate that, IκB-α/NF-κB pathway contributes to the effect of Syk inhibition on hypotension and inflammation induced by LPS and zymosan in septic and non-septic shock, respectively.

This study is supported by a grant from the Research Foundation of Mersin University (BAP-ECZ F EMBF (SŞF) 2012-6 B).

Keywords: septic shock, non-septic shock, BAY 61-3606, Syk, NF-κB

P012: Effects of fresh coconut milk consumption for 6 weeks on vascular functions in middle-aged male rats

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Coconut milk (CCM) has been an important cooking ingredient in the Asia-Pacific region since ancient times. However, its high content of saturated fats may facilitate the development of cardiovascular disease, so some people are reluctant to consume it. The present study is aimed to test if the consumption of fresh coconut milk by middle-aged male rats affected vascular functions and lipid profiles. Mature coconut kernel was grated and compressed to receive fresh coconut milk followed by lyophilization. Rats received either CCM (3 g/kg) or distilled water, orally once a day for 6 weeks. Feeding with CCM caused a lowering of the maximal contraction of the endothelium-intact thoracic aortic rings to phenylephrine that was eliminated by N^G-nitro-L-arginine (L-NA) or by the disruption of the endothelium. It also caused a higher relaxation to acetylcholine, but not to glyceryl trinitrate on phenylephrine precontracted thoracic aortic rings. DL-propargylglycine caused a small increase in the baseline tension of the L-NA treated, endothelium denuded-thoracic aortic rings of the CCM-treated rats and produced a higher contractile response of the thoracic aortic ring to low concentrations of phenylephrine than the control group. The expression of eNOS- and cystathionine-γ-lyase (CSE) by the thoracic aorta of the CCM-treated rats was also higher than that of the control. These results indicated that CCM-treatment of middle-aged rats caused an upregulation of eNOS and CSE protein expression that resulted in an increased production of NO and H_2S from the blood vessels and opposed the vascular contraction to phenylephrine with an increased relaxation to acetylcholine. CCM also caused a decreased serum glucose level. Thus, from these results CCM consumption could be beneficial to prevent and/or to reduce the development of cardiovascular disease in the middle-aged human.

Keywords: coconut, thoracic aorta, middle-aged rat, NO, H_2S

P013: Protective properties of grape polyphenols concentrate on the model of hypertensive syndrome in rats

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We aimed to investigate the properties of polyphenol concentrate obtained from Kazakhstan selection Cabernet Sauvignon grape type on the model of hypertensive syndrome in rats

The study was conducted on 30 outbred male rats weighing 180 ± 20 g. Animals held on a normal diet and free access to water and food. Animals were randomly divided into three groups: intact group (n = 6, tap water as a water source), control group (n = 12) and experimental group (n = 12), where the drinking water was 0.8% solution of NaCl. 10 days prior to the experiment, rats in the experimental group received the concentrate of grape polyphenols in a dose of 0.5 ml/kg. Rats in the control group received saline at the same volume. Prior to the experiment and after ten days, systolic blood pressure was measured using non-invasive device for measuring blood pressure in rats (Leica, Germany). Statistical results are showed as arithmetic mean and standard error of the arithmetic mean.

Using a 0.8% NaCl solution in place of drinking water yielded hypertensive syndrome in rats in the control group: starting BP - 150 ± 18.3 mmHg and after 10 days of receiving 0.8% NaCl - 219 ± 13.6 mmHg (p=0.008). In the experimental group, a statistically significant increase in blood pressure could not be obtained: the initial BP - 159 ± 16.5 mmHg after 10 days of receiving 0.8% NaCl - 171 ± 15.7 mmHg (p=0.09). The difference between the level of blood pressure in control and group receiving polyphenol extract were 219 ± 13.6 mmHg and 171 ± 15,7 mmHg (p=0.03), respectively.

We can assume that polyphenol concentrate from Kazakhstan selection Cabernet Sauvignon grape prevents the formation of hypertensive syndrome in rats provoked by excessive administration of sodium chloride.

Keywords: hypertension, polyphenols, cytoprotectors

P014: AMG9810 inhibits TRPV1 channel mediated vascular responses

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TRPV1 was originally identified as a pain mediating sensory neuronal receptor, steaming a significant effort to develop antagonists as analgesic drugs. However, TRPV1 was also identified in non-neuronal cells in the past decade. Among these non-neuronal TRPV1 populations vascular smooth muscle cells are of particular importance for three reasons: (1) Agonists can evoke robust vasoconstriction; (2) vascular TRPV1 expression and function appears to be tightly regulated. (3) vascular on-target side effects may complicate the clinical success of TRPV1 antagonists developed as analgesics. Here we describe the vascular effects of a potent TRPV1 antagonist (AMG9810) in the rat.

Capsaicin and AMG9810 were purchased from Tocris Bioscience.

AMG9810 effects were tested on isolated skeletal muscle arteries in vitro and on blood pressure, plasma extravasation in vivo.

AMG9810 (0.3 and 1.0 µM) was without effects on the arteriolar diameter of isolated skeletal arteries of the rat. Capsaicin (1 nM–10 µM) evoked a significant constriction (from 193±8 µm to 40±6 µm), with a half maximal effect at 100 nM (48±16% constriction). This 100 nM capsaicin evoked constriction was antagonized by AMG9810 in a concentration dependent manner (constrictions: 31±24, 20±12 and 16±10%, at 100, 300 and 1000 nM, respectively). AMG9810 alone reduced the blood pressure transiently, and inhibited the pulmonary chemoreflex evoked by intravenously administered capsaicin (blood pressure reduction systolic: 88±13, 107±10, diastolic: 55±6, 66±6 ΔmmHg at 1 and 3 mg/kg intravenous dose of AMG9810, respectively). The half maximal dose (ED_{50}) of capsaicin was 0.8, 5.1 and 6.9 µg/kg at 0, 1 and 3 mg/kg AMG9810. In contrast AMG9810 was unable to antagonize capsaicin evoked plasma extravasation.

AMG9810 dose dependently blocks non-neuronal TRPV1 receptors and in a low dose reduces the capsaicin-evoked pulmonary chemoreflex.

Keywords: AMG9810, capsaicin, TRPV1, vasoconstriction, chemoreflex

P015: Impact of diabetes and obesity in control of blood pressure in patients under pharmacological antihypertensive treatment

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The objective of this study is assessing the impact of diabetes and obesity on the control of blood pressure (BP) in patients under pharmacological antihypertensive treatment.

A systematic review of articles that used multivariable logistic regression models to identify predictive factors of not achieving adequate BP control in patients under pharmacological antihypertensive treatment was done by searching in PubMed, Scielo and Scopus (Jan-2015). Only original research articles written in Roman characters were included. Literature selection was done in two steps, following Cochrane and PRISMA recommendations. Data were extracted independently by two researchers. Two different meta-analyses (MA) (random effects model) were done: one assessing diabetes as risk factor to uncontrolled BP in treated patients and another assessing body mass index (BMI) as risk factor to uncontrolled BP in treated patients. Heterogeneity was assessed by the inconsistency index (I square).

A total of 2905 articles resulted from the search, excluding 2699 in the screening phase and 152 in the full-text phase, resulting in 54 articles included for qualitative extraction and 43 articles for meta-analyses. Of these, 8 present Odds Ratio (OR) and 95% Confidence Interval (95%CI) of diabetes as a risk factors for having high BP among treated patients and 7 present OR and 95%CI of BMI as a risk factors for having high BP among treated patients.

In the Diabetes MA, a pool OR 3.187 (95%IC: 1.849 - 5.495) was obtained, with Z=4.171; p<0.001 and I-square= 96%. In Obesity MA, a pool OR 1.048 (95%IC: 1.028 - 1.070) was obtained, with Z=4.599; p<0.001 and I-square= 60%.

Both comorbidities have a negative influence on the control of blood pressure in patients under pharmacological antihypertensive treatment, however, the impact of diabetes as comorbidity is 3 times higher than obesity.

Keywords: hypertension control, treatment failure, comorbidities, risk factors

P016: Protective effects of ellagic acid on cardiovascular damage caused by hypertension in rats

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Ellagic acid (EA) is a natural phenol found in numerous fruits (mainly in red fruits and nuts) which is described as having antioxidant and antiproliferative properties. Hence, it was hypothesized that EA could improve the cardiovascular damages caused by hypertension. The hypertension was induced in male Wistar rats (200-230g, n=6 in all groups) by oral administration with L-NAME (60 mg/Kg/day in drinking water) for six weeks. EA was administered orally (10 or 30 mg/Kg/day by gavage) six weeks along with oral treatment of L-NAME. The control group received only drinking water and vehicle (distilled water). The blood pressure was recorded every week by tail-cuff plethysmography. After six weeks, the rats were anesthetized, sacrificed and the blood was collected to quantify nitrate/nitrite. Aortas were isolated and set up to isometric recordings in an organ bath and for histological assay with hematoxylin/eosin to evaluate vascular remodeling. The high level of blood pressure (205.4±21.4 mmHg) was reduced ($p<0.01$) by treatment with AE 10 or 30 mg/Kg (175.1±16.6 and 172.3±11.2 mmHg, respectively) but not reached the level observed in control group (119.2±8.6mmHg). The blood concentration of nitrate/nitrite (NO metabolites) was significantly ($p<0.05$) reduced in hypertensive rats (12.5±2.7 µmol/L) vs. control rats (26.2±2.1 µmol/L). The treatment with either 10 or 30 mg/Kg EA significantly restored nitrate/nitrite production (36.7±11.2 and 35.9±9.8 µmol/L). The vascular relaxation to acetylcholine was impaired in hypertensive group (43.4±6.3%) vs. control group (95.7±3.4%). The treatment with either 10 or 30 mg/Kg EA significantly improved the vascular relaxation (57.9±3.6 and 95.7±9.7%) and resulted in lesser aortic wall thickening. EA attenuates hypertension possibly improving the NO biodisponibility. The relaxation endothelium-dependant is impaired by hypertension and improved after treatment with AE. Moreover, the vascular remodeling during hypertension was attenuated by treatment with AE.

This study is supported by FAPEG, CNPq.

Keywords: ellagic acid, hypertension, vascular remodeling, relaxation

P017: Vasorelaxing effect of resveratrol on bovine retinal arteries

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Resveratrol is red wine polyphenol that causes vasorelaxation, which could be of interest in the treatment or prevention of eye diseases with an impaired blood flow, such as glaucoma, age-related macular degeneration and diabetic retinopathy. In this study, the vasorelaxant capacity of cis- and trans-resveratrol was tested on bovine retinal arteries. Its mechanism was investigated and its influence on the continuously released retinal relaxing factor (RRF) was examined. The investigation was performed by mounting isolated bovine retinal arteries into wire myographs for isometric tension measurements. Concentration-response curves of cis- and trans-resveratrol were constructed to investigate the vasorelaxant capacity. Concentration-response curves of resveratrol in absence or presence of the endothelium or different inhibitors were constructed to elucidate the vasorelaxing mechanism. The influence of resveratrol on the RRF was examined on mice femoral arteries by comparing the relaxations, elicited by RRF, with and without resveratrol incubation. Both resveratrol isomers caused a similar strong concentration-dependent relaxation. Removal of the endothelium or blocking endothelium-dependent pathways did not change the relaxation. Also K^+ channels blockers did not reduce the relaxation, except the 120 mM K^+ Krebs Ringer bicarbonate solution. Phorbol 12-myristate 13-acetate and phorbol 12,13-dibutyrate blocked the relaxation partially, and so did the inhibition of heme oxygenase-1. Blocking adenylyl cyclase, AMP-activated protein kinase, estrogen receptors, sirtuin 1 or sarco/endoplasmic reticulum Ca^{2+} ATPase did not have an effect. The relaxation caused by the RRF was not altered by resveratrol incubation. It is concluded that cis- and trans-resveratrol relax bovine retinal arteries similarly and concentration-dependently. The main relaxation mechanism remains unclear, but K^+ channels, CO and the myosin phosphatase pathway may be involved. Resveratrol does not have an influence on the RRF.

Keywords: resveratrol, retinal artery, vasorelaxation

P018: Protective effect of resveratrol and quercetin on *in vitro*-induced diabetic mouse corpus cavernosum

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Hyperglycemia and increased levels of methylglyoxal (MGO) can trigger the development of vascular complications in diabetes. Resveratrol and quercetin are red wine polyphenols with known beneficial cardiovascular properties, including an antioxidant capacity. This study evaluated whether resveratrol and/or quercetin could prevent in vitro-induced diabetic changes in neurogenic and vascular relaxant responses of mouse arteries and corpora cavernosa. Isometric tension of isolated aorta, mesenteric arteries and corpora cavernosa was measured using organ bath systems. Diabetic conditions were mimicked in vitro by co-incubating the tissues for 2 h with high glucose (HG, 30 mM) and MGO (120 µM). The presence of HG and MGO significantly blunted acetylcholine (Ach)-induced relaxations in corpora cavernosa and mesenteric arteries but not in aorta. Electrical field stimulated (EFS) responses of corpora cavernosa were also significantly inhibited by these diabetic conditions. In corpora cavernosa 2 h co-incubation with resveratrol (30 µM) or quercetin (30 µM) significantly attenuated HG and MGO-induced deficits in Ach- and EFS-responses. Our study demonstrates that in mouse arteries, HG and MGO rather affect endothelium derived hyperpolarizing factor-mediated than nitric oxide (NO)-mediated relaxations. In corpora cavernosa HG and MGO interfere with NO release. Resveratrol and quercetin protect mouse corpora cavernosa from diabetic-induced damage to NO-mediated relaxant responses. This might rely on their antioxidant capacity.

Keywords: resveratrol, quercetin, diabetes, erectile dysfunction

P019: Ketamine treatment reverses age-related vascular dysfunction in thoracic aorta

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Ketamine is a general anesthetic which has a variety of cardiovascular effects such as increases in cardiac output, arterial blood pressure, and heart rate. It has been also suggested that ketamine induces systemic hypotension. There are several in vitro studies which has been reported its direct vasodilatory effect on vascular smooth muscles isolated from aorta and mesenteric, femoral, cerebral arteries. It was the aim of this study to answer the question whether chronic treatment of ketamine reverses the adverse effect of age on vascular function in thoracic aorta.

Thirty rats were randomly divided into 5 groups: 4 month control (n=6), 18 month control (n=6), ketamine-treated 18 month group (n=6), 24 month control (n=6) and ketamine-treated 24 month group (n=6). Ketamine-treated groups received intraperitoneal injection of 10 mg/kg/day of ketamine for 14 days. At the end of 14 days, the effect of chronic treatment with ketamine was investigated on carbachol (10^{-8}-10^{-4} M) and sodium nitroprusside (SNP) (10^{-8}-10^{-4} M) after preincubation with phenylephrine (10^{-6} M) in organ chambers. Statistical comparisions between the groups were performed using analysis of variance followed by Tukey's test.

Carbachol-induced relaxation of the thoracic aorta of the 18 month and 24 month control groups were significantly decreased compared to young control group ($p<0.05$). The impaired carbachol-induced relaxations of aortic rings were markedly improved by treatment of ketamine. There were no changes in the relaxant response to SNP between the groups.

The findings of the current study showed that age-related dysfunction in endothelium-dependent vasodilation in thoracic aorta can be reversed using ketamine treatment.

Keywords: ketamine, vascular dysfunction, aging

P020: Protective effects of necrostatin-1 on doxorubicin induced cardiotoxicity

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The clinical use of Doxorubicin (Dox) is limited by its cardiotoxic side effects. This study was performed to investigate the protective effects of necrostatin-1 (Nec-1)-an inhibitor of necroptosis -on doxorubicin induced cardiotoxicity.

Male Sprague-Dawley rats were divided into four groups. Groups were defined as; 1:control, 2:Dox group: A single dose of Dox (10 mg/kg, i,p), 3:Dox+Nec-1 group; A single dose of Dox (10 mg/kg,i,p) + Nec-1 (1,65 mg/kg, i.p, for seven days), 4:Nec-1 group (1,65 mg/kg, i.p, for seven days).In all groups, isolated hearts were perfused by Langendorff system. Perfusion pressure (PP), left ventricular developed pressure (LVDP) and heart rate per minute(HR),LV(dP/dt)max and LV (dP/dt)min which shows systole and diastole rate were recorded. Cardiac tissues were used for histological examination and for measurement of malondialdehide (MDA) levels and expression levels of bcl-2, bax, casp-3, nox-2 genes.

In Dox group versus to control group; PP significantly increased ($p<0.001$), HR, LVDP, LV (dP/dt)max and LV (dP/dt)min significantly decreased ($p<0.001$). However, in Dox+Nec-1 group versus to Dox group; PP significantly decreased ($p<0.05$). HR, LVDP, LV (dP/dt)max and LV(dP/dt)min significantly increased ($p<0.05$). MDA levels in Dox group versus to control group significantly increased ($p< 0.001$), in Dox+Nec-1 group versus to Dox group significantly decreased ($p< 0.05$). Histological examinations showed that Nec-1 reduced the cellular injury induced by doxorubicin. Expression level of bcl-2 significantly decreased in Dox group versus to control group ($p<0.001$), in Dox+Nec-1 group versus to Dox group significantly increased ($p< 0.001$). Expression levels of bax, casp-3 and nox-2 in Dox group versus to control group significantly increased ($p<0.001$), in Dox+Nec-1 group versus to Dox group significantly decreased (bax; $p<0.05$, casp-3 and nox-2: $p<0.001$).

It is concluded that Nec-1 has protective effects on cardiotoxicity induced by doxorubicin.
Keywords: doxorubicin, cardiotoxicity, necrostatin-1, nox-2

P021: Resveratrol treatment reversed vascular dysfunction induced by chronic fluorosis in rats

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Fluoride is a naturally occurring contaminant in the water and essential for normal maintenance of teeth and bones. However, prolonged exposure to fluoride is found to be deleterious. In the present study, effects of resveratrol on vascular responses were examined in rats exposed to chronic fluorosis.

Chronic fluorosis was induced through NaF (10 mg/L/day) by adding to drinking water in male and female Sprague Dawley rats (200-250g). Resveratrol (50 mg/L/day) was also administrated in drinking water. Rats were monitored by measuring blood pressures for 90 days. At the end of the experiment, serum fluor level was measured in plasma samples and responses of isolated thoracic aorta were recorded in organ bath.

Serum fluoride level was elevated in all NaF administered rats. In both male and female rats, NaF-induced blood pressure elevation was reversed by resveratrol treatment. Phenylephrine-stimulated contraction of aortic smooth muscle was also increased by NaF toxicity and resveratrol inhibited it. Fluorosis caused decrease in acethylcholine-induced endothelium-dependent relaxations of vessels and this detrimental effect was reversed by resveratrol treatment. Sodium nitroprusside-induced endothelium-independent relaxations were similar in all groups.

The results of this study indicated that beneficial effect of resveratrol on vascular dysfunctions in chronic fluorosis is related to protection of vessel functions in rats.

Keywords: fluorosis, resveratrol, blood pressure, vascular relaxation

P022: Role of the nitric oxide on rosuvastatin-induced relaxation of the calf cardiac vein during cooling

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3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors including rosuvastatin do not only lower plasma cholesterol but also have non-cholesterol lowering effects on the vessel wall, which decrease cardiovascular complications. The aim of the present study was to evaluate the effect of cooling (to 28°C) on the responses induced by rosuvastatin (10^{-9}-$3x10^{-4}$ M) on serotonin (5-HT, 10^{-6} M) precontracted calf cardiac vein and the role of nitric oxide (NO) in these effects.

Ring preparations of veins obtained from calf hearts were suspended in organ baths containing 25 ml of Krebs-Henseleit solution, maintained at 37°C and continuously gassed with 95% O_2-5% CO_2. After a resting period, preparations were contracted with 5-HT at 37°C and rosuvastatin was added cumulatively. After the first concentration-response curve was completed, preparations were washed and allowed to reestablish a resting tension. After the contractile responses to 5-HT, the temperature was changed from 37 to 28°C. Cooling was rapidly achieved and preparations were allowed to equilibrate at this temperature for 30 min. The influence of NO on relaxations to rosuvastatin was specifically addressed by pre-treating the rings with N^G nitro-L-arginine methyl esther (L-NAME, 10^{-4} M). Again, after the contractile response to 5-HT, the temperature was changed from 37 to 28°C. L-NAME was added to the organ bath 20 min before concentration-response curves were obtained.

Rosuvastatin produced concentration-dependent relaxation of calf cardiac vein precontracted with 5-HT. During cooling, the pIC_{50} value, but not the maximal response, to rosuvastatin was significantly higher than at 37°C. Cooling to 28°C in the presence of L-NAME decreased the pIC_{50} values to rosuvastatin.

The results suggested that rosuvastatin induced relaxation of calf cardiac vein and NO played an essential role on the decreased sensitivity to rosuvastatin during cooling.

Keywords: cardiac vein, cooling, nitric oxide, rosuvastatin

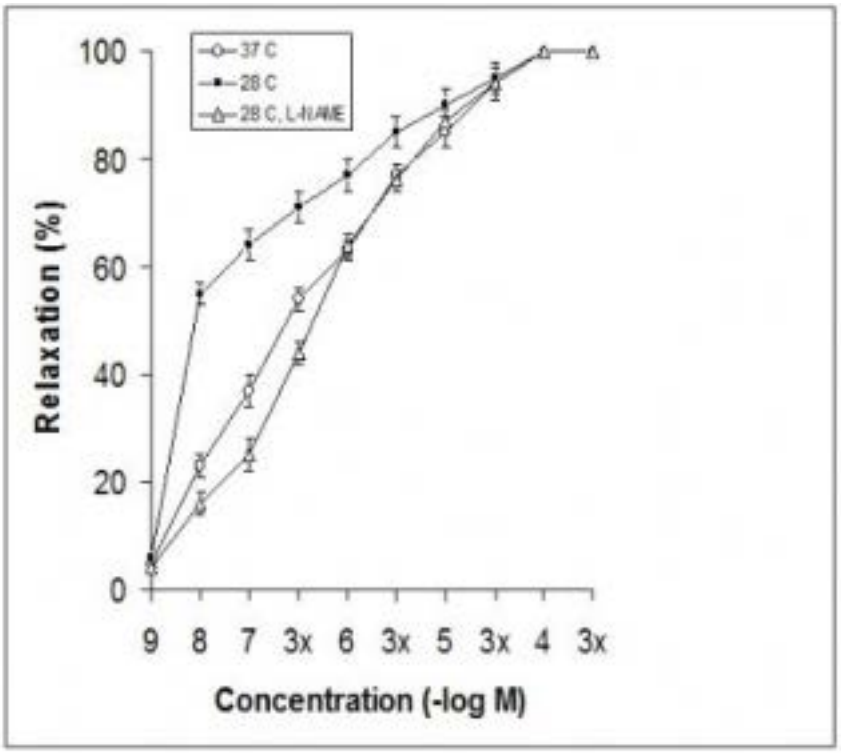

Figure 1. Concentration-response curves of rosuvastatin in calf cardiac veins precontracted with 5-HT at 37°C, 28°C and 28°C in the presence of L-NAME. Each point is the mean ± SEM of six experiments.

P023: Relationship with urotensin-II peptide and cyclosporine-A due to endothelial dysfunction and nephrotoxicity in rats

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Cyclosporine is an immunosupressive drug used after organ transplantation. Side effects of the drug are nephrotoxicity and cardiotoxicity. Urotensin is a potent vasoconstrictor peptide, which plays an important role in renal and vascular disorders.

The aim of the present study is to establish the role of urotensin peptide in cyclosporine induced nephrotoxicity and vascular dysfunction, as well as to investigate the effect of palosuran, a urotensin receptor antagonist, on such complications.

Rats weighing 180-200 grams were used in the study. Daily single dose of 30 mg/kg cyclosporine was injected introperitoneally for three consecutive weeks. Palosuran 150 mg/kg twice a day was administered to the treatment group for three weeks. Relaxation responses to acethylcholine and sodium nitroprusside, and contraction responses to phenylephrine, L-NAME and urotensin were evaluated in the thoracic aorta. Serum and renal tissue SOD, catalase, MDA, TBARS and MPO levels, serum urotensin levels were measured. Protein expressions of these parameters were investigated via western blot in renal and aortic tissues. Furthermore, iNOS and urotensin expressions were evaluated by immunohistochemistry.

While cyclosporine reduced endothelium-related relaxation responses, palosuran ameliorated them by increasing nitric oxide production. In the cyclosporine-treated group, urotensin-mediated contraction responses were decreased. Creatine clearance decreased in rats that developed cyclosporine associated nephropathy. In this group, palosuran significantly improved the renal function. No remarkable change could be detected in the antioxidant capacity, but a significant reduction was observed in the peroxidation capacity and palosuran amended this pathology. Immunohistochemical studies revealed that, cyclosporine damaged renal glomerular and tubular structures and increased iNOS, as well as urotensin expressions in the kidney, aorta and renal arteries. Yet, it was observed that palosuran had a strong impact on returning these expressions to normal control levels.

Urotensin system plays an important role in cyclosporine induced nephrotoxicity and vasculopathy.

Keywords: cyclosporin-A, urotensin-II peptide, palosuran, endothelial dysfuntion, nephrotoxicity

P024: The effects of melatonin on protection of postconditioning in myocardial ischemia reperfusion induced infarct size: Role of mitochondrial uncoupling protein 3

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Ischemic postconditioning (PostC) is a strong endogenous cardioprotective phenomenon which targets the increased tolerance of the myocardium against continuous ischemia when the myocardium is subjected to short intervals of ischemia-reperfusion (IR) at the beginning of reperfusion. It has been reported that protective effects of PostC decrease/disappear with age and chronic heart diseases. Similarly low serum levels of melatonin have been reported in the same risk groups. The aim of this study was to investigate the effects of physiological and pharmacological concentration of melatonin on protection of PostC in IR induced infarct size and role of mitochondrial Uncoupling Protein 3 (UCP3) using an in vivo model of myocardial IR injury.

Rats were pinealectomized (Px) or sham-operated (non-Px) 2 months before the IR studies. To produce cardiac damage, the left main coronary artery was occluded for 30 min followed by 120 min reperfusion in anesthetized rats. PostC induced with 3 cycles of RI (10 s each) after 30 min ischemia.

Infarct size, expressed as the percentage of the risk zone was found significantly higher in Px rats (54,68±1,5%) than in the control group (35,1±2,5%). PostC and melatonin administration (10 mg/kg) to Non-Px rats significantly reduced the infarct size. Level of UCP3 decreased with IR and Px, increased by PostC and melatonin. On the other hand, PostC does not create significant effect in Px rats but protection was provided with melatonin.

These results suggest that physiological and pharmacological concentrations of melatonin are important in protection of PostC. As protection of PostC reduced with Px, melatonin levels have been reported to decrease with age, melatonin replacement therapy may attenuate IR-induced myocardial injury and increased protective effetcs of PostC. This study also showed that the PostC and melatonin has mitochondrial protective effects on UCP3 upregulated mechanism.

This project is supported by TUBITAK (115S323).

Keywords: myocardial ischemic postconditioning, melatonin, UCP3

P025: Effects of endoplasmic reticulum stress on hypertension induced endothelial dysfunction in rat

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Hypertension is one of the most frequent health threatening diseases and also it is very expensive among all cardiovascular diseases. Endoplasmic reticulum stress (ERS) which occurs as a result of the accumulation of unfolded or misfolded proteins in endoplasmic reticulum, may be involved with the pathogenesis of cardiovascular diseases. In present study, the effects of ERS inhibition on hypertension induced endothelial dysfunction were examined.

Hypertension was induced through unilateral nephrectomy, deoxycorticosteroid asetate (DOCA) injection (20 mg/kg, twice a week) and 1 % NaCl with 0.2 % KCl by adding to drinking water in male Wistar albino rats (8 weeks old). Animals were monitored by measuring blood pressures for 12 weeks. An ERS inhibitor tauroursodeoxycolic acid (TUDCA) (150 mg/kg/day, i.p.), was administrated to animals for last four weeks. At the end of the experiment, contraction and relaxation responses of isolated thoracic aortas were recorded. “Total antioxidant capacity” (TAC) and “nitrite” levels, as an indicator of nitric oxide (NO), were measured spectrophotometrically in plasma samples.

TUDCA administration significantly decreased systolic blood pressures and improved acethylcholine-induced endothelium-dependent vasorelaxations in hypertensive rats. Sodium nitroprusside-induced endothelium-independent relaxations were reduced in hypertensive rats and reversed by TUDCA. Potassium chloride (KCl) induced vasoconstrictions were enhanced by TUDCA in the endothelium-intact aortas of hypertensive and normotensive rats. TUDCA treatment increased KCl induced vasoconstrictions in the endothelium-denuded aortas of hypertensive rats. In both hypertensive and normotensive rats TUDCA treatment enhanced phenylephrine-induced vasoconstrictions which obtained in medium with or without calcium. TUDCA treatment increased plasma NO levels. Plasma TAC levels were increased in hypertensive rats and reduced by TUDCA administration.

These results suggest that the beneficial effects of ERS inhibition on hypertension may be related to the protection of vessel functions.

Keywords: hypertension, endothelial dysfunction, endoplasmic reticulum stress, nitric oxide, tauroursodeoxycolic acid (TUDCA)

P026: Effects of LXR agonist GW3965 on vascular reactivity in DOCA-salt induced hypertension in rat

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Liver X receptors (LXRs) are nuclear receptors that involve in the regulation of cholesterol, fatty acid and glucose metabolism and inflammation process. LXR pathway has been defined as a potential target for cardiovascular disease in recent studies. LXRs are expressed and functional in vascular smooth muscle cells and important participants in the pathophysiology of hypertension. In present study, the effects of LXR agonist GW3965 on vascular reactivity in DOCA-Salt induced hypertension were examined.

Hypertension was induced through deoxycorticosteroid asetate (DOCA) injection (20 mg/kg, twice a week) and 1 % NaCl with 0.2 % KCI by adding to drinking water following the unilateral nephrectomy in male Wistar albino rats (8 weeks old). Blood pressures were measured with tail-cuff method for 6 weeks. An LXR agonist GW3965 (10 mg/kg/day, i.p.) was administered to animals for last seven days. Contraction and relaxation responses of isolated thoracic aorta were recorded. Total antioxidant capacity (TAC) and nitrite levels, as an indicator of nitric oxide, were measured spectrophotometrically in plasma samples.

GW3965 treatment significantly reduced systolic blood pressures in hypertensive rats. Acethylcholine-induced endothelium-dependent and sodium nitroprusside-induced endothelium-independent vasorelaxations were significantly decreased in hypertensive rats but not affected by GW3965. Potassium chloride (KCl) induced vasocontractions were enhanced in the endothelium-intact aortas of hypertensive rats but GW3965 did not change these contractions. KCl or phenylephrine (Phe) induced vasocontractions were reduced in the endothelium-denuded aortas of hypertensive groups and increased with GW3965 treatment. In hypertensive rats, GW3965 did not change Phe-induced vasocontractions in the Ca^{2+}-free medium. Plasma nitrite levels did not change in hypertensive group. GW3965 treatment enhanced nitrite levels in both normotensive and hypertensive rats. GW3965 increased plasma TAC levels in normotensive rats.

These results suggest that LXR agonists may exhibit a beneficial effect on impaired contractile activity of vessels in hypertension.

Keywords: hypertension, liver X receptors, GW3965

P027: Cilostazol-induced relaxation of calf cardiac vein and coronary artery during cooling

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Most of the previous studies examining the effect of cooling on smooth muscle responses have focused on the cutaneous vessels and information about deep vessels is rather limited. The aim of the present study is to evaluate the influence of cooling (to 28°C) on the vasodilatation induced by cilostazol (10^{-9}–$3x10^{-4}$M) on carbachol (10^{-6} M)-precontracted calf cardiac vein and coronary artery and the role of nitric oxide (NO) in these effects.

Ring preparations of great cardiac vein and the anterior interventricular branch of left coronary artery were mounted in 25 ml organ baths containing Krebs-Henseleit Solution, aerated with 95 % O_2 and 5 % CO_2. Preparations were contracted with carbachol, cilostazol was added to the organ bath cumulatively. In another part of the study, after the contractile responses to carbachol, the temperature was changed from 37 to 28°C and preparations were allowed to equilibrate at this temperature for 30 mins then cilostazol was added both in the absence and presence of N^G-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M). In another series of experiments, the relaxant effect of acethylcholine (ACh) was investigated in preparations precontracted with serotonin (5-HT) at 37 and 28°C. In a different series of the study, the action of sodium nitroprusside in preparations precontracted by carbachol was investigated at 37 and 28°C.

Cilostazol produced concentration-dependent relaxation of calf cardiac vein and coronary artery rings precontracted with carbachol. During cooling, the pIC_{50} values, but not the maximal responses, to cilostazol were significantly lower than at 37°C in both preparations. Cooling to 28°C in the presence of L-NAME did not modify the effect of temperature both in cardiac vein and coronary artery.

These results demonstrate that in calf cardiac vein and coronary artery cooling increased the sensitivity to cilostazol independently on NO.

Keywords: cardiac vein, cilostazol, cooling, coronary artery, nitric oxide

P028: Effect of peroxynitrite on the anticontractile activity of perivascular adipose tissue

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Perivascular adipose tissue (PVAT) releases multiple vasoactive substances and regulates vascular tone. The anticontractile effect of PVAT is impaired in mice lacking the alpha1 isoform of AMPK-activated protein kinase (AMPK$\alpha^{-}/^{-}$). Furthermore, nitrotyrosine; a marker of peroxynitrite ($ONOO^{-}$) formation in vivo is more highly expressed in the aortic PVAT of AMPK$\alpha^{-}/^{-}$ mice compared to wild-type control mice. In this study we sought to investigate whether $ONOO^{-}$ can influence the anticontractile activity of PVAT in wild-type mice.

Endothelium-denuded C57BL/6 mouse aortic rings with and without PVAT were mounted on a small artery wire myograph. In some experiments either the ring or the isolated PVAT was treated with $ONOO^{-}$ prior to contracting it with U46619. Cromakalim was then added to induce relaxation of the aortic rings.

Relaxation responses to cromakalim were significantly enhanced in the presence of PVAT (43.73±7.36% *vs* PVAT(-) 21.12±0.92%, $p<0.01$). Pre-treatment of PVAT (+) rings with $ONOO^{-}$ did not have any effect on the relaxation to cromakalim, however, transferring $ONOO^{-}$-treated PVAT to PVAT (-) rings enhanced the relaxation to cromakalim (64.45±9.21%, $p<0.0001$). Incubation of PVAT (-) rings with conditioned media from $ONOO^{-}$-treated PVAT also induced significantly more relaxation to cromakalim (68.15±9.22%, $p<0.0001$). The augmented relaxation observed following addition of $ONOO^{-}$-conditioned medium or $ONOO^{-}$-treated PVAT to PVAT (-) rings was greater than that caused by the presence of PVAT(+) alone ($p<0.001$).

Peroxynitrite increases the anticontractile activity of PVAT, likely through increased release of an anticontractile factor, but this effect is reduced when the PVAT remains attached to the vessel.

Keywords: perivascular adipose tissue, peroxynitrite, AMPK, cromakalim

P029: The transcription factor ETS-2 a possible marker of early instability in CABG patients

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In patients with cardiovascular disease (CVD), the endothelial progenitor cells (EPCs) play a key role in endothelial repair processes. It has hypothesized that Ets2 transcription factor could be involved so active in the instability of CVD/hyperlipidemia. Our main objective was to determine the degree of expression of transcription factor Ets2 and endothelial repair factors (Endoglin-CXCR4), in peripheral blood mononuclear cells and relate the data found with clinical parameters from patients undergoing coronary artery-bypass grafting (CABG). Seventy patients undergoing CABG from the cardiac surgery service were selected for the study and categorized into five CVD stages. The Expression of Ets-2, CXCR4, and endoglin were measured by Western blot. An increase was observed in the expression of the transcription factor Ets-2, in patients without CV predictor risk factor associating with early stages of CV instability (GI: 2,16 ± 1,4), and its expression was decreased in patients with longer evolution of CVD (GII: 1,16±0,8; GIII1,06±0,5; GIV:0,91±0,3; GV:0,56±0,3). A direct association was found in the expression of Ets-2 with age (p=0,04) and endoglin expression (p=0,008), and indirectly with the evolution of CVD (p=0.008) and hyperlipidemia (p=0.03). The silent of expression of Ets-2 in EPCs in culture by SiRNA, decreased the expression of endoglin (p=0.001). The transcription factor Ets-2 could be an early marker of cardiovascular instability associated with states of hyperlipidemia. Our data suggesting that a poor functionality of circulating EPCs could be associated with decreased expression of the transcription factor Ets-2 in advanced stages of cardiovascular disease.

This study is supported by FIS PI12/00590 (plan estatal I+D+I 2013-2015).

Keywords: ETS2, endothelial progenitor cells, patients undergoing coronary artery-bypass grafting

P030: Study of sitagliptin (inhibitor of dipeptidyl peptidase 4) on the proliferation and apoptosis of vascular smooth muscle cells of diabetic and non-diabetic patients

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Sitagliptin is a drug used for the treatment of the diabetes. It is a selective inhibitor of dipeptidil peptidasa 4 (DPP-IV). It has been reported that DDP-4 would lead to NFκβ activation, which is a regulator of the vascular smooth muscle cells (CMLV) proliferation. We investigated the effect of sitagliptin on the proliferation and apoptosis of CMLV of diabetic patients (D) and non-diabetics patients (ND).

The CMLV were obtained of mesenteric arteries from abdominal surgery of diabetic patients and non-diabetics patients, and cultivated with sitagliptin in increasing concentrations (1µM, 2µM, 5µM, 25µM, 50µM, 100µM). For the study of cell viability the cell count was performed to the 24h, 72h, 7-14days. Cellular proliferation was assessed by a BrdU incorporation kit. Apoptosis was measured by DNA fragmentation ELISA (n=3).

In non-diabetic patients there were a decrease in the number of cells (cells counter) at 72h in the concentrations of sitagilptin of 25, 50 and 100µM ($p<0.0001$). Morover the apoptosis increased significantly to the concentrations 5-100µM ($p<0.0001$). Cellular proliferation (BrdU incorporation) decreased gradually at all concentrations, being more pronounced at higher concentrations (50 and 100µM, $p<0.001$). In diabetic patients sitagliptin causes a significant decrease in the number of cells in all concentrations at 24hours. The apoptosis significantly increases and the proliferation diminishes significantly in all drug concentrations ($p<0.0001$).

Sitagliptin diminishes the cellular proliferation and increases apoptosis both in cells of diabetic and non-diabetic patients, being these effects more pronounced in patient diabetics.

This study is supported by FIS PI1200590 (Plan Estatal I+D+I 2013-2015).

Keywords: sitagliptin, proliferation and apoptosis of vascular smooth muscle cells, diabetic and non-diabetic patients

P031: The in vitro effects of botilinum toxin A and papaverine on radial artery grafts

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Radial artery grafts are frequently used during coronary artery bypass graft surgeries. It is important to prevent spasm of the grafts. This study aims to assess the vasodilator effect of botilinum toxin A (BTA) and papaverine on human radial artery grafts.

After institutional approval, discarded human radial artery rings were suspended in Krebs-Henseleit solution at 37^{O}C bath, bubbled with 95% O_2 - 5% CO_2. At the end of the 60 min resting period, 80mM KCl was added to the bath and control contractions were recorded. After 40min of incubation with 10^{-6} M or 10^{-8} M BTA, cumulative responses for 10^{-12}-10^{-8} M endothelin-1 (ET-1), or 10^{-9}-10^{-6} M serotonin (5HT) were recorded (0-hour). Cumulative responses were repeated at the 1st and 2nd hours. The same procedures were repeated with 10^{-4} M or 10^{-6} M papaverine. The results of the vasoconstrictors were presented as % of control KCl contraction. Additionally, maximal responses of the groups were standardized by expressing as % contraction of the vasoconstrictor agent. Two-way repeated measures of ANOVA was used to compare the contraction responses by time and groups and independent samples t-test for comparing standardized contraction responses of BTA and papaverine. $p<0.05$=significant

Significant inhibition was observed with BTA and papaverine in different concentrations of 5HT and ET-1 induced contractions. Inhibition by BTA did not show time dependent reduction, while papaverine inhibition was reduced with time. At low and high concentrations, papaverine resulted in higher inhibition than BTA in maximal 5-HT induced contractions at all times. High concentrations of both BTA and papaverine similarly inhibited ET-1 induced maximal contractions whereas low concentrations of both the inhibitors' responses were similar at 0-hour but BTA resulted in higher inhibition at the 2nd hour (Table 1).

BTA inhibits ET-1 and 5-HT induced contractions. This inhibition shows difference according to the vasoconstrictor agent and contrary to papaverine does not decrease with time.

Keywords: botilinum toxin A, papaverive, radial artery, in vitro

Table 1. Comparison of the effects of botilinum toxin A and papaverine on maximum 5-HT and ET-1 induced contractions.

Vasoconstrictor	Inhibitor agent (concentration)	0-hour	2nd hour
5HT	BTA 10^{-8}M	%87.3±14.1	%80.0±7.1
5HT	Pap 10^{-6}M	%35.2±10.1*	%51.9±6.3*
5HT	BTA 10^{-6}M♯	%67.8±7.5	%61.6±6.3
5HT	Pap 10^{-4}M	%11.3±6.1Ψ	%19.7±6.4Ψ
ET-1	BTA 10^{-8}M	%54.3±12.3	%46.5±10.4
ET-1	Pap 10^{-6}M	%61.0±14.2	%84.2±23.1*
ET-1	BTA 10^{-6}M	%46.5±10.7	%44.4±16.8
ET-1	Pap 10^{-4}M	%41.0±13.2	%67.4±23.7

*BTA: botilinum toxin A, Pap: papaverine. All groups n=6 (♯n=7). Results are % of vasoconstrictor (5HT or ET-1) elicited maximal control contractions. Mean±SD. Ψp<0.05 compared to same hour BTA 10^{-6}M, *p<0.05 compared to same hour BTA 10^{-8}M.*

P032: The effect of 5-lipoxygenase inhibition and its interaction with cyclooxygenase pathway in a rat model of myocardial infarction

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Our objective is to evaluate the effect of 5-lipoxygenase (LOX) inhibition alone or along with cyclooxygenase (COX) inhibition on inflammatory parameters in a rat model of myocardial infarction. Male Wistar rats (200-250 g) were treated with 5-LOX inhibitor zileuton (Zil; 5 mg/kg; per oral; twice daily) alone or along with the non-selective COX inhibitor indomethacin (Indo; 5 mg/kg; intraperitoneally), COX-2 inhibitor nimesulide (Nim; 5 mg/kg; subcutaneously) or COX-1 inhibitor ketorolac (Ket; 10 mg/kg; subcutaneously) for 3 days and subjected to ischemia/reperfusion (I/R). I/R was established by ligating the left anterior coronary artery for 30 minutes and then followed by reperfusion for 2 hours. Control group underwent sham operation. At the end of reperfusion, the animals were decapitated. Trunk blood was collected for the measurement of cytokines (TNF-alpha and IL-1β) and left ventricles were examined microscopically and stored for biochemical assays. Serum cytokine levels increased in I/R group in comparison to control group ($p<0.05$); however, they did not change significantly in the treatment groups. Tissue MPO activity was found to be lower in the I/R group compared to control group ($p<0.05$) and this effect continued in other groups. Tissue MDA level of the I/R group was higher in comparison to control group ($p<0.001$) and did not show significant changes in treatment groups. Increase in tissue GSH level of the I/R group in comparison to control group was reversed by Zil treatment. Increase in tissue NF-κB level in the I/R group was prevented by all treatments ($p<0.001$). Microscopic score of the left ventricle was higher in all groups compared to control group ($p<0.001$). Extent of tissue damage and inflammatory parameters except for the tissue NF-κB level did not seem to be protected by 5-LOX inhibition in rat model of myocardial infarction.

Keywords: zileuton, lipoxygenase, cyclooxygenase, myocardial infarction

P033: Expressions and functions of phosphodiesterase enzymes are different in different region of the rat heart

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Phosphodiesterase (PDE) enzymes are responsible for the breakdown of cAMP and cGMP and adjust the levels of cyclic nucleotides in heart. Alteration of PDE enzymes expression in different tissues caused conflicts between functional and clinical effects of PDE inhibitors. Therefore the aim of this study was to investigate the functional role and gene/protein expression of PDE enzymes in atrial and ventricular regions of the rat heart.

The spontenously beating right atrium and electrically stimulated left papillary muscles from Wistar albino rats were placed in organ bath. The effects of PDE1, 2, 3, 4, 5 inhibitors (Vinpocetine, EHNA, Milrinone, Rolipram Sildenafil) on functions were recorded. The expressions of PDE's in tissues as well as in enzymatically isolated atrial and ventricular cells were examined by Real Time PCR and Western blot.

Right atrial contractions have been increased with Vinpocetine while suppressed by EHNA, Milrinone, Rolipram and Sildenafil. Unlike Milrinone and Sildenafil, Vinpocetine increases the heart rate whereas EHNA and Rolipram have not any significant effect. Left papillary muscle contractions have been increased only with the Vinpocetine while other PDE inhibitors have not been the emergence of a significant effect. The mRNA and protein levels of PDE enzymes were significantly different in atrial and ventricular intact tissues or isolated cells.

PDE-1, 2, 3, 4 and 5 enzymes show functional and regional differences in the rat heart. Therefore, these differences should be taken into account in the experimental or therapeutic approaches of the heart.

Keywords: phosphodiesterases, right atrium, papillary muscle

P034: The role of Liver X Receptors on cardiac dysfunction induced by hypertension

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Liver X receptors (LXRs) regulate genes involved in lipid and glucose metabolism. Although, LXRs were expressed in cardiac tissues, their function in hypertension was not determined. In this study, the effects of LXR agonist GW3965 treatment on hypertension induced functional alterations and tissue oxidative status were examined in the rat heart.

Hypertension was induced through deoxycorticosterone acetate injection (20 mg/kg, sc, twice a week) and 1% NaCl with 0.2% KCl by adding to drinking water following the unilateral nephrectomy in male Wistar albino rats (8-weeks-old) for 6 weeks. GW3965 (10mg/kg/day) was intraperitoneally administered last one week. At the end of treatment, right atrium (RA) and left papillary muscle (LPM) were isolated and rhythmic activity and contractions were recorded. Total antioxidant capacity (TAC) of tissue was measured spectrophotometrically. Tissue 4-hydroxynonenal (4-HNE) level and plasma lipids were determined using commercial kits.

GW3965 reduced systolic blood pressure in hypertensive group. Noradrenaline-induced contractions of RA were not different in all groups, whereas heart rate was higher in hypertensive group. Noradrenaline-stimulated contraction of LPM was elevated by GW3965 in hypertensive animals. In Ca^{2+}-free medium, noradrenaline-induced contractions and rhythmicity of RA were not different in all groups. While, additional Ca^{2+}-induced contraction of RA was lower, sinus rate was elevated in hypertensive group and GW3965 treatment reversed these responses. In hypertensive animals, tissue TAC levels were higher, but GW3965 treatment did not alter. Tissue 4-HNE levels decreased in hypertensive animals but were not affected by GW3965 treatment. Plasma total cholesterol, HDL and LDL levels were higher in hypertensive animals but triglyceride levels did not change. GW3965 treatment did not affect plasma lipid levels in all groups.

These results suggest that LXR stimulation by GW3965 improved blood pressure and cardiac functions that would be a potential target therapy for hypertension.

Keywords: hypertension, cardiac dysfunction, liver X receptors, GW3965

P035: Effect of endoplasmic reticulum stress inhibition on cardiac remodelling induced by hypertension

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Hypertension leads to cardiac remodelling including impaired cardiac contractility as well as fibrosis. Various factors lead to accumulation of unfolded proteins in endoplasmic reticulum (ER), a condition referred to as ER stress (ERS). Effect of ERS inhibition on cardiac functions, expression of functional proteins and structural organization in hypertension were examined in this study.

Hypertension was induced by deoxycorticosterone acetate-salt (20mg/kg, s.c, twice a week and 1 % NaCl with 0.2 % KCl in drinking water) treatment and unilateral nephrectomy in male 8-week-old Wistar albino rats for 12 weeks. Blood pressure was measured weekly. ERS inhibitor tauroursodeoxycolic acid (TUDCA) (150mg/kg/day, ip) was given in last four weeks. At the end of treatment, right atrium (RA) and left papillary muscle (LPM) were isolated and rhythmic activity and contractions of tissues were recorded. Expressions of functional proteins were studied in ventricular tissue. In histopathologic examination, Masson's trichrome staining was used to assess the interstitial and perivascular fibrosis.

TUDCA reduced systolic blood pressure in hypertensive group. In Ca^{2+}-free medium, noradrenaline-stimulated and additional Ca^{2+}-induced contractions and rhythmic activity of cardiac tissues were similar in all groups. Ca^{2+}-mediated contractions of RA (developed tension) in Ca^{2+}-free medium were higher in hypertensive group. Effect of ryanodine on contractions and sinus rate of RA were not different in all groups, but LPM contractions were higher in hypertensive group. ERS markers GRP78 and p-PERK expression increased with hypertension and TUDCA treatment reversed this elevation. Ryanodine receptor-2 expression in cardiac tissues was similar in all groups. Interstitial and perivascular collagen deposition were increased by hypertension and TUDCA treatment prevented perivascular collagen deposition.

These findings demonstrated that hypertension caused ERS in the rat heart and inhibition of ERS by TUDCA might improve the functional and structural alterations in the cardiac tissues.

Keywords: hypertension, cardiac dysfunction, endoplasmic reticulum stress, tauroursodeoxycolic acid (TUDCA)

P036: The effect of flecainide and ranolazine on calcium transients in rat cardiomyocytes

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Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a genetic condition that results in an increased propensity for arrhythmias. It is derived from mutations in either the ryanodine receptors (RYR2) or calsequestrin, both of which are involved in intracellular calcium handling in cardiac myocytes. Currently, CPVT is generally managed by beta-blockers, although flecainide, an established class 1c anti-arrhythmic, and has recently been demonstrated as having significant clinical benefit for CPVT due to its peak in a blocking properties. Ranolazine, an anti-anginal, has also gained prominence due to its late Ina blocking properties, and therefore potentially represents a greater role in managing arrhythmias in failing hearts.

Our study aimed to determine the effect of flecainide and ranolazine on calcium transients in healthy and failing rat cardiomyocytes.

Ventricular myocytes were isolated from healthy and 16-week MI rats. Wide-field epi-fluorescence microscopy was used to image waves, and Ca^{2+} transients were recorded using Fluo-4 AM loaded cells. Cardiomyocytes were infused with 5µM of flecainide or 10µM ranolazine, and paced at 0.5 Hz. 20 mM caffeine spritz was used to assess SR Ca content.

Both flecainide and ranolazine infusion in healthy cardiomyocytes showed no significant change in calcium transient height (0.41±0.06 *vs*. 0.26±0.06, p=0.13, and 0.41±0.06 *vs.* 0.26±0.06, p= 0.13 respectively). Ranolazine infusion in failing cells also resulted in no significant reduction in transient height (0.45±0.03 vs. 0.07±0.03, p=0.25). However flecainide led to a significant transient height reduction in the failing cardiomyocytes (0.65±0.12 *vs*. 0.17±0.02, p<0.5).

Ranolazine exposure had no significant effect in reducing calcium transients in both healthy and failing cardiomyocytes. Flecainide however seemed to have a significant effect on cells isolated from failing cardiomyocytes, which indicates a greater role for it in such situations.

Keywords: anti-arrythmics, cpvt, flecainide, ranolazine

P037: The role of a reduction in calcium sensitivity in H_2S induced relaxation in bovine retinal artery

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Recently, we have shown the vasorelaxing effect of H_2S on isolated bovine retinal artery which was partially related to the activation of K_{ir} and K_v channels. Herein, we aimed to investigate the probable role of Ca^{++} related mechanisms on relaxation response of H_2S in isolated bovine retinal arteries.

Sodium hydrosulphide (NaSH) was used as the donor of H_2S. To evaluate the role of Ca^{++} related mechanisms in the vasorelaxant effect of NaSH, we performed experiments both in Ca^{2+} free and Ca^{2+} containing mediums as well as in the presence and absence of the inhibitors of Rho-Kinase, Y-27632 (10^{-5}M, 20 min) or myosin light chain kinase (MLCK), ML-9 (10^{-5}M,20 min). Thereafter, the concentration-dependent contraction responses of $CaCl_2$ (10^{-5}-10^{-2}M) were estimated in the presence and absence of NaSH ($3x10^{-3}$M, 20 min). The relaxant effect of NaHS (10^{-5}-$3x10^{-3}$M) was also determined in isolated bovine retinal arteries contracted with $CaCl_2$ (10^{-2}M).

The relaxation responses of NaHS in PGF2α ($3x10^{-5}$M) precontracted retinal arteries both in Ca^{2+} free and Ca^{2+} containing medium were found comparable. However, the presence of NaHS significantly decreased the maximum contractions to $CaCl_2$ (10^{-5}-10^{-2}M) (+NaHS E_{max}: 0.20±0.05mN/mm vs control E_{max}: 0.45±0.05mN/mm, n=11; $p<0.05$). Besides, a relaxation response was observed to NaHS in $CaCl_2$ (10^{-2}M) contracted arteries. On the other hand, a significant difference was not observed in relaxation response of NaHS either in the presence of Rho kinase or MLCK inhibitor (+Y-27632; E_{max}: 70.56±2.86%, vs control; E_{max}: 60.36±7.29%, n=7; $p>0.05$; and +ML-9; E_{max}: 62.75±9.05%, *vs* control; 66.46±5.85%, n=10; $p>0.05$).

Our study suggested that H_2S induced relaxation in does not seem to be related to the presence of extracellular calcium or the calcium sensitization mechanism, RhoA/Rho kinase pathway. However, the relaxing effect of H_2S in bovine retinal arteries may be produced partially via reducing the calcium sensitivity of contractile proteins.

Keywords: hydrogen sulfide, retinal artery, vasorelaxation, calcium sensitization, contractile proteins

P038: Levosimendan confers protection against doxorubicin induced acute cardiotoxicity through induction of anti-oxidant and anti-apoptotic mechanisms

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Doxorubicin (DXR) is a antineoplastic agent accompanied by a dose-dependent induction of cardiomyopathy. Levosimendan (Levo) is a clinically used inotropic and vasodilator drug indicated for treatment of heart failure. We sought to investigate the effect of Levo on acute doxorubicin-induced cardiotoxicity and the underlying signaling cascade. In vitro: PC3 cell line was treated with DXR (10-200 nM), Levo (1-50 uM) as well as with a combination of the two compounds in order to investigate the possible inhibition of DXR antitumor activity by Levo. In vivo: Male rats were randomized into the following groups: 1) Control group, 2) DXR group (DXR 20mg/kg) 3) DXR+LEVOA group (DXR 20mg/kg + LEVO 12mg/kg) 4) DXR+LEVOB group (DXR 20mg/kg + LEVO 24mg/kg). On the third day of administration, echocardiography was performed and rats were sacrificed. The hearts were excised for histological evaluation, determination of nitroxidative stress biomarkers (MDA, PCs, Nitrotyrosine), MMP2, TGF-β1 mRNA levels and expression of iNOS, phospho-Akt/Akt, phospho-ERKs, MnSOD, IL-6 and NOX-4. Concerning our findings; in vitro: Levo amplified the antitumor effect of DXR. In vivo: DXR reduced the fractional shortening (FS) ($p < 0.001$ vs Control), while no change was observed by Levo administration. Myocardium exhibited morphological changes only in DXR group including cytoplasmic vacuolization (CV) and infiltrations of immune cells. Levo reduced oxidative stresss biomarkers and IL-6 dose-dependently (DXR+LEVOB vs DXR, $p<0.05$). Moreover, at the higher dose it decreased mRNA levels of MMP2 and NOX-4; it reduced the expression of iNOS in both doses. An increase of MnSOD expression and phosphorylation of Akt was observed in LEVOB group. No changes were observed in the levels of TGF-β1 and in the phosphorylation of ERKs. Levosimendan exhibited structural and biochemical beneficial effects on the myocardium by regulation of oxidative stress and activation of antiapoptotic mechanisms, whilst no effects on FS are observed.

Keywords: doxorubicin, levosimendan, oxidative stresss, acute cardiotoxicity

P039: Anti-atherosclerotic and cardioprotective properties of *Crocus sativus L.* aqueous extract in ApoE-/- deficient mice

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Saffron is an antioxidant herbal derivative with potential anti-atherosclerotic and cardioprotective properties. We investigated the effects of a standardized saffron aqueous extract (SFE), on the development and stability of atheroma in diabetic diet apolipoprotein-E deficient (Apo-E-/-) mice and its cardioprotective properties against ischemia/reperfusion injury. 40 male Apo-E-/- mice receiving high-fat diet for 12 weeks were randomized to receive SFE 30 mg/kg (Saffron Group 1, SF1, n=10), 60mg/kg (SF2, n=10), 90 mg/kg (SF3, n=10) or equal volume of water for injection (WFI) (control group, COG, n=10) for 4 weeks. Afterwards, mice underwent glucose tolerance test and respective area under concentration *vs* time curve (AUC), as well as blood lipid and glucose profiles were assayed. In aortic tissue mean plaque area (MPA), relative plaque elastin and collagen content, and fibrous cap (FC) thickness were measured. Moreover, male Apo-E-/- mice following normal-diet were subjected to 30 min ischemia followed by 3 hrs reperfusion, with the following interventions: 1) COG (n=6): WFI, 2) SFE (n=6): orally SFE (60 mg/kg, 4-weeks). Ischemic area/area at risk ratio was measured and ischemic myocardial tissue was collected up to 10th min of reperfusion (n=6) for assessment of p-eNOS, eNOS, p-Akt, Akt, p-p42/p44, p42/p44, iNOS, IL-6 and MnSOD expression. MPA was smaller in SF groups vs COG. Atherosclerotic lesions from SFE treated mice showed increased collagen ($p<0.001$) and elastin ($p<0.001$) content, thicker FC ($p=0.023$) and fewer Internal Elastic Lamina ruptures per mm of arterial girth ($p<0.001$) vs COG. Finally, all doses of SFE ameliorated glucose levels and AUC. SFE reduced infarct size vs COG (16.8±1.1 vs 45.3±2.0, $p<0.0001$), while eNOS, Akt and p-44/p-42 phosphorylation, upregulation of MnSOD and reduced IL-6 expression was observed in the same group. SFE exerted anti-diabetic, anti-atherosclerotic and cardioprotective effects and promoted plaque stability in Apo-E-/- mice in a dose-dependent manner.

Keywords: Apo-E (-/-) mice, atherosclerosis, oxidative stresss, ischemia-reperfusion injury

P040: Timolol improves vascular dysfunction via inhibition of oxidative stresss in DOCA-salt hypertensive rats

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Oxidative stress plays an important role in the pathogenesis of many cardiovascular diseases including hypertension. In experimental studies, the interventions lowering oxidative stresss seems to be beneficial to prevent hypertension.
Systhemic usage of timolol, nonselective beta-blocker, was reported to prevent the structural, functional and electrophysiological defects occuring in the heart from diabetic and aging rats. These beneficial effects of timolol were associated with its antioxidant properties. Therefore, the aim of this study was to examine the effects of timolol on blood pressure and vascular system related to its possible antioxidant properties in the deoxycorticosterone acetate (DOCA)-salt hypertensive rats.
Left uninefrectomized male Sprague Dawley rats were divided into three groups including Control (n=6), DOCA-salt (n=6, 40mg/kg/week DOCA,sc), DOCA-Tim (n=6, 5mg/kg/d timolol,sc). SBP and heart rate were measured weekly by tail-cuff method. After 8 weeks, thoracic aortas were removed. Cumulative concentration-response curves were performed for phenylephrine, acetylcholine and sodiumnitroprusside in the presence of submaximal contraction to phenylephrine. Oxidative stress was evaluated by measuring plasma 8-isoprostan, superoxide dismutase (SOD) and catalase (CAT). Data were analysed using One-way ANOVA followed by Post Hoc test.
In consistant with decreased oxidative stresss, timolol treatment significantly lowered DOCA-salt-induced hypertension. Timolol treatment also resulted in a marked negative chronotropic effect (Table 1).
Thoracic aortic rings showed unaltered maximal responses to phenylephrine in all groups (Fig.1A). Timolol treatment significantly improved both acetylcholine- and sodiumnitroprusside-induced relaxations impaired in DOCA-salt group (Fig.1B-C).
These results show that the beneficial effects of timolol on SBP and vascular function in DOCA-salt hypertensive rats may be releated with decreased oxidative stresss level. However it should not be ruled out the possibility of its negative chronotropic effect in lowering blood pressure.
Keywords: deoxycorticosterone asetate, timolol, hypertension, oxidative stresss, aorta

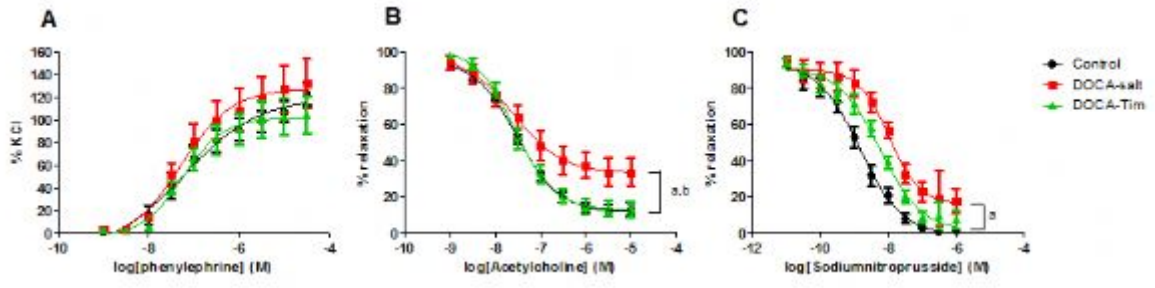

Figure 1. The concentration-response curve to phenyleprine(A), acetylcholine(B) and sodiumnitroprusside(C) in thoracic aortas from Control, DOCA-salt and DOCA-Tim rats. p<0.05 a-significantly different from control group. b- significantly different from DOCA-salt group.

Table 1. The changing on Body weight, SBP, Heart rate and Plasma 8-isoprostane, SOD and CAT levels in Control, DOCA-salt and DOCA-Tim groups. p<0.05 a-significantly different from control group. b-significantly different from DOCA-salt group.

	Control	**DOCA-salt**	**DOCA-Tim**
Body weight (g)	368±6	263±23 [a]	309±9
Systolic BP (mmHg)	120±2	174±4 [a]	123±5 [b]
Heart rate (bpm)	372±7	397±13	324±10 [a,b]
8-isoprostane (pg/ml)	89±9	157±15 [a]	106±13 [b]
SOD (ng/ml)	3.6±0.2	2.9±0.1 [a]	3.8±0.2 [b]
CAT (ng/ml)	52.7±3.0	52.6±3.4	50.8±3.6

P041: Pannexin channels do not influence endothelial function in porcine coronary arteries

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Pannexins are a family of proteins that form channels in cell membranes to allow passage of ATP as an intercellular messenger. It has recently been suggested that pannexins contribute to endothelium-dependent regulation of arterial tone, by facilitating endothelium-dependent hyperpolarizing (EDH) responses. This study examined this possibility in porcine coronary arteries (PCA).

PCAs were obtained from a local abattoir and prepared for isometric tension recording. After raising tone with U46619, responses to the endothelium-dependent vasorelaxant, bradykinin (BK), were assessed in the absence and presence of the pannexin inhibitors, carbenoxolone (100 µM), mefloquine (20 µM) or probenecid (1 mM). Similar experiments were carried out in the presence of the nitric oxide synthase inhibitor L-NAME (100 µM) and cyclooxygenase inhibitor, indomethacin (10 µM). $P<0.05$ was considered significant.

Under control conditions, BK produced a concentration-dependent vasorelaxation ($-\log EC_{50}$ 7.79±0.49; R_{max}=82±23%, n=4). The response to BK was increased in the presence of carbenoxolone ($-\log EC_{50}$=8.31±0.11; R_{max}=102±5%, n=4), unaffected by probenecid ($-\log EC_{50}$=8.34±0.30; R_{max}= 69±9%, n=4) but reduced by mefloquine ($-\log EC_{50}$=7.05±0.30; R_{max}=63±22%, n=4). BK caused a concentration-dependent relaxation in preparations exposed to L-NAME and indomethacin, indicating an EDH response ($-\log EC_{50}$=7.58±0.27; R_{max}=53±9%, n=4). This response was not affected by either carbenoxolone ($-\log EC_{50}$=7.12±1.63; R_{max}=41±29%, n=4) or probenecid ($-\log EC_{50}$=7.46±0.70; R_{max}=64±22%, n=4) but reduced by mefloquine ($-\log EC_{50}$=6.87±2.10; R_{max}=29±15%, n=4).

Thus, BK induces vasorelaxation in the PCA, in part, by causing an EDH response. The use of three different pannexin inhibitors produced conflicting effects. Only mefloquine inhibited EDH responses to BK; probenecid and carbenoxolone had no effect. Thus we cannot conclude that pannexins are involved in mediating EDH responses in the PCA.

Keywords: pannexin, channels, endothelial

P042: Cyclophilin-D-independent effects of H_2S donors in cardioprotection

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We have previously shown that H_2S salts (NaHS and Na_2S), GYY4137 (a slowly releasing agent), thiovaline (a donor with intermediate H_2S releasing rate) and AP39 (a mitochondrial-targeted H_2S donor) all reduce infarct after LAD ligation and we have observed that the effects of Na_2S, but not those of AP39, are associated with enhanced eNOS-phosphorylation on Ser1176. In line with these observations, herein we report that administration of the endothelial nitric oxide synthase (eNOS) inhibitor N-nitro-L-arginine-methyl-ester (L-NAME) reversed the infarct-limiting effects of Na_2S in ischemia-reperfusion (I/R) injury (infarct/risk area 17.8+ 1.8% for Na_2S vs 32.9+ 2.4% for L-NAME+Na_2S), while the cardioprotective effects of AP39, GYY-4137 and thiovaline were not affected by NOS inhibition. Treatment of animals with this Na_2S lead to enhanced vasodilator-stimulated phosphoprotein (VASP) phosphorylation, a marker of cGMP-dependent protein kinase (PKG) activation. The protective effect of Na_2S was limited by the PKG inhibitor DT-2 (29.9+1.8% for DT-2+Na_2S). In contrast, we did not observe VASP phosphorylation on Ser239 after AP39, nor was the infarct-limiting effect of AP39 affected by PKG inhibition (16.5+2.3% vs 14.4+3.7% for AP39 and DT-2+AP39). Opening of the mitochondrial permeability transition pore (mPTP) is considered to be a major cause of cell death in ischemia-reperfusion injury of the heart. Therefore, we tested the effects of Na_2S and AP39 in mice lacking cyclophilin-D (CyD), a key regulator of mPTP. CyD knockout mice exhibited smaller infarcts after LAD ligation compared to wild-type mice (39.2+1.8% vs 17.7+2.2% for wild-type and CyD KO, respectively). Administration of both Na_2S and AP39 further reduced infarct size in CyD KO animals (8.9+3.1% vs 10.4+3.6% for Na_2S and AP39). We conclude that Na_2S limits infarct size in a NO/cGMP/PKG-dependent pathway, while AP39 exhibits NO-independent cardioprotection; both agents salvage the myocardium by acting at a site different than CyD.

Keywords: cardioprotection, H_2S, infarct

P043: Investigation of the effect of anacardic acid on ICAM-1, VCAM-1 and NF-κB in human saphenous vein graft endothelial culture induced with TNF-alpha

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Atherosclerosis is a chronic inflammatory disease, with cellular and humoral immune responses. The cells going through the atherosclerotic process secrete cytokines and they also have the ability of being activated by the cytokines. It is demonstrated that inflammation generates accumulation of the monocytes and/or lymphocytes as a result of the expression of adhesion molecules, cytokines and chemokines after activation of the endothelium, which consequently results in infiltration of the subendothelium.

Anacardic acid (AA) is a bioactive phytochemical found in the nutshell of the Anacardium occidentale. AA may provide better protection against several pathophysiological disorders, such as oxidative damage or cancer. Several studies on NF-κB activation pathways by using AA and on apoptosis and inflammation mechanisms of NF-κB regulatory gene products suggest that AA suppresses NF-κB, which is activated by the growth factors and inflammatory responses.

In our study, we aimed to investigate the relation between AA and ICAM-1, VCAM-1 and NF-κB, which are associated with atherosclerotic process and with formation of atheroma plaque, by using the inflammation model of human saphenous vein endothelial culture constituted by TNF-alpha stimulation.

In our study, the acute effect of the AA on adhesion molecules and on NF-κB has been determined by applying TNF-alpha on endothelial cells after acute application of AA. Afterwards, changes in mRNA levels of ICAM-1, VCAM-1 and NF-κB genes have been determined by using qRT-PCR method. Double immunofluorescence procedure has been applied to NF-κB and to ICAM-1 proteins. The cytotocixity test has been applied to all groups. As a consequence of our study, it is considered that AA can be effective on prevention of the development of saphenous vein graft disease and on its treatment by altering the expression of adhesion molecules through NF-κB pathway.

Keywords: atherosclerosis, saphenous vein, anacardic acid, inflammation

P044: The effects of pitavastatin on nuclear factor-kappa B (NF-κB) and adhesion molecules in human saphenous vein graft endothelial culture

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We aimed to study pitavastatin's effects on Nuclear Factor-kappa B (NF-κB) and adhesion molecules in human saphenous vein graft endothelial culture indicating its pleotropic properties.

Low-dose (0.1 µM/L) and high-dose (1 µM/L) pitavastatin calcium were administered as a frontline therapy in human saphenous endothelial cell culture, followed by induction of inflammation by TNF-alpha and determination of mRNA level alterations of VCAM-1, ICAM-1, and NF-κB genes of endothelial cells using the qRT-PCR method. Additionally, immunofluorescence method was used to show the expression of NF-κB and ICAM-1. Finally, LDH levels were determined by the ELISA method to quantify cytotoxicity.

ICAM-1 mRNA expression in the low-dose pitavastatin+TNF-alpha group was significantly higher than that in the TNF-alpha group and significantly lower than that in the high-dose pitavastatin+TNF-alpha group (for all comparisons, P=0.001). VCAM-1 mRNA expression in the low-dose pitavastatin+TNF-alpha group was higher than those of the TNF-alpha and high-dose pitavastatin+TNF-alpha groups (for all comparisons, P = 0.001). VCAM-1 mRNA expression was significantly lower in the high-dose pitavastatin+TNF-alpha group than the TNF-alpha group. The low-dose pitavastatin+TNF-alpha group had a similar NF-κB mRNA expression with TNF-alpha and high-dose pitavastatin+TNF-alpha groups.

Pitavastatin increases ICAM-1 mRNA expression in saphenous vein endothelial cells. However, it increases VCAM-1 in low dose but decreases it in high dose. Furthermore, the effect of pitavastatin on adhesion molecules appears independent of NF-κB. Novel studies are needed in this field.

Keywords: pitavastatin, saphenous vein, nuclear factor kappa B, pleiotropic effect, inflammation

P045: Role of MMP-2 on vascular reactivity changes in early atherosclerosis

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Matrix metalloproteinase 2 (MMP-2) that degrades extracellular matrix plays a crucial role in physiological and pathological processes. The decreased contraction in collar-induced atherosclerosis is attributed to transformation of phenotype of the vascular smooth muscle cell to the synthetic form with increased MMP-2 expressions. We investigated the effects of selective MMP-2 inhibition by ARP-100 on vascular smooth muscle contractions, nitric oxide dependent and independent relaxations in the collar-induced atherosclerosis as a vascular dysfunction model, which accompanies to the increased MMP-2 expression.

Perivascular silicon collar was placed around carotid arteries of rabbits for 14 day to induce early atherosclerosis. Intimal thickening was demonstrated by haematoxylin/eosin staining. Isolated organ bath experiments were performed to investigate vascular reactivity changes to relaxation to acetylcholine and nitroglycerin as well as contraction to phenylephrine, serotonin and potassium chloride.

A significant intimal thickening was observed on collared arteries. The collar significantly decreased the contractions induced by potassium chloride, phenylephrine and serotonin. ARP-110 (48 nM, 12 hour) reversed the decreased contractions in collared arteries indicating the specific role of MMP-2 on diminished contractile responses in the collar model. The collar induced an increase in acetylcholine relaxation in carotid arteries pre-contracted with potassium chloride; in another terms augmented nitric oxide derived relaxation. However, ARP-100 (48 nM, 1 or 12 hour) did not change nitroglycerin or acetylcholine relaxations.

We suggest that MMP-2 has a role in vascular hypo-contractility in the collar model at least partially and this model could be useful to investigate new therapy approaches in pathological conditions with vascular hypo-contractility

This study is supported by 10ECZ007, TUBITAK 109s453 and Pharmaceutical Research Center; FABAL.

Keywords: early atherosclerosis, MMP-2, vascular reactivity, collar, hypo-contractility

P046: Taurine relaxes human radial artery in vitro

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Taurine is a sulfur-containing amino acid-like endogenous compound found in substantial amounts in mammalian tissues. The present study was designed to observe the effects of taurine on the contractions induced by depolarization and serotonin (5-hydroxytriptamine, 5-HT) in human radial artery (RA) in vitro and to get an inside into its mechanism(s).

RA rings were suspended in isolated organ baths and tension was recorded isometrically. First, a precontraction was achieved by adding potassium chloride (KCl, 45 mM) or 5-HT (30 µM) to organ baths. When the precontractions were stable, taurine (20, 40, 80 mM) was added cumulatively to the baths. Antagonistic effect of taurine (20, 40, 80 mM) on calcium chloride ($CaCl_2$, 10 µM to 10 mM) –induced contractions was also investigated. Taurine -induced relaxations were also tested in the presence of the large conductance Ca^{2+}-activated K^+ channel inhibitor tetraethylammonium (TEA, 1 mM), ATP-sensitive K^+ channel inhibitor glibenclamide (GLI, 10 µM) and the voltage-sensitive K^+ channel inhibitor 4-aminopyridine (4-AP, 1 mM).

Preincubation with taurine (20, 40, 80 mM) did not affect the basal tone but inhibited the contraction induced by 5-HT and KCl in RA. $CaCl_2$–induced contractions were significintly inhibited in the presence of taurine (20, 40, 80 mM) ($p<0.05$). The relaxation to taurine in RA was not affected by GLI and 4-AP. But, TEA inhibited taurine-induced relaxations significantly ($p<0.05$). Comparison among multiple groups was made by using a one-way ANOVA followed by Scheffe's post hoc procedure to determine significant differences among the means of the data groups.

Present experiments show that taurine relaxes contracted RA and inhibits the 5-HT and KCl –induced contractions, and suggest that a mechanism related to activation of large conductance Ca^{2+} -activated K^+channels may be involved in the action of taurine.

Keywords: taurine, radial artery, vasodilation, potassium channel

P047: Modulation of vascular tone by omega-3 polyunsaturated fatty acids in human saphenous vein

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Dietary intake of omega-3 polyunsaturated fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has been reported to have health benefits, ensuing studies have revealed their cardio-protective, anti-inflammatory and anti-carcinogenic properties. However, little is known about the effect of omega-3 polyunsatutared fatty acids on the regulation of vascular tone. Therefore, in this study we aimed to evaluate the effect of EPA and DHA on human bypass graft vessel, saphenous vein (SV) under normal or inflammatory conditions.

Isolated human SV was cultured (18h) in the presence (Inflammation) or absence (Normal) of both interleukin-1beta (IL-1β) and lipopolysaccharide (LPS). Subsequently, vascular preparations were set up in organ bath system and contracted with potassium chloride (KCl, 40mM). Two concentration–response curves of noradrenaline (NA) separated with an incubation period of 40 min without (Control) or with EPA or DHA (100 µM) were established. The maximal effect (E_{max}) expressed as % of KCl (40mM) contraction and sensitivity (pEC_{50}) of the vessels to NA were calculated from each concentration-response curve and expressed as –log M.

In SV preparations under normal conditions, incubation with EPA or DHA significantly decreased vascular reactivity to NA (Control: E_{max}=156.7±9.2%, EPA: E_{max}=115.1±16%, $p<0.05$; DHA: E_{max}=120.0±3.2%, $p<0.05$, n= 3-5) and pEC_{50} (Control: pEC_{50}=6.9±0.18, EPA: pEC_{50}=6.5±0.08, DHA: pEC_{50}=6.5±0.04, $p<0.05$). Under inflammatory conditions, the maximum contraction induced by NA was attenuated and DHA or EPA pretreatment significantly decreased vascular reactivity induced by NA (+Inflammation, Control: E_{max}=128.2±5.8%, EPA: E_{max}=98.7±9.3, $P<0.01$; DHA: E_{max}=102.6±13.2%, $p<0.01$, n=3-5) and pEC_{50} (Control: pEC_{50}=6.6±0.15, EPA: pEC_{50}=6.3±0.09, DHA: pEC_{50}=6.3±0.05, $p<0.01$).

Our results showed that DHA and EPA decreased vascular tone induced by NA in human SV under normal and inflammatory conditions. These results suggest that omega-3 polyunsaturated fatty acids including EPA and DHA might constitute therapeutic target to prevent graft vasospasm.

Keywords: omega-3 polyunsaturated fatty acids, human saphenous vein, vascular tone, inflammation

P048: The assessment of the relaxant effect of S-nitrosoglutathione on isolated human saphenous vein

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S-nitrosothiols (RSNOs) are thought to represent the circulating reservoir of nitric oxide (NO). S-nitrosoglutathione (GSNO) is an endogenous S-nitrosothiol which suggested to be a potent vasodilator with a prolonged relaxant effect compared to the current NO donors that clinically used. There are limited studies about its vascular effects on human vessels while no data is available on its mechanism of action. In this study, we aimed to investigate the acute effect of GSNO on human saphenous vein rings as well as the possible underlying mechanisms.

Isolated human saphenous veins obtained from coronary artery bypass surgery, were mounted in an organ bath system, aerated with %5CO_2 + %95^{o}2 at 37^{o}C with a resting tension of 2g. The effect of GSNO (10^{-8}-10^{-4}M) were studied in a concentration-dependent manner on rings precontracted submaximally with phenylephrine ($3x10^{-5}$M). In order to analyse its mechanism of action, the effects of GSNO were studied in the absence and presence of NO synthase inhibitor, L-NAME (10^{-4}M, 30min), soluble guanylyl cyclase (sGC) inhibitor, ODQ (10^{-5}M, 30min) or a selective inhibitor of ATP-sensitive K^+ channels (K_{ATP}), glibenclamide (10^{-5}M, 30min).

GSNO produced concentration-dependent relaxant effects on precontracted human saphenous veins (E_{max}: 102,40±1,37%). The prominent relaxant influence of GSNO was not altered in the presence of the inhibitor of NO synthase or K_{ATP} channels. While, a significant decrease was observed with ODQ (ODQ-E_{max}: 43,73±8,61%; Control-E_{max}:108,4±4,76%, $p<0.001$, n=5)

Our results indicate that acute relaxant effects of GSNO in isolated human saphenous vein were neither mediated by K_{ATP} channel activation nor endogenous NO. Whereas, the activation of sGC pathway is likely be involved in this response. A better understanding of the mechanism regulating the vasorelaxant effect of GSNO and its possible role as a new antispasmodic agent for bypass graft spasms will provide us new therapeutic opportunities.

Keywords: S-nitrosoglutathione, nitric oxide, coronary artery bypass graft, human saphenous vein

P049: The effect of sirolimus and everolimus on human saphenous vein

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Studies showing the direct effects of the agents used in drug-eluting stents (DES) on vascular smooth muscle and endothelial function are inadequate. In addition, contradictory results are noteworthy in those experimental studies. In this study, we aimed to investigate the direct effects of these agents (sirolimus and everolimus) involved in drug-eluting stents on a coronary bypass graft material, namely human saphenous vein and the possible mechanisms mediating their effects.

Isolated human saphenous veins obtained from coronary artery bypass surgery, were mounted in an organ bath system, aerated with %5 CO_2+%95 O_2 at 37^0C with a resting tension of 2g. After an equlibrium period, vessel segments were standardized with KCl (40 mM) and then endothelial functions were tested by acetylcholine (10^{-5}-10^{-4} M). Thereafter, the direct effects of everolimus (10^{-8}-10^{-5}M) and sirolimus (10^{-8}-10^{-5}M) were studied in a concentration-dependent manner on rings precontracted submaximally with phenylephrine (10^{-5}M). In parallel, the effect of dimethyl sulfoxide (DMSO) (<0,1%), the solvent of both sirolimus and everolimus was also studied. At the end of each experiment, smooth muscle relaxation capacity of the vessels was tested with sodium nitroprusside (SNP, 10^{-4} M).

Sirolimus and everolimus produced modest relaxations on human saphenous veins (E_{max}: for sirolimus= 22,43%±4,45 and for everolimus= 29,47%±5,06, n=5). However, this effect was not found significantly ($p>0.05$) different from control (DMSO) group.

In contrast to previously published articles showing the acute relaxant effect of sirolimus on vascular tissues, our findings strongly suggest that sirolimus and everolimus do not have prominent direct relaxant effects on isolated human saphenous veins. Further studies which aimed to evaluate the influence of pre-treatment with sirolimus and everolimus on the vascular smooth muscle reactivity and endothelial function of human saphenous vein will possibly clarify their impact on the graft vessel tone.

This study is supported by Istanbul University Scientific Research Project No: 24947.
Keywords: coronary bypass graft, drug eluting stent, everolimus, human saphenous vein, sirolimus

P050: Trimetazidine prevents left ventricular remodeling in rats with chronic alcohol-induced cardiomyopathy

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Alcoholic cardiomyopathy (ACM) refers to a group of specific dilated cardiomyopathy. The aim of this study was to develop a translational model of ACM in rats and to determine whether pharmacologic inhibition of 3-ketoacyl-coenzyme A thiolase (3-KAT), which catalyzes the final step of fatty acid oxidation could improve alcohol-induced cardiomyopathy.

Adult male albino rats were given 10% ethanol as the only source of fluid and ad libitum access to food during 24 weeks. Echocardiographic (ECG), morphological and electrophysiological tests were applied to assess ACM. Further a 4-week treatment with 3-KAT inhibitor trimetazidine was investigated in ethanol-deprived rats.

ECG revealed decrease in fractional shortening (FS, 43%) and ejection fraction (EF, 28%) and increase in end-systolic (ESD, 95%) and end-diastolic (EDD, 31%) dimensions in ethanol-experienced rats in comparison with ethanol-naïve rats of the same age, indicating heart failure and cardiac remodeling. The increase of the left and right ventricle (197% and 65% respectively) squares and presence of fatty myocardial degeneration contributed to the establishment of and ACM model system. The ventricle electrical fibrillation threshold was reduced in the ethanol-experienced rats (43%). Trimetazidine after 28 days i.p. administration at cardioprotective dose restored the pathophysiological changes induced by long-term ethanol consumption, increasing FS ($p<0,0001$) and EF ($p<0,0001$), decreasing ESD ($p<0.05$) and square of left ventricle ($p<0,0001$) in ethanol-experienced rats as compared with control group.

After 24 weeks of alcohol intake dilated heart failure, fatty myocardial degeneration and declined electrical stability of the myocardium were observed in adult rats similar to those reported in humans with ACM. Therefore, targeting cardiac fatty acid oxidation may be a novel therapeutic approach to alleviate the growing burden of ACM.

Keywords: alcoholic cardiomyopathy, rats, ECG, fatty myocardial degeneration, trimetazidine

P051: Vasorelaxant effect of arctigenin in human saphenous vein

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Arctigenin (ATG) is a phenylpropanoid dibenzylbutyrolactone lignan with antioxidant, antiinflammatory, bronchodilatatory, antidiabetic, anti-carcinogenic and cardioprotective properties. However, there is little available information concerning pharmacological activities of ATG in the direct vascular activity. Therefore, in this study we aimed to evaluate the effect of ATG on human bypass graft vessel, saphenous vein (SV) under normal or inflammatory conditions. Isolated human SV was cultured (18h) in the presence (Inflammation) or absence (Normal) of both interleukin-1beta (IL-1β) and lipopolysaccharide (LPS). Subsequently, vascular preparations were precontracted with norepinephrine (NE) 1 µM, and when the plateau were reached ATG (0.1-100 µM) were added in cumulative manner to the baths. To determine involvement of ATG induced vasorelaxation previously, these preparations were incubated separately for 20 minutes with different inhibitors of; nitric oxide synthase, L-NOARG (100 µM), soluble guanylyl cyclase, 1H-

[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 10 µM), cyclooxygenase, indomethacin (10 µM), ATP-sensitive K^+ channel, glibenclamide (GLI, 10 µM), voltage sensitive K^+ channel, 4-aminopyridine (4AP, 1mM) and inward rectifier K^+channel, barium chloride ($BaCl_2$, 100 µM). The effects of ATG were normalized (%) with respect to the initial precontraction. ATG have shown concentration dependent relaxation (-Inflammation, ATG: E_{max} 100±1.9%; +Inflammation, ATG: E_{max}=104±1.2%, n=7) and pEC_{50} (ATG: pEC_{50}=4.62±0.13, ATG: pEC_{50}=5.17±0.04, P<0.001). The relaxation of SV was not affected by GLI, 4AP, $BaCl_2$, Indomethacin, L-NOARG and ODQ.

Our results showed that ATG exhibits relaxant response induced by NE in human SV under normal and inflammatory conditions. These results suggest that ATG might constitute therapeutic target to prevent graft vasospasm.

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Keywords: arctigenin, human saphenous vein, vascular tone, inflammation

Vascular Endothelium

P052: Increased Nox5 expression and RhoA/p38MAPK/NFκB activation are involved in cigarette smoke-induced functional arterial CXCL16 expression

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Cardiovascular disease (CVD) is a major co-morbidity in chronic obstructive pulmonary disease (COPD), however the mechanism by which it is developed are poorly understood. Chemokine synthesis and expression by endothelial cells is likely an important process underlying cell recruitment within the cardiovascular system in airway inflammatory diseases caused by cigarette smoke (CS). Therefore, the potential link between CXCL16 and CS-induced endothelial dysfunction were investigated. Flow cytometry and immunofluorescence were used to determine CXCL16 expression on human umbilical artery endothelial cells (HUAEC). A flow chamber assay was employed to measure leukocyte arrest under dynamic conditions. Intravital microscopy was used to evaluate arteriolar leukocyte recruitment in animals exposed for 3 days to CS. CS aqueous extract (CSE) at 1% induced CXCL16 expression on the arterial endothelium and its neutralization resulted in a significant inhibition of CSE-induced mononuclear leukocyte-HUAEC interactions (62%). CSE-induced CXCL16 expression was found to be dependent on Nox5 expression and subsequent RhoA/p38-MAPK/NFκB activation. In vivo, mice CS exposure for 3 days provoked a mild inflammatory response in the lung and enhanced CXCL16 expression in the cremasteric arterioles, resulting in leukocyte-arteriolar endothelial cell adhesion, which was significantly reduced in animals with a nonfunctional CXCR6 receptor. CXCL16/CXCR6 axis blockade might be a new therapeutic target in the prevention and treatment of COPD-associated CVD.

Keywords: cigarette smoke, signalling pathways, chemokines, endothelium, mononuclear cells

P053: Protective effects of PPARβ/δ activation on endothelial dysfunction induced by plasma from systemic lupus erythematosus patients in human endothelial cells

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We tested whether plasma from of systemic lupus erythematosus (SLE) patients alters human endothelial cells function and whether GW0742, a peroxisome proliferator activator receptor beta/delta (PPARβ/δ) agonist, ameliorates endothelial dysfunction.

12 nonpregnant women with SLE ≥ 18 years old participated in the study. In addition, a control group (n = 5) matched for sex and age was recruited. Cytokines and double-stranded DNA autoantibodies (anti-dsDNA) levels were tested in plasma samples. Endothelial cells, isolated from human umbilical cord veins, (HUVECs) were used to measure nitric oxide (NO), and reactive oxygen species (ROS) production, and NADPH oxidase activity, after 24 h of incubation in medium 199 plus 10% plasma, in the absence or in the presence of GW0742 and/or the PPARβ/δ antagonist GSK0660. Statistical analyses were performed using Graph Pad Prism 5 software.

Interferon-γ, interleukin-6, and interleukin-12 levels were significantly ($P < 0.05$) increased in plasma from SLE patients with active nephritis (AN) (n = 6), as compared to both SLE patients with inactive nephritis (IN) (n=6), and control group. The NO production stimulated by the calcium ionophore A23187, was significantly reduced (≈ 25%, $P < 0.05$) in HUVECs incubated with plasma from AN-SLE patients as compared with control group. Interestingly, plasma from IN-SLE patients did not modify A23187-stimulated NO production. In addition, increased ROS production and NADPH oxidase activity were found in HUVECs incubated with plasma from AN-SLE, which were suppressed by the NADPH oxidase inhibitor apocynin. GW0742 incubation restored the impaired NO production and the increased ROS levels induced by plasma from AN-SLE patients. These protective effects were abolished by GSK0660.

PPARβ/δ activation may be an important target to control endothelial dysfunction in SLE patients.

Keywords: systemic lupus erythematosus, PPARβ/δ, HUVECs, nitric oxide, reactive oxygen species

P054: Effect of adipokines visfatin and IL (interleukin)-1β in the vascular damage in mice mesenteric microvessels

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Vascular aging is associated with high circulating levels of pro-inflammatory adipokines, like IL (interleukin)-1β or visfatin, which is a compound with enzymatic activity nicotinamide phosphoribosyltransferase (Nampt). These adipokines are over-secreted by adipose tissue of patients with metabolic diseases such as obesity and type 2 diabetes mellitus, leading to functional and structural changes associated to vascular complications. This study aimed to determine *in vivo* whether these adipokines may be directly related to vascular damage.

Osmotic mini-pumps were implanted subcutaneously for seven days in C57/BL6 three-month old male mice, infusing visfatin and/or APO866 (specific inhibitor of Nampt), IL-1β or vehicle. After sacrifice, the vascular reactivity of mesenteric microvessels was studied using a wire myograph system. The vascular segments were contracted with NA (noradrenaline, 10^{-3} M) and endothelium-dependent relaxations were induced with increasing and cumulative concentrations of ACh (acetylcholine; 10^{-5} M - 10^{-6} M), while endothelium-independent vasodilatation were produced with SNP (sodium nitroprusside; 10^{-9} M - 10^{-5} M). Additionally, microvessels from mice treated with visfatin were preincubated *ex vivo* with SQ-29.548 (thromboxane receptor antagonist A2, 10^{-5} M).

After infusion with visfatin (100 ng/kg/day) or IL-1β (12 µg/kg/day), an impairment of endothelium-dependent vascular relaxations to ACh were observed. This negative effect of visfatin was prevented by APO866 (2,4 mg/kg/day). Moreover, endothelium impairment was also reversed when the microvessels obtained from mice infused with visfatin, were preincubated with SQ-29.548. The endothelium-independent relaxations to SNP were not affected by either visfatin, IL-1β, APO866, or SQ-29.548.

In vivo subacute infusion of pro-inflammatory adipokines, like visfatin or IL-1β, produce endothelial dysfunction in mice mesenteric microvessels, supporting its role as mediators of the vascular damage associated to cardiometabolic diseases. The effect of visfatin could be due by prostanoid vasoconstrictors released from the vascular wall by a mechanism that involves Nampt activity.

Keywords: visfatin, IL-1β, endothelial dysfunction, mesenteric microvessels, mice

Gasotransmitters

P055: The relaxant effects of slow releasing hydrogen sulfide donors GYY4137 and AP66 on human saphenous veins and the role of caveolae

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Hydrogen sulfide (H_2S) plays an important role in many physiological and pathological processes. It has been suggested that traditional sulfide salt H_2S-donors (e.g.Na_2S/NaHS) are inadequate to study the cellular physiology of H_2S since they generate H_2S rapidly and thus, slow releasing H_2S donors offer a more physiological relevant alternative. Caveolae are described as plasma membrane invaginations function in a variety of cellular processes including endocytosis, transcytosis, calcium signaling and signal transduction. Endothelial nitric oxide synthase (eNOS) and heme oxygenase-1 (HO-1), enzymes responsible for synthesis of two other gasotransmitters nitric oxide (NO) and carbon monoxide (CO), respectively, were localized in caveolae and the activity of these enzymes were negatively regulated by caveolin-1, the major coat protein of caveolae. In this study, we aimed to investigate concentration-dependent effects of slow releasing H_2S donors, GYY4137 and AP66 on isolated human saphenous veins and evaluate the role of caveolar integrity in these effects.

The effects of slow releasing H_2S donors, GYY4137 (10^{-7}-10^{-3}M) and AP66 (10^{-7}-10^{-4}M) were evaluated in noradrenaline precontracted human saphenous veins, harvested during coronary artery bypass surgery.

GYY4137 and AP66 have induced concentration-dependent relaxations with slower onset in precontracted human saphenous veins (E_{max}: 45.92±4.43%, n=5; E_{max}: 41.65±4.70%, n=5, respectively). Disruption of the caveolae with a cholesterol-depleting agent, methyl β-cyclodextrin (MβCD, 10^{-2} M, 1h), significantly reduced GYY4137 and AP66 induced relaxations (E_{max}: 32.28±6.08%, $p<0.05$ *vs* caveolae intact vessels; E_{max}: 19.11±8.98%, $p<0.05$ *vs* caveolae intact vessels, respectively).

Caveolar structure includes various proteins and ion channels (e.g. K_{ATP} channels) which play important roles in signal transduction. Therefore, the decrease in the relaxant effects of slow releasing H_2S donors in caveolae depleted human saphenous veins suggests a crucial role for the integrity of caveolar structure on the responses induced by H_2S.

Keywords: GYY4137, AP66, hydrogen sulfide, relaxation, saphenous vein

P056: Mechanism of restoration of carbachol-induced contractions by H_2S in detrusor smooth muscle in acute cystitis

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Interstitial cystitis is characterized by bladder inflammation in which agonist-induced contractions can vary[1,2]. H_2S donor diallyl trisulfide has been shown to protect rat hepatocytes against cyclophosphamide toxicity[3]. The aim of this study is to examine the pathways involved in carbachol-induced contractions after NaHS treatment in β-escin permeabilized mice detrusor smooth muscle having cyclophosphamide-induced acute cystitis.

Cyclophosphamide (300 mg/kg) was injected to mice intraperitoneally 4h before the bladder dissection. NaHS (10 mg/kg) was injected intraperitoneally 30 min before cyclophosphamide. Detrusor strips mounted in 1 ml organ baths containing modified Krebs' solution were permeabilized with 100 µM β-escin (30 min). Contractions were expressed (mean±S.E.M) as % of 80 mM K^+. $P<0.05$ was accepted as significant.

Carbachol (50 µM)-induced contractions that were significantly increased from 18.8±6.1% (control,n=8) to 44.3±9.3% in cystitis (n=6, $P<0.05$) were restored by NaHS treatment (18.1±6.1%; n=7, $P<0.05$). In another group of experiments, carbachol-induced contractions were significantly decreased with Rho kinase inhibitor Y-27632 (5 µM) (12,1±4,5%, n=4) or protein kinase C inhibitor GF109203X (5 µM) (11,4±4%, n=3) in cystitis group. Furthermore, carbachol-induced contractions were decreased in the presence of these two inhibitors in control group, as well as after NaHS treatment. NaHS was also able to reverse the increase in carbachol-induced contractions caused by cyclophosphamide in the presence of Y-27632 or GF109203X.

NaHS treatment prevents the increase in carbachol-induced contractions in mice detrusor with acute interstitial cystitis. The activation of Rho kinase and protein kinase C pathways are of importance in increased carbachol contractions in cystitis. Based on our preliminary data, we suggest that both Rho kinase and protein kinase pathways are not involved in the protective mechanism of H_2S against cystitis in detrusor smooth muscle.

[1]*Weng et al.(2005)Toxicol Appl Pharmacol,208,163-169.*
[2]*Mok et al.(2000)J Auton Nerv Syst,80(3),130-136.*
[3]*Deng et al.(1994)Biomed Environ Sci,7(1),85-90.*

Keywords: H_2S, bladder detrusor, carbachol, permeabilized

Respiratory Pharmacology

P057: Hydroxysafflor yellow a attenuates bleomycin-induced pulmonary fibrosis in mice

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Hydroxysafflor yellow A (HSYA) is an active ingredient of traditional Chinese medicine Carthamus tinctorius L. which has been used in the treatment of cardiovascular diseases for over 2000 years in China. We want to investigate whether HSYA attenuates pulmonary fibrosis induced by bleomycin (BLM) in mice. The mice received BLM via oropharyngeal aspiration and 26.7, 40, 60 mg/kg/d HSYA was intraperitoneally injected once daily. After 21 days arterial blood gas analysis was performed and the mice were killed. HE staining and Masson's trichrome stain were used to observe morphological changes and hydroxyproline content was measured. mRNA expression of transforming growth factor-β1 (TGF-β1), connective tissue growth factor (CTGF), alpha-smooth muscle actin (α-SMA), and collagen I were measured by real-time PCR. α-SMA-positive cells in lung tissues were detected by immunohistochemical staining. A549 cell was cultured and cellular changes were observed after TGF-β1 and HSYA treatment. Phosphorylation of Smad3 was evaluated by western blotting. The blood gas changes due to BLM were attenuated by 40 or 60mg/kg/d HSYA($P<0.05$). HSYA decreased the BLM induced lung consolidation area and collagen deposition in mice with pulmonary fibrosis. Lung hydroxyproline content increase after BLM administration was inhibited by 60mg/kg/d HSYA($P<0.05$). 60mg/kg/d HSYA also alleviated the BLM-induced increase of TGF-β1, CTGF, α-SMA, and collagen I mRNA levels($P<0.05$). 40 or 60mg/kg/d HSYA treatment inhibited BLM induced increase of α-SMA protein expression($P<0.05$), and attenuated the pulmonary morphological changes in lung tissue. 5 or 50μmol/L HSYA inhibited A549 cells morphological changes, collagen I ($P<0.01$)mRNA expression increase and Smad3 phosphorylation($P<0.05$) after TGF-β1 stimulation. Mice pulmonary fibrosis induced by BLM can be attenuated by HSYA ip and the inhibition of TGF-β1 induced Smads pathway activation may be one of the important mechanisms.

Keywords: hydroxysafflor yellow A, pulmonary fibrosis, bleomycin, transforming growth factor-β1, alpha-smooth muscle actin

P058: Protective effect of hydroxysafflor yellow A on inflammatory injury in chronic obstructive pulmonary disease rats

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To investigate the attenuating effect of Hydroxysafflor Yellow A (HSYA) on inflammatory injury during chronic obstructive pulmonary disease(COPD). Passive cigarette smoke and intratracheal instillation of lipopolysaccharide were used to establish a COPD model in rats. Hematoxylin and eosin staining of lung tissue sections was applied, real-time PCR was used to assay mRNA levels of some cytokines in lung tissues, the cytokines in bronchoalveolar lavage fluid(BALF) were measured by enzyme linked immunosorbent assay, western blot analysis was applied to determine phosphorylated p38mitogen activated protein kinase(p38 MAPK) levels in lung tissues, and Nuclear Factor (NF)-κB p65 protein levels in lung tissues were detected by immunohistochemical assay. Lung alveolar septa destruction, alveolus fusion, inflammatory cell infiltration, and bronchiole exudation were observed. These above pathological changes were alleviated in the HSYA+COPD groups. The mRNA expression of tumor necrosis factor(TNF) α, interleukin(IL)-1β, IL-6, intercellular adhesion molecule(ICAM)-1, and Vascular Cellular Adhesion Molecule(VCAM-1) were significantly increased in lung tissues of COPD rats (all $p<0.001$) and they were inhibited by 48 or 76.8 mg/kg HSYA(all $p<0.01$). Levels of TNF-alpha, IL-1β and IL-6 protein levels in BALF of COPD rats were significantly increased (all $p<0.001$) and this increase was inhibited by 48 or 76.8 mg/kg HSYA(all $p<0.01$). The levels of phosphorylated p38MAPK and NF-κB p65 in lung tissues of COPD rats were significantly increased(all $p<0.001$) and they were attenuated by 48 or 76.8 mg/kg HSYA(all $p<0.05$). All of these above pharmacological effects of HSYA were dosage dependent. HSYA can alleviate inflammatory cell infiltration and other pathological changes in lungs of COPD rats. HSYA inhibited inflammatory cytokine expression elevation, and increased phosphorylation of p38MAPK and NF-κB p65 expression in lungs of COPD rats. In conclusion, the protective mechanism of HSYA to inhibit COPD inflammation might be attenuating NF-κB and p38MAPK signal transduction.

Keywords: hydroxysafflor yellow A, chronic obstructive pulmonary disease, inflammation, p38 MAPK, NF-κB

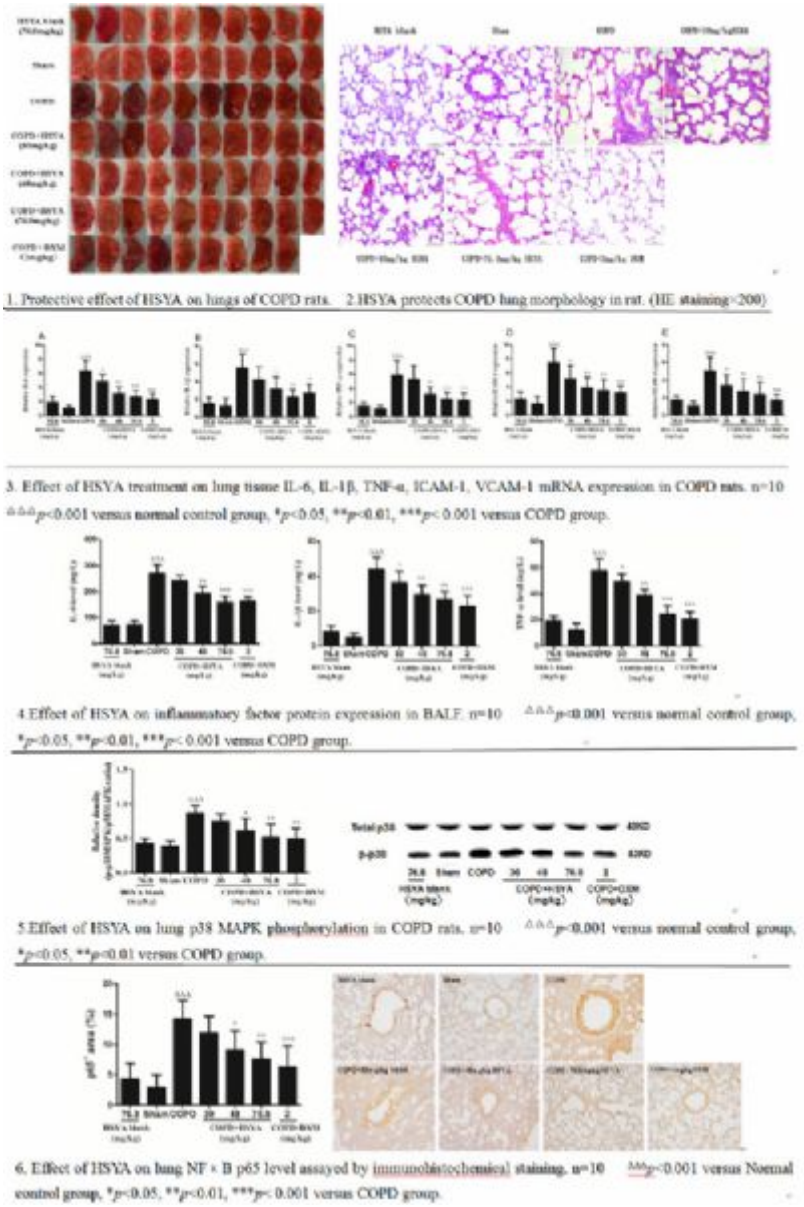

Figure 1. Protective effect of hydroxysafflor yellow A on inflammatory injury in chronic obstructive pulmonary disease rats. *HSYA=hydroxysafflor yellow A; COPD=chronic obstructive pulmonary disease; DXM= Dexamethasone*

P059: Evaluation of the mechanism of tracheal hyperreactivity to 5-hydroxytriptamine in lipopolysaccharide-induced airway inflammation

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Airway inflammation is associated with bronchial hyperreactivity to pharmacologic agonists and various irritant compounds. In the present study, we have investigated the mechanism of the hyperreactivity elicited with 5-hydroxytriptamine (5-HT) in lipopolysaccharide (LPS)-induced airway inflammation in mice (female, CD1). For this purpose, 60 µl LPS (0,1 mg/ml), was administered intranasally (i.n.) to the LPS group and control group of mice received 60 µl PBS (i.n.). 48 h after LPS or PBS treatments, mice were euthanised by cervical dislocation and the tracheas were isolated and mounted in organ baths. The change in tension was measured using an isometric force transducer and cumulative concentration–dependent contractile responses to carbachol and 5-HT were obtained. Carbachol (10^{-8}-10^{-4}M)-induced contraction responses were not changed, whereas 5-HT-(10^{-8}-10^{-4}M) contractions were enhanced in the LPS group when compared to control group. Incubation with muscarinic receptor antagonist atropine (10^{-6}M) inhibited 5-HT-induced contractions to the same level in both groups. 5-HT_{2A} receptor antagonist ketanserin (10^{-9}-10^{-6}M) antagonized 5-HT contractions in a competitive manner with a pA_2 value of 8.92 in the control group. However, inhibitory effect of ketanserin on 5-HT contractions was more pronounced and inhibition was in a non-competitive manner in the LPS group. 5-HT_3 receptor antagonist alosetron (10^{-9}M, 10^{-7}M) elicited a slight inhibitory effect on 5-HT contractions only in the LPS group. Electrical field stimulation (EFS) (0.25-20 Hz)-induced contraction responses were also obtained in isolated tracheas from LPS- and control group of mice. EFS-induced frequency-dependent contraction responses were not altered with LPS application when compared to that in the control group. In conclusion, tracheal 5-HT hyperreactivity seems to be mediated by 5-HT_{2A} receptors and occur by the enhanced acetylcholine release from cholinergic nerves in LPS-induced airway inflammation.

Keywords: airway, airway inflammation, 5-hydroxytriptamine, lipopolysaccharide, mice

Endocrine Pharmacology

P060: Investigation of *PTEN* and *NOS* gene expressions in Hashimoto's thyroiditis

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Hashimoto's thyroiditis (HT) is the most common autoimmune disease in humans frequently leading to hypothyroidism. It is defined by the presence of goiter and serum thyroid autoantibodies. The etiology of HT is currently understood to be multifactorial. However, the pathogenesis of HT is still not fully known. The aim of this study was to investigate the possible contribution of phosphatase and tensin homolog (*PTEN*) and nitric oxide synthase (*NOS*) gene expressions in patients with HT.

A total of 41 patients with HT and 42 healthy control subjects with similar age and sex were included to this study. mRNA's from blood samples and paraffin thyroid sections were extracted, and real time polymerase chain reaction on the BioMark HD dynamic array system (Fluidigm, South San Francisco, Calif., USA) was performed for the gene expression analysis. For calculation of the significance of differences in gene expressions, the Student's t-test was used.

Gene expression analysis showed that *NOS1* mRNA content in thyroid tissue was markedly augmented (p=0.022). However, *NOS1* gene expression was not significantly modified in leukocytes. Although *PTEN (PTEN1)* gene expression was decreased in leukocytes (p=0.031), it was elevated in thyroid tissue (p=0.041). Additionally, no marked changes were noted in *TPTE (PTEN2)*, *NOS2*, and *NOS3* gene expressions in leukocytes or in thyroid tissue.

To the best of our knowledge, these results are the first to demonstrate the contribution *PTEN* and *NOS* gene expressions in HT. Our data showed that *PTEN (PTEN1)* and *NOS1* gene expressions are differentially regulated in peripheral leukocytes and thyroid tissue. These genes may contribute to the pathology of HT.

Keywords: gene expression, nitric oxide synthase, phosphatase and tensin homolog, thyroiditis

P061: High fructose consumption causes pancreatic inflammation in rats

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High-fructose consumption has been shown to cause metabolic disturbances. However, limited data is available on pancreatitis due to fructose feeding in rats. The aim of the present study was to investigate the influence of dietary high fructose on pancreatic inflammatory parameters including TNF-alpha, IL-1β, NFκB, Nrf2 and iNOS.

Four-week-old Wistar male rats were divided into two groups: control and fructose. Fructose was given to rats in drinking water (20 %) for 15 weeks. Body weights were monitored weekly and plasma triglyceride and glucose levels were measured. TNF-alpha and IL-1β pancreatic levels were measured with ELISA kits. Real Time-PCR was utilized to quantify the pancreatic levels of NFκB, Nrf2 and iNOS mRNA expressions.

Dietary fructose increased glucose (control: 109±2.5 mg/dl; fructose: 159±7 mg/dl) and triglyceride (control: 127±7.8 mg/dl; fructose: 287±35 mg/dl) levels in plasma of rats. Fructose consumption significantly elevated TNF-alpha and IL-1β levels as well as NFκB and iNOS mRNA levels in pancreas, in contrary this treatment led to a reduction in Nrf2 mRNA expression.

High fructose-induced metabolic changes are positively correlated with the increases in pancreatic TNF-alpha, IL-1β, NFκB and iNOS. Moreover, dietary fructose caused a seriously decrease in cytoprotective factor, Nrf2 mRNA expression.

Keywords: fructose, pancreatic inflammation, TNF-alpha, IL-1β, NFκB

P062: Evaluation of efficiency of French maritime pine bark (*Pinus maritima*) extract in high-fat diet and streptozotocin-induced diabetes in rats

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Type 2 Diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by insulin resistance. Increased oxidative damage due to diabetes plays an important role in complications. Renal failure following diabetic nephropathy is one of the main causes of morbidity and mortality in DM. PBE (Pine bark extract) is a complex mixture of bioflavonoids, with oligometric proanthocyanidins (OPC) as the major constituents which has been reported to be one of the strongest antioxidants. This herbal medication has been shown to have beneficial effects on DM in a few experimental and clinical studies.

In this study, high-fat diet (HFD) (for 4 weeks) and following low-dose streptozotocin (STZ) (35 mg/kg, ip) were used to create experimental T2DM in rats. The animals were randomly divided to one of the experimental groups as follow: Control group (standard diet), HFD group, HFD and STZ-induced diabetic groups. Diabetic groups were also divided into subgroups: Rats were given orally either serum physiologic (diabetic control) or metformin (300 mg/kg/day, positive control) or PBE (10, 50 or 100 mg/kg/day) or combination of PBE (50 mg/kg/day) and metformin (300 mg/kg/day) for 4 weeks.

At the end of the study, fasting glucose, insulin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, HbA1c, urea, creatinine, C-reactive protein, serum nitrite/nitrate and malondialdehide (MDA) levels in the serum of rats were analyzed. Endothelial functions of isolated thoracic aortas, lipid peroxidation (MDA levels) in kidneys and histopathologic evaluations of pancreas and kidneys were also evaluated.

PBE improved the increased fasting blood glucose, HbA1c and HOMA-IR levels in diabetic rats significantly. Combined treatment was found to be more effective to prevent diabetic injury in histopathologic studies of pancreas and kidneys.

Beneficial effects of PBE obtained from this study may contribute to using PBE, alone or in combination with other antidiabetic agents, in order to prevent the complications of diabetes mellitus.

Keywords: French maritime pine bark extract, diabetes mellitus, diabetic nephropathy, endothelial function, streptozotocin

P063: Effects of insulin on serum uridine levels in rats

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The aim of the present study was to determine the effects of insulin on serum uridine levels in rats.

Adult male Sprague Dawley rats (250–300 g) were used in the present study with the experimental protocols approved by the Animal Care and Use Committee of Uludag University (Approval ID: 2014-03/04). In the first study, levels of serum uridine were determined in fasting or fed situation. Rats fasted for 24 hours were randomized into two groups: rats in the first group were sacrificed immediately and those in the second group were sacrificed 2 h after feeding, and blood samples were obtained in order to determine serum levels of uridine, glucose and insulin. In the second study, serum uridine, insulin and glucose levels were determined after subcutaneous injection of different doses (0.1, 0.5 and 1 IU/kg) of insulin from blood samples obtained at different time points through a cannula inserted into left femoral artery. In the third study, levels of serum uridine were investigated in the hyperinsulinemic-euglycemic clamp method performed by infusion of insulin and dextrose (which compensated for the insulin effect on blood glucose) through a cannula inserted into the femoral vein and blood was collected for uridine, glucose and insulin analyses through the femoral artery cannula.

Our findings showed, for the first time, that serum uridine concentrations were decreased after feeding, as well as insulin administration in a dose- and time-dependent manner. We also found that the fall in serum uridine after exogenous insulin was not associated with glucose uptake.

We conclude that insulin lowers serum uridine independent of its effect on glucose uptake. Hence, serum uridine concentrations may represent a new indicator of insulin resistance.

Keywords: uridine, insulin, hyperinsulinemic-euglycemic clamp

P064: Renin-angiotensin system in pathophysiology and pharmacotherapy of osteoporosis and sarcopenia

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Osteoporosis (low mineral bone density) and sarcopenia (loss of skeletal muscle mass and function) are two age-related conditions that often coexist and may lead to higher risk of fall, fractures, disability, mortality. They share a number of predisposing factors and pathogenetic mechanisms including increased activity of renin-angiotensin system (RAS). They are also co-morbid with other conditions associated with higher RAS activity – hypertension, heart failure, diabetes type 2, obesity, etc.

The aim is to summarize data on the importance of RAS and its inhibitors for the pathogenesis and treatment of osteoporosis and sarcopenia. For this purpose, the literature is reviewed.

Circulating RAS regulates blood pressure and electrolyte homeostasis. Local, tissue RASs are important in the regulation of the cell growth, regeneration, apoptosis, inflammation and angiogenesis. In the bone local RAS can play an important role in both metabolism and remodelling independently of the systemic RAS. Activation of local RAS via angiotensin-II (AT-II) – AT1receptor (AT1R) lead to an osteopenic phenotype with bone resorption and progression of osteoporosis. RAS inhibitors attenuate this process with superiority of AT1R-blockers comparing to ACE inhibitors.

Recent evidences focus on the physiological role of the RAS on skeletal muscle. Regulation of local perfusion by AT-II is fundamental for its metabolic activity and function. In animal models AT-II induces skeletal muscle wasting through enhanced protein degradation and apoptosis and decreased protein synthesis. Recently, the attention is focused on muscle satellite cells (muscle progenitor cells) as main target of the RAS activation or blockade. AT-II reduces satellite cell migration, differentiation and growth through AT1R. Blockade of the AT-II/AT1R axis may be potentially useful in sarcopenia.

Identification of pathways affecting both bone and muscle will facilitate the development of pharmacological agents aiming at concomitantly treating osteoporosis and sarcopenia.

Keywords: renin-angiotensin system, sarcopenia, osteoporosis

P065: Protective effect of dexpanthenol against streptozotocin-induced lung injury in rats

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Diabetes mellitus (DM) is a serious metabolic health problem. Hyperglycemia can induce oxidative stresss and attenuate antioxidative mechanisms through non-enzymatic glycation of antioxidant enzymes.

In the literature, there is limited data regarding the lung specific changes of overall antioxidant capacity and the underlying mechanism in early diabetes. Therefore, this hypothesis was tested in an estab¬lished rat model of streptozotocin (STZ)-induced type 1 diabetes. Also, we aimed to investigate the protective effect of dexpanthenol (Dex), an alcoholic analogue of pantothenic acid, on STZ-induced lung injury.

The rats were weighted and diabetes was induced in rats by a single intraperitoneally (i.p.) injection of STZ (50 mg/kg). After a blood sample was obtained through the tail vein, animals with glucose levels higher than 250 mg/dl on the 5th day after STZ was administered were included in the study. The animals were divided into 4 groups each with 8 rats, according to their experimental treatment as follows: (1) control group rats were treated with vehicle only (i.p.) (2) Dex group; 500 mg/kg Dex was continued by i.p. for 6 weeks, (3) STZ group and 4) STZ+Dex-treated group; 500 mg/kg Dex was applied 1 h before the STZ injection and continued for 6 weeks by i.p. After scarification, right section of the lung was used for thiobarbituric acid-reactive substances (TBARS), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH) contents.

The results were presented in the Table 1. Briefly, lung tissue TBARS levels were found to be significantly higher in the STZ group, whereas the values of CAT, GPx and GSH contents were lower when compared to the control group. Dex treatment significantly ameliorated all of these detrimental changse in the lung tissue.

According to our biochemical results, Dex exerts protective effects on the lung injury induced by STZ application which reflects type 1 DM.

Keywords: streptozotocin, diabetes, dexpanthenol, lung, oxidative stresss

Table 1. Biochemical results in the lung tissue.

Groups	Lung-Weight (mg)	TBARS (nmol/g tissue)	CAT (k/g prot)	GPx (U/mg prot)	GSH (µmol/g tissue)
STZ	19.23±2.96*	21.29±5.31*	1.96±0.62*	13.96±3.75*	6.77±0.82*
Control	16.03±2.16	14.68±3.30	4.29±1.15	22.87±3.77	10.78±2.88
STZ+Dex	15.03±1.41**	15.02±3.53**	4.43±1.31**	21.04±3.19**	9.88±2.92**
Dex	13.54±1.40**	13.48±2.28**	4.65±1.75**	23.45±4.19**	10.13±1.25**

*Dex: Dexpanthenol. *p<0.05 versus Control; **p<0.05 versus STZ*

Obesity and Metabolic Diseases

P066: Alterations in small molecule metabolites of cardiac muscle in experimental type 2 diabetic cardiomyopathy rat model

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The small molecule metabolites of cardiac muscle were analyzed in experimental type 2 diabetic cardiomyopathy (DC) rat model induced by feeding high-sucrose/fat diet (HSFD) and streptozotocin (30 mg/kg, i.p.). The metabolomic analysis of myocardial tissue was conducted using the Ultra high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLCQ-TOF/MS). The remarkable differences between control and type 2 DC rats were analyzed by combination of analysis of the metabolomics profile and principal components. At the 4th, 8th, 12th, 16th, and 22nd time points, cardiac function data were collected using MP150 systems, fast plasma glucose and lipids were measured by ultraviolet spectrophtometric method. Compared with control rats, the metabolic profile of myocardial tissue of model rats was significantly altered. Specifically, the levels of PC (20:2/18:2), PC(18:0:16:0) and PC(20:4/18:0) were significantly up-regulated. In addition, rats treated with HSFD/streptozotocin showed systolic and diastolic dysfunction from the 16th week, and blood glucose and lipids significantly increased. These results indicated that rats induced by high sugar/high fat diet and STZ showed similar hemodynamic characteristic of clinic diabetic cardiomyopathy, and some small molecule metabolites of cardiac muscle were significantly changed, in which might be potential targets for therapeutic intervention of diabetic cardiomyopathy and biomarkers for clinical diagnosis.

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Keywords: diabetic cardiomyopathy, small molecule metabolites, cardiac muscle

P067: Characterization of Streptozotocin (STZ)-induced diabetic rats, a Type 1 diabetes model with associated co-morbidity

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The metabolic profile, associated pathological markers and pharmacological validity of the rat streptozotocin (STZ) model of type 1 diabetes was characterized. Male Wistar rats received a single intraperitoneal injection of STZ (45 mg/kg). Body weight, basal glycaemia and insulinemia, response to oral glucose challenge (OGTT), food and water intake, kidney function, intestinal motility and pain sensitivity were measured over a 15-week period.

Body weight was reduced in diabetic rats (305.3 ± 18.6 vs. 559.0 ± 17.7 g in controls, 15 weeks after STZ, $p < 0.001$). Daily food and water intake was increased in diabetic rats (+163% and +1089% respectively, $p < 0.001$).

Basal glycaemia was increased in diabetic rats (30.4 ± 1.3 vs. 4.8 ± 0.2 mmol/L in controls, $p < 0.001$) and glycaemia remained elevated 180 minutes after OGTT whereas it returned to normal levels in non-diabetic rats. A five-day oral treatment with metformin (200 mg/kg) inhibited OGTT-induced hyperglycaemia. Insulinemia was decreased following STZ (0.15 ± 0.02 vs. 0.73 ± 0.15 ng/ml, $p < 0.001$) and remained low after OGTT (0.19 ± 0.04 vs. 3.66 ± 0.31 ng/ml, $p < 0.001$).

Daily urine production was higher in diabetic rats (10.2 ± 0.9 vs. 5.0 ± 0.5 ml/100 g in controls, $p < 0.001$) whereas the glomerular filtration rate was decreased (1.68 ± 0.16 vs. 2.81 ± 0.18 ml/min, $p < 0.001$).

Intestinal transit was decreased in diabetic rats 15 weeks after STZ (-42%, $p < 0.001$) and gastric emptying displayed a similar trend.

Diabetic rats showed hypersensitivity to tactile stimulations 22 and 35 days after STZ, although they displayed normal responses to thermal stimulation.

These observations confirm that the STZ-induced diabetes model is representative of the polymorphism of type 1 metabolic syndrome and can therefore be a useful tool for the pharmacological evaluation of antidiabetic drug candidates.

Keywords: diabetes, rat, renal function, gastro-intestinal functions, pain sensibility

P068: Effects of chronic etanercept, a TNF-alpha inhibitor, on vascular reactivity in cafeteria diet-fed rats

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Current studies suggested that inflammation is associated with cardiovascular diseases caused by obesity. In this study, we aimed to investigate the effects of etanercept, a TNF-alpha inhibitor, on vascular smooth muscle reactivity in cafeteria diet (CD)-fed rats.

Male weanling Wistar Albino rats (50–70g, 30 days after birth) were divided into three groups (n=8): First group of rats (control) was fed a standard pelleted diet. The second group of animals (obese) was fed on CD which is a high-fat diet and composed by a mixture of pate, bacon, chips, cookies, chocolate and chow with proportions of 2:1:1:1:1:1, respectively, and was given daily. The third group (etanercept-treated) was also fed on CD and treated with etanercept (0.8 mg/kg/weekly/subcutaneously) during 12 weeks. The body weights of animals were measured weekly. After 12 weeks, the aortas of the rats were removed and placed in the organ chambers for isometric tension measurements. The tissues were contracted with phenylephrine (10^{-6}M) and then relaxation responses to cumulative concentrations of carbachol (10^{-8}-10^{-5} M) or sodium nitroprusside (10^{-8} -10^{-4} M) were measured.

After 12 weeks, the body weight of obese group was higher than control ($p<0.0001$) and etanercept-treated group was lower than obese group ($p<0.0001$). The contractile responses to 80 mM KCl did not change in all groups. Endothelium-dependent relaxation in response to carbachol was significantly decreased in the obese group compared with the control group ($p<0.05$). In etanercept-treated group, the impairment of relaxation returned to the control group ($p>0.05$). Relaxation of the aortic tissues in response to sodium nitroprusside was similar in all groups ($p>0.05$).

Our study confirm that obesity causes endothelium-dependent vascular dysfunction and etanercept treatment prevented this impairment. Thus, TNF-alpha-mediated inflammation may have an important role on endothelial dysfunction induced by obesity which develops at young ages.

Keywords: cafeteria diet, obesity, TNF-alpha, etanercept, vascular reactivity

P069: The effect of "Boldine" on metabolic syndrome induced erectile dysfunction

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Erectile dysfunction (ED) is highly seen in patients with Metabolic Syndrome (MetS) and characterized with endothelial dysfunction (EndD). Factors such as insulin resistance, hyperglycemia, hyperinsulinemia and hyperlipidemia seen in MetS cause EndD with a decrease in bioavailibity of nitric oxide. Boldine is an antioxidant alkoloid which protects endothelial functions. The goal of this study is to investigate erectile functions in high fructose-induced Mets model and the effect of in vivo boldine treatment on Mets-induced ED.

Wistar rats (Weight 180-200g) were randomly divided into 3 groups as; control

[standart diet (SD)-fed group,n=8], MetS [60% high fructose diet (HFD)-fed group,n=8] and boldine treatment [60% HFD- fed with boldine 40mg/kg/day group,n=8]. Animals were fed with 60% HFD and SD for 12 weeks in MetS and control groups respectively. Boldine treatment was started in the 10th week of HFD and was administered with i.p. gavage for 2 weeks. In order to evaluate erectile functions, intracavernous pressure (ICP) /mean arterial pressure (MAP) ratio and area under curve (AUC) were performed during cavernous nerve stimulation. MetS model was evaluated with biochemical analyses [serum triglyceride (TG), uric acid (UA), blood glucose, insulin levels], waist circumference/tibial length ratio and HOMA index.

ICP/MAP and AUC values which were decreased in MetS animals ($p<0.01$), were improved in the boldine treatment group ($p<0.001$ and $p<0.01$, respectively)(Figure 1). In MetS group TG, UA, insulin levels, weight, waist circumference(WC)/tibia length(TL) ratio and HOMA index were significantly increased when compared to control group ($p<0.05$, $p<0.01$, $p<0.05$, $p<0.05$, $p<0.0001$, $p<0.0001$, $p<0.05$, respectively) (Table1). Boldine treatment caused an increase in glucose levels but did not change other parameters (MetS vs MetS+boldine,$p<0.05$).

Preserved erectile functions in rats by boldine treatment might be the consequence of improvement in endothelial response. Further studies are needed to clarify the mechanism.

This study is supported by TUBITAK (114S792).
Keywords: metabolic syndrome, erectile dysfunction, endothelial dysfunction, boldine

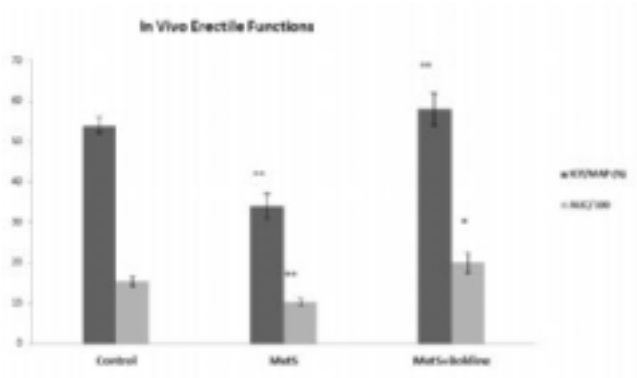

Figure 1. Measurement of erectile functions during electrical stimulation of cavernous nerve. *Percentage of intracavernous pressure /mean arterial pressure ratio (ICP/MAP), area under curve (AUC). AUC values represent as AUC/100 in the figure. Statistical analysis was performed by ANOVA followed by Tukey-Kramer post-hoc test and Student's t-test (Graphpad, San Diego, CA). P<0.05 was considered as statistically significant. ICP/MAP and AUC, Control vs Mets group **p<0.01; ICP/MAP and AUC, Mets group vs MetS+Boldine ***p<0.001 and **p<0.01, respectively.*

Table 1. Biochemical parameters, weight, waist circumference(WC)/tibial length(TL) and HOMA index in all groups at the end of 12th weeks.

	TG(mg/dl)	UA(mg/dl)	Insulin(mU/L)	Glucose(mg/dl)	Weight(gr)	WC/T(cm)	HOMA index
Control(n=8)	33,9±4,2	1,4±0,2	2,7±0,82	236,9±30	346±9,8	3.7±0.04	0,04±0.01
MetS (n=8)	51,8±6,4*	2,4±0,2**	8,9±2,5*	287,1±39,2	451±16,8***	4.3±0.06***	0,2±0,1*
MetS+Boldine (n=8)	55,6±6,4	2,04±0,2	10,0±3,8	395,1±39,4*	432±14,5	4.2±0.05	0,3±0,1

*Statistical analysis was performed by ANOVA followed by Tukey-Kramer post-hoc test and Student's t-test (Graphpad, San Diego, CA). P<0.05 was considered as statistically significant. Control vs Mets group TG*p<0.05; UA **p<0.01; Insulin *p<0.05; Weight ***p<0.0001; WC/TL ***p<0.0001 and *p<0.05. Mets vs MetS+boldine Glucose p<0.05.*

P070: Hypocholesterolaemic effects of probiotics

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Due to health-promoting effects in humans, probiotic bacteria have been already used to treat or prevent a wide range of diseases. One beneficial effect that has been suggested to result from a human consumption of probiotic bacteria is a reduction in serum cholesterol levels, as confirmed by a number of in vivo and clinical studies. It is well-known that elevated blood cholesterol is a major risk factor for coronary heart diseases. Accordingly, the aim of this work was to evaluate the mechanisms of cholesterol-lowering effects of probiotics.

We have analysed original and review articles published until January 2016 in various databases, using the keywords such as probiotics, cholesterol, hypercholesterolemia, bile salt hydrolase.

A number of cholesterol lowering molecular mechanisms by different probiotic strains have been proposed. One of the most important mechanisms is through the bile salt hydrolase (enzyme BSH, EC 3.5.1.24), the enzyme responsible for bile salt deconjugation in the enterohepatic circulation and subsequent increase of bile salts excretion. The hypocholesterolemic effect has also been attributed to their ability to bind the cholesterol to the surface of probiotic cells. Cholesterol may be also removed by probiotics by incorporation into the cellular membranes during growth. Furthermore, one of the suggested mechanisms is the conversion of cholesterol to coprostanol by intestinal bacteria, which decreases the amount of cholesterol being absorbed, leading to a reduced concentration in the physiological cholesterol pool.

Numerous mechanisms for cholesterol-lowering effects of probiotics have been suggested, based mostly on in vitro evidence. Thus, future studies are required in order to confirm these assumptions and to address issues like dosage and viability of probiotic strains, industrial standardization and safety aspects.

This study is supported by Ministry of Education, Science and Technological Development of Serbia, Grant No. 41012.

Keywords: probiotics, cholesterol, hypercholesterolemia, bile salt hydrolase

P071: Targeting adipocyte differentiation with Hydro Alcoholic Extract of *Myristica fragrans* seeds for the treatment of obesity

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Myristica fragrans is a culinary Indian spice known for its medicinal value in Indian traditional medicine. Its constituents like myrcene and gama terpinine shown to have anti-inflammatory and LDL antioxidant activities. The role of Hydro Alcoholic extract of *Myristica fragrans* (MFSHAE) on obesity is unexplored. This study is aimed to evaluate the effect of MFSHAE on adipocyte differentiation using 3T3L1 pre-adipocytes.

MFSHAE was characterised by LC-MS/MS. 3T3L1 pre adipocytes were exposed to Differentiation-Cocktail (DC) in the presence and absence of MFSHAE (50µg and 100µg) or GW9662 (5µM) or myrcene (M) (25µM and 50 µM). Degree of differentiation was assessed by oil red staining and tri glyceride content. Adipolysis potential was evaluated by glycerol content. Immunoblot analysis of adipogenic regulators like PPAR γ, Adiponectin, perilipin and CEBP alpha was performed. Glucose uptake assay was performed to assess the effect of MFSHAE and Myrcene on insulin resistance.

LC-MS/MS revealed the presence of myrcene as the major phyto-constituent. MFSHAE and myrcene decreased triglyceride content in dose dependent manner ($p = 0.05$, 0.01 MFSHAE 50µg, 100µg Vs DC, $p = 0.01$, 0.01 M 50 µM Vs DC) and futher confirmed with oil red staining. Adipolysis potential was found to be increased with MFSHAE and myrcene (Table 1).

Immunoblot analysis showed decreased expression of PPAR γ ($p < 0.01$ MFSHAE, $p < 0.01$ M), CEBP α ($p < 0.05$ MFSHAE, $p < 0.01$ M), perilipin ($p < 0.05$ MFSHAE, $p < 0.05$ M) and increased expression of Adiponectin ($p < 0.01$ MFSHAE, $p < 0.01$ M). Glucose uptake was found to be unaltered with treatments ($p = 0.1256$, 0.1584 MFSHAE 50µg, 100µg Vs DC, $p = 0.2568$, 0.1695 M 50 µM Vs DC).

In summary the observations of this study identify the inhibitory effect of MFSHAE and myrcene on adipocyte differentiation with out inducing insulin resistance

Keywords: *Myristica fragrans* , adipocyte differentiation, PPAR γ antagonism, insulin resistance

Table 1. Effect of MFSHAE and myrcene on adipolysis by estimating the glycerol content

S.No	Treatment Group	Glycerol concentration(nM/mL)
1.	Blank	0.14 ± 0.025
2.	DC	21.28 ± 4.15
3.	MFSHAE 50µg	131.61 ± 16.21 #
4.	MFSHAE 100µg	248.59 ± 21.14 ##
5.	Myrcene 25 µM	214.85 ± 12.54 ##
6.	GW9662 5µM	218.14 ± 16.34 ##

 # p < 0.01 vs DC, ## p < 0.001 vs DC

P072: Fractionation of wheat germ by high pressure extraction methods and evaluation of its antioxidant properties in extracts and dry plant material

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Wheat germ has high contents of functional ingredients and vitamins1. The total content of Phenolic compounds (TPC) and Antioxidant capacity indicators were evaluated for the extracts Of Wheat germ consecutively isolated by supercritical carbon dioxide (SFE-CO2) and pressurized liquid extraction with acetone, methanol and water. Antioxidant properties of solid plant material were evaluated by the direct antioxidant capacity measurement by the so-called QUENCHER method.

After the SFE-CO2 of 0.5 mm grained wheat germs, the oil was collected and we continued to extract using PLE. TPC was measured by Folin method2 for extracts and plant material. Antioxidant activity was assessed using ABTS3, DDPH4 and ORAC5 methods for extracts and plant material. TROLOX was used as standard.

SFE-CO2 extract had the lower antioxidant activity and TPC. PLE–Acetone and PLE–Methanol extractions achieved extracts with higher antioxidant activity, as measured with ABTS – ORAC – and DDPH (Tab 1). Of note, TPC was not related with antioxidant activity, in particular in PLE – H2O. The latter had TPC similar to that PLE – Methanol, but lower antioxidant activity (Tab 1).

Antioxidant capacity values by QUENCHER method were determined in the dried plant materials after SFE-CO2 extraction.It may be observed That TPC determened by Quencher method is higher than TPC in SFE CO2 extract. It can be explained by the fact that polyphenolic compounds are poorly soluble in CO2 and most likely treatment of stems at high pressure during SFE-CO2 resulted in some changes, which made some groups better accessible in the reaction with Folin-Cisocalteu reagent.

PLE achieved extracts with higher TPC, as compared to SC-CO2. However, the antioxidant activity in wheat germ seems attributable to compounds with medium polarity. Further studies are in progress on the antioxidant activity of these extracts in cellular models.

Keywords: wheat germ, antioxidant, plant extraction, antioxidant capacity measurement

Table 1.

		ABTS$^{\bullet+}$	ORAC	TPC	DDPH
Quencher (in µmol TE g-1 DW)		167.01±7.22	275.80±10.91	13.03±1.98	4.67±0.06
Extracts	SC–CO2	260.19±14.84	445.10±34.40	1.21±0.36	32.45±0.31
	PLE – Acetone	347.59±8.70	814.34±18.43	16.94±0.15	87.60±4.03
	PLE – Methanol	321.44±2.94	725.77±50.53	13.17±0.15	97.72±1.59
	PLE – H2O	287.95±3.18	573.15±27.31	12.47±0.51	32.24±0.96

Gastrointestinal Pharmacology

P073: Intestinal anti-inflammatory effects of a *Theobroma grandiflorum schum*. (cupuaçu) enriched diet in the trinitrobenzenesulfonic acid model of rat colitis

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Dietary products are among the therapeutic approaches used to modify intestinal microflora and to promote protective effects during the intestinal inflammatory process. Cupuaçu is one of the most consumed exotic tropical fruits of brazilian Amazon rainforest, its rich composition in polyunsaturated fatty acids and dietary fibers, which is used by colonic microbiota for the anaerobic production of the short-chain fatty acids that serve as a major fuel source for colonocytes, may contribute for its prebiotic potencial. The aim of this study is to evaluate the intestinal anti-inflammatory effects of a enriched diet with pulp of this fruit in trinitrobenzenesulfonic acid (TNBS) model of rat colitis.

Cupuaçu pulp was incorporated in wistar rat's normal diet in two different concentrations (5% and 10%) and were given *ad libitum* thirty five days before colitis induction. Non-colitic and TNBS-control groups (n=7) were also included. Animals were sacrificed 48 hours after the induction of the inflammatory process. Body weight, colonic weight/length and production of inflammatory and oxidative stresss markers were evaluated. Statistical significance was set at $p<0.05$.

Cupuaçu at 5% was capable to prevent the depletion of endogenous antioxidant glutathione and cupuaçu at 10% reduced the activity of myeloperoxidase and also reduced the levels of pro-inflammatory cytokines IL-1β and IL-6, compared with TNBS-control groups.

Cupuaçu, at both concentrations, was able to ameliorate the alterations induced by TNBS in inflammatory and oxidative stresss markers. The dietary use of this fruit constitutes an important dietary supplement and complementary medicine product to prevention and treatment of human inflammatory bowel disease.

Keywords: prebiotics, cupuaçu, TNBS, dietary fibers, inflammatory bowel disease

P074: Intestinal anti-inflammatory effect of olive leaf extract in the DSS model of mouse colitis

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Extracts from olive (Olea europea) leaves are used in Mediterranean traditional medicine as an anti-inflammatory remedy. They contain antioxidant phenolic compounds, like oleuropeoside, which could be interesting for the treatment of inflammatory conditions associated with oxidative stresss in humans, including inflammatory bowel disease.

We aimed to evaluate the intestinal anti-inflammatory properties of an olive leaf extract in the dextran sodium sulfate (DSS) model of mouse colitis, which resembles human IBD.

Male C57BL/6J mice were assigned into five groups: non-colitic, colitic control and colitic treated groups with olive leaf extract (0.1-0.5-1 mg/kg) from the day of colitis induction (3% DSS in the drinking water for 5 days) until one week after the establishment of the colitic process. The inflammatory status was evaluated macroscopically by a disease activity index (DAI), and biochemically by colonic determination of mediators of inflammation and intestinal epithelial barrier function. In vitro immunomodulatory properties of the extract (0.1-100 mg/ml) were determined in LPS-stimulated cell lines.

According to the DAI values, the treatment improved the recovery of the colitic mice, maybe through a reduction of the colonic expression of pro-inflammatory mediators (IL-1β, IL-6, TNF-alpha, ICAM-1 and MIP-2), and significantly up-regulating key players of the intestinal epithelial integrity (occludin, ZO-1 and MUC-3). Besides, it displayed immunomodulatory properties in vitro since it inhibited LPS-induced nitrite production in RAW cells and decreased IL-6 production in LPS-stimulated CMT-93 cells.

The olive leaf extract showed intestinal anti-inflammatory activity in the DSS model of mouse colitis, maybe be related to its antioxidant properties as well as the downregulation of the immune response. The extract could also improve the intestinal epithelial barrier and also have a direct effect on immune cells, as demonstrated in the in vitro studies.

Keywords: olive, DSS, colitis, inflammation

P075: Protective effect of lazariod U-74389 G on experimental colitis in Wistar rats

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Lazaroid U-74389G is a synthetic 21-aminosteroids with free radical-scavenging and anti-inflammatory effects. This study was designed to evaluate the anti-inflammatory activity of U-74389G on experimental 2,4-dinitrobenzenesulfonic acid hydrate (DNBS)-induced colitis in Wistar rats.

Three experimental groups were formed: Group 1 (control group, n=6) treated with 0.25 ml of 50% ethanol intrarectally; Group 2 treated with DNBS (30 mg in 0.25 ml of 50% ethanol administered intrarectally (n=6); Group 3 treated with DNBS and U-74389G (n=6). Animals received U-74389G at a daily dose 15 mg/kg i.p. for 6 days, starting 1 day before colitis induction. During the experiment, the body weight of the rats and food intake were recorded as markers of their clinical condition. On day 6 colonic tissues were excised and scored for macroscopic and histological damage. Microscopic changes were assessed by light microscopy on hematoxylin/eosin-stained histological slices. Blood samples were taken to measure levels of cytokines by ELISA methods.

DNBS decreased significantly body weight (from 238.75±7.9 g to 206.25±6.09 g, p=0.04). Rats treated with U-74389G showed greater food intake and weight gain, comparable to changes in the control group. U-74389G reduced macroscopic damages: U-74389G score was 1.42±0.39 and DNBS score – 3.87±0.60; $p<0.05$. All other assessed macroscopic parameters were significantly improved in rats treated with U-74389G. The microscopic tests showed that U-74389G group had significantly lower score compared with DNBS group. The levels of inflammatory cytokines IL-1, IL-6 and tumour necrosis factor alpha were significantly higher than those of DNBS group, while the level of anti-inflammatory IL-10 was significantly elevated by U-74389G.

These findings indicate that U-74389G inhibits significantly colonic inflammatory damages in a rat model of inflammatory bowel disease.

Keywords: cytokines, experimental colitis, inflammatory bowel disease, lazaroid U-74389G

Genitourinary and Reproductive Pharmacology

P076: HGF-cMET pathway has a role in testicular damage in diabetes induced by streptozotocin

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HGF/c-Met system has a role in germ cell homeostasis and male fertility. We aimed to investigate the role of HGF/c-Met pathway in testis tissues of diabetic rats and the effects of insulin treatment on the HGF/c-Met system.
Twenty one male Wistar rats were randomised into three groups: control (C), diabetic (D) and insulin treated diabetic groups (D+I). Diabetes was induced by 45 mg/kg Streptozotocin (STZ) injection. NPH insulin (6 U/kg/day) was administered subcutaneously for eight weeks to D+I groups. Testicular damage was examined by histologically and Johnsen's score was evaluated. Immunohistochemical stainings of HGF and c-Met was analysed by using antibodies against HGF and c-Met.

Diabetes caused degeneration in seminiferous tubule epithelium and disorganization of cell lines in testicular tissue. Insulin treatment decreased the testicular damage induced by diabetes. Similarly decreased Johnsen's score in D group was increased in D+I group ($p<0.001$) but it was stil low compared the C group ($p<0.005$). The immunohistochemical staining score of HGF and c-Met was increased in D group compared to C group ($p<0.001$) and it was decreased in D+I group compared to D group ($p<0.01$). But the score was stil high compared to C group in c-Met staining ($p<0.001$).

HGF/c-Met pathway might have a role in diabetes induced testicular damage. Although insulin is essential treatment for diabetes it might be inadequate to prevent diabetes induced testicular dysfunction. Drugs acting on HGF/c-Met pathway might be effective to return the testicular damage induced by diabetes.

Keywords: streptozotocin induced diabetes, HGF/c-Met, testis tissue, insulin treatment

P077: Ambroxol improves cyclophosphamide-induced oxydative damage and contractility in mice urinary bladder

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Chemotherapy-induced hemorrhagic cystitis (HC) is a life threatening and common clinical disorder in cancer patients which are treated with alkylating agents like cyclophosphamide (CP). It has been shown that CP-induced releasing of local inflammatory cytokines and reactive oxygen species are main components leading to CP–related HC.

Ambroxol (AMB) is a mucolytic agent which has been used for the treatment of some respiratory disorders. It is thought that mucolytic action of AMB is due to its antioxidant and anti-inflammatory properties. Previous experiments revealed that AMB significantly attenuated proinflammatory cytokines, total protein and lipid peroxidation *in vivo* and *in vitro*.

We undertook the present study to elucidate the effect of AMB on CP-induced oxidative urinary damage and detrusor contractility. Swiss albino mice (25-40g) were divided into 5 groups (n=6) of control, CP (300mg/kg,i.p.), CP+AMB30 (300mg/kg+30mg/kg,i.p.), CP+AMB70 (300mg/kg+70mg/kg,i.p.), CP+AMB100 (300mg/kg+100mg/kg,i.p.). AMB was administered once a day for 3 days. Single dose of CP was administered on the third day, 2h after the last dose of AMB. 6h after CP administration, the bladder was removed for MDA, catalase (CAT) analyses and detrusor contractility studies.

We demonstrated that MDA level was significantly elevated in CP group compared with the control ($p<0.05$). Morever, pretreatment with AMB70 and AMB100 significantly prevented the elevation of MDA level ($p<0.05$). A significant decrease in CAT activity was observed in CP group compared with the control and AMB100 treatment prevented the decrease in CAT activity ($p<0.05$).

CP treatment caused a significant decrease on cumulative acetylcholine contraction compared with control ($p<0.05$). However KCl induced contraction was not to be significant among all groups.

Our results suggest that AMB has a potential to be a therapeutic intervention for CP-induced HC.

Keywords: hemorrhagic cystitis, cyclophosphamide, ambroxol, oxidative stresss, detrusor contractility

P078: Effect of fructose rich diet on rat detrusor smooth muscle contractility

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The increase in high-energy food intake, decreased physical activity, sedentary lifestyle, lack of exercise, development of transportation systems and urbanization threaten the public health all over the world. Overweight and obesity are defined as abnormal or excessive fat accumulation which presents health risk. In urinary system, renal failure, kidney tumors, kidney stone formation, infertility and voiding dysfunction have been demonstrated in association with obesity. In this study we aimed to investigate the effect of fructose rich diet on detrusor contractility in rats.

Rats were randomized into 2 groups. The control group received regular food and water ad libitum. The obesity group received fructose 37-40% diet. Six weeks later, animals were weighed and sacrified by cervical dislocation and detrusor muscle was evaluated for in vitro and histopathologic studies.

In control and obesity groups, mean body weight was 237.1±4.8 g and 293.8±9.3 g. respectively. Difference between control and obesity groups was found to be statistically significant ($p<0.001$). Acetylcholine (ACh) at 10^{-5}-10^{-3} M concentrations produced dose-dependent contractions at detrusor smooth muscle strips in control and obesity groups, whereas, obesity group revealed significantly decreased contractile response ($p<0.01$). Similarly, electrical field stimulation (EFS), produced dose-dependent contraction in control group detrusor strips (2-64 Hz). Contrary to control group, EFS in frequency of 2, 4 and 8 Hz did not change the detrusor contractions but, 16 Hz ($p<0.05$), 32 Hz ($p<0.05$) and 64 Hz ($p<0.01$) induced diminished contractions when compared the control group. Microscopic appearence of the detrusor revealed normal cellular arrangement in control group but, in obesity group, marked subephitelial fibrosis was encountered with Masson tricrome stain.

This study shows that, fructose rich diet causes marked subephitelial fibrosis which is an important risk factor for detrusor muscle contractility.

Keywords: fructose, diet, detrussor, contractility, rat

P079: The effect of atorvastatin and gemfibrozil on mouse corpus cavernosum tissue

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Most of the drugs used in the treatment of cardiovascular diseases cause unfavourable effects on erectile functions. In this study, the effect of atorvastatin and gemfibrozil, which has different hypolipidemic mechanisms of action, on the erectile functions observed in mouse corpus cavernosum tissues are evaluated in vitro.

Mouse corpus cavernosum tissues are dissected under ketamine and xylazine anesthesia. Vessels were suspended in 30 ml organ baths filled with Krebs solution and aerated with carbogen (95% O_2, 5% CO_2) at 37°C. An initial tension of 500 mg was applied to the suspended tissue strips. After a stabilisation period of 90 minutes, the protocols were applied to the tissue.

Atorvastatin and gemfibrozil showed no direct contractile or relaxant effect on corpus cavernosum tissues. Both drugs caused a dose–dependent relaxation in tissues precontracted with phenylephrine. While the relaxant effect of atorvastatin is inhibited 40% by L-NAME, this relaxations are totally inhibited by atropine. The relaxations caused by gemfibrozil are inhibited both by L-NAME and atropine. No change was observed in responses of the tissues to acethylcholine, nitroprusside and electrical field stimulation when incubated with atorvastatin or gemfibrozil.

As a conlusion, both drugs showed similar effects on corpus cavernosum tissues. Atorvastatin and gemfibrozil caused these effects via endothelial nitric oxide. When all the results are evaluated, not only the two drugs showed no unfavorable effects but also may have some beneficial effects on erectile functions.

Keywords: corpus cavernosum, erectile function, atorvastatin, gemfibrozil

P080: Chronic effects of atypic antipsychotics on vas deferens contractions in mice

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Antipsychotic drugs are known to be commonly associated with sexual dysfunction. However, the effects of the newer atypical antipsychotics have been largely unexamined. Serotonine is one of the important neurotransmitters on sexual dysfunction that inhibits sexual function. The aim of this study to investigate the effects of sertindole, asenapine and ziprasidone on serotonin and potassium chlorid (KCl)-induced contractions of the vas deferens in mice.

Male inbred mice were used in this study. The mice were randomly divided in to experimental groups (n=7) as follows: saline; sertindole 1.3 mg/kg; sertindole 2.5 mg/kg; asenapine 0.05 mg/kg; asenapine 0.075 mg/kg; ziprasidone 1 mg/kg; ziprasidone 2 mg/kg. Mice were treated by ip injection of drugs during 21 days. Mice receiving only the vehicle (0.9% saline, i.p.) during 21 days served as control group (n=7).

After 21 days of treatment, the effects of drugs were investigated on serotonin (10-8 to 10-4 M) and 80mM KCl-induced contractile responses in the epididymal and prostatic portions of mice vas deferens strips.

Serotonin-induced contractile responses were significantly increased in the epididymal and prostatic portion of the vas deferens obtained from the sertindole-, asenapine-and ziprasidone-treated groups. Sertindole, asenapine and ziprasidone treatments had no effect on KCl-induced contractions of the vas deferens in both portions. There were no significant differences in KCl-induced contractile responses among the groups.

These results showed that serotonin-induced contractions of vas deferens were affected by the chronic treatments of sertindole, asenapine and ziprasidone. Serotonergic receptors may contribute to changes in vas deferens contractions in mice with chronic treatment of sertindole, asenapine and ziprasidone. Thus, our results may explain one of the causes of sexual dysfunction of sertindole, asenapine and ziprasidone.

Keywords: asenapine, sertindole, sexual dysfunction, ziprasidon

P081: The effects of Ferula eleaochytris root extract on erectile dysfunction in diabetic rats

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Ferulago eleaochytris is an endemic plant known as 'ÇAKŞIR' in our country. This plant is used as an aphrodisiac in the South and Southeast Anatolia region (Kahramanmaraş). There are a few scientific studies shown that the root extract of Ferulago eleaochytris has been shown experimentally to increase the relaxation responses on human penile erectile tissue. Potent antioxidant and anti-inflammatory properties of this plant have also been suggested. In our previous studies, the preventive and regenerative effects of the various antioxidants on diabetic erectile dysfunction have been shown. However there is no study about the effect of Ferulago eleaochytris on diabet. The aim of this study is to investigate the effects of the root extract of Ferula eleaochytris in vitro and in vivo on diabetes-induced erectile dysfunction in rats.

In this study, Wistar albino male rats (n=30) were randomly divided into five groups; control group, diabetic group (60mg/kg; streptozotocin; STZ), Ferulago eleaochytris group (40 mg/kg), diabetes + Ferulago eleaochytris treatment group (40 mg/kg), and ethyl alcohol group. Ferula eleaochytris was dissolved in ethyl alcohol. Ferulago eleaochytris and ethyl alcohol were given through oral gavage for 8 weeks. STZ was applied as a single intraperitoneal injection to induce diabetic group. Intracavernous pressure (ICP) and nitrergic relaxations were measured to evaluate the erectile function respectively in vivo and in vitro. The data obtained in this study were presented as mean ± standard deviation (SD) by using GraphPad Prism software (San Diego, CA, USA). Statistical analysis were evaluated by Two-way analysis of variance (ANOVA) programme.

In conclusion, in in vitro experimental studies, nitrergic relaxations significantly decreased in the diabetic groups were partly restorated by the treatment of Ferulago eleaochytris. In in vivo groups, Ferulago eleaochytris treatment also partly inhibited the ICP decreased by STZ in diabetic rats.

This study is supported by Çukurova University Research Fund (TDK-2015-1966) and TUBİTAK 2211-C (2014-1).

Keywords: diabetes mellitus, erectil dysfunction, ferulago eleaochytris, intracavernosal pressure, nitrergic relaxations

P082: Investigation concerning protective and therapeutic effects of agomelatine on renal ischemia-reperfusion injury in rats

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Ischemic renal injury can occur as a result of renal transplantation, partial nephrectomy, and trauma among other reasons. Although restoration of blood flow is the only way to save renal tissue from eventual damage, reperfusion often exacerbates kidney dysfunction induced by reperfusion injury, which is called the oxygen paradox. This study was designed to investigate the protective and therapeutic effects of agomelatine, a novel antioxidant, on renal damage induced by renal ischemia-reperfusion (I/R) in rats.

Thirty-two Wistar albino rats were randomly divided into four groups: (1) sham group, in which the rats only underwent right nephrectomy (n=8); (2) right nephrectomy and left kidney ischemia (1h) and reperfusion (24h) group (I/R) (n=8); (3) right nephrectomy and 25 mg/kg agomelatine by tube before left kidney ischemia (1h) and reperfusion (24h) group (AGO+I/R) (n=8); (4) after right nephrectomy, left kidney ischemia (1h) and 25 mg/kg agomelatine by tube before reperfusion (24h) group (I/R+AGO) (n=8). At the end of the study, malondialdehyde (MDA), total oxidative status (TOS), total antioxidant capacity (TAC), oxidative stresss index (OSI), protein carbonyl (PC) contents were assayed in the kidney tissue. Also, blood levels of urea nitrogen (BUN) and creatinine (Cr) were determined.

Tissue MDA, TOS, OSI and PC levels were found to be significantly higher in the I/R group, whereas TAC levels were lower when compared to the sham group. Also in this group BUN and Cr levels were found as significantly elevated. Agomelatine treatment significant decrease in the BUN, Cr, TOS, MDA and PC production when compared to the renal I/R group. Additionally, TAC was significantly increased before or after renal I/R in the agomelatine-treated groups.

Our results indicated agomelatine provides preventive and therapeutic effects on I/R-induced renal injury in rats by inhibiting reactive oxygen species production and enhancing antioxidant status.

Keywords: agomelatine, oxidative stresss, renal ischemia-reperfusion, rat

Table 1. Oxidative and antioxidative status of the kidney and the levels of BUN and Cr.

Groups	MDA nmol/g tissue	TOS mmol H2O2 equivalent/ g protein	TAC mmol Trolox equivalent/L	OSI Arbitrary unit	PC nmol/mg protein	BUN (mg/dL) Mean ±SD	Cr (mg/dL) Mean ±SD
1. Sham	5.54±0.91	2.71±0.07	0.83±0.07	3.26±0.83	0.63±0.06	23.55±1.14	0.67±0.20
2. I/R	12.39±3.48*	5.87±0.15*	0.51±0.15*	12.21±2.70*	1.51±0.42*	99.46±6.31*	3.60±0.94*
3. AGO+I/R	5.92±0.90**	2.65±0.10**	0.79±0.10**	3.50±1.25**	0.74±0.13**	25.20±2.36**	0.69±0.41**
4. I/R+AGO	5.88±1.68**	3.02±0.09**	0.76±0.09**	4.05±1.16**	0.63±0.10**	29.17±3.49**	0.72±0.82**

**p<0.05 vs Sham; **p<0.05 vs I/R. Results are presented as mean±SD. AGO: Agomelatine; MDA: Malondialdehyde; TOS: Total oxidative stresss; TAC: Total antioxidative capacity; OSI: Oxidative stress index; PC: Protein carbonyl, BUN: Blood urea nitrogen; Cr: Creatinine.*

P083: Effects of long-term treatment with paliperidone, iloperidone and loxapine on mice isolated vas deferens

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Schizophrenia is a chronic psychiatric disease which is treated by antipsychotic drugs. Antipsychotics-related sexual dysfunction is a quite frequent is sue mainly in chronic treatments affecting both quality of life and compliance. This study aimed to investigate the long-term treatments of paliperidone, iloperidone and loxapine on serotonin, adenosine triphosphate (ATP) and KCl-induced contractions of the mice vas deferens.

Male inbred mice were randomly divided into seven equal experimental groups (n=7) as follows: Saline; Paliperidone 0.25 mg/kg; Paliperidone 0.5mg/kg; Iloperidone 0.5 mg/kg; Iloperidone 1 mg/kg; Loxapine 2.5mg/kg; Loxapine 5mg/kg. All drugs were dissolved in 0.9 % saline. Mice were treated by ip. injection of drugs or saline during 21 days. After 21 days of treatment, the effects of paliperidone (0.25 and0.5 mg/kg), iloperidone (0.5 and 1 mg/kg) and loxapine (2.5 and 5 mg/kg) were investigated on serotonin

[10^{-8} to 10^{-4} M], ATP

[10^{-8} to 10^{-4} M)] and 80 mM KCl-induced contractile responses in the epididymal and prostatic portions of mice isolated vas deferens strips.

Serotonin-induced contractile responses were significantly increased in the epididymal and prostatic portion of the vas deferens obtained from the paliperidone-, iloperidone and loxapine-treatment group. All used drugs significantly inhibited ATP-induced contractions of the prostatic and epididymal portions of the mice vas deferens, but had no effect on KCl-induced contractions. There were no significant differences in KCl-induced contractile responses among the groups.

These results show that long-term treatments with paliperidone, iloperidone and loxapine affect vas deferens motility. Serotonergic and purinergic receptors may be responsible for the impairment of motility in long-term treatment of paliperidone, iloperidone and loxapine. Implications for future research about sexual dysfunction, in all new treatments, should be strongly taken into account.

Keywords: iloperidone, paliperidone, loxapine, sexual dysfunction

P084: Decorin administration improves bladder function in partial bladder outlet obstruction in rabbits

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In partial bladder outlet obstruction, the bladder wall undergoes hypertrophic changes and the contractile function decreases. Decorin, located in extracelluler matrix, regulates collagen fibrils. In this study, we aimed to test whether bladder function can be protected by decorin administration in partial bladder outlet obstruction (pBOO) in rabbits.

42 male rabbits were divided into 3 groups, pBOO group, pBOO + intradetrusor decorin injected (IDI) group and control group. Both pBOO and pBOO+IDI groups were divided into 3 sub-groups (n=6 each). All but control group initially underwent pBOO procedure, pBOO and pBOO+IDI groups were sacrified on the 2th, 4th and 8th week, and control group (n=6) was sacrified on the 4th week of the obstruction and bladders were removed. 2x10 mm strips were cut and mounted in organ baths containing Krebs-Henseleit solution gassed with 95% O_2 and 5%CO_2 at 37°C. Isotonic contractions were recorded with Grass model P122 ploygraph. After equilibration, concentration-response curves (CRC) to carbachol (10^{-8}-3×10^{-4}M) were then constructed for each tissue. Thereafter, strips were incubated with antagonists (Pirenzepine, M1 and M3 muscarinic receptor antagonist, 2×10^{-6}M; 4-DAMP, M3 muscarinic receptor antagonist, 3×10^{-8}M) for 30 min before repeating CRC to carbachol. Strips were also subjected to electrical field stimulation EFS (80 V, 1 ms, 1-128 Hz, 20 s) every 4 min with Grass S88 stimulator.

CRC to carbachol in the control and pBOO+IDI groups were greater than the pBOO groups ($p<0.01$, ANOVA). In all groups, pirenzepine and 4-DAMP carbachol CRC's to the right ($p<0.01$, ANOVA). EFS values in control and pBOO+IDI groups were slightly but not significantly higher than pBOO group ($p>0.05$, ANOVA).

Decorin increases bladder function in pBOO in rabbits. The increase in the carbachol-induced contractions are caused by the increase in the bladder smooth muscle functional M_3 receptors.

Keywords: decorin, partial bladder outlet obstruction, rabbit

P085: Protective and therapeutic effects of dexpanthenol on isoproterenol-induced renal damage in rats

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The aim of this study was to determine the protective and therapeutic effects of dexpanthenol (DEX) on isoproterenol (ISO)-induced renal damage.
Forty rats were distributed into four groups: group I (Control); group II (ISO); ISO (150 mg/kg/day) was given to rats once a day for 2 consecutive days with an interval of 24 hours; group III (DEX+ISO): DEX (250 mg/kg) was applied 30 minutes before the first ISO administration and continued in the next two days after second ISO administration; group IV (ISO+DEX): After the ISO treatment at 1st and 2nd days, DEX was given at 3rd and 4th days. At the end of the experiment protocol, renal tissue levels of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), reduced glutathione (GSH), total oxidant status (TOS); total antioxidant capacity (TAC), oxidative and stress index (OSI) were determined.

SOD, CAT, TAC, GPX, GSH values indicated a significant decrease in the ISO group whereas MDA, TOS, and OSI levels were significantly higher when compared to the control group. DEX treatment significantly reduced all of these parameters in the both treatment groups (Table 1).

Our results showed that DEX provides preventive and therapeutic effects on ISO-induced renal damage in rats by inhibiting reactive oxygen species production, and enhancing antioxidant status.

Keywords: dexpanthenol, kidney, isoproterenol, oxidative stresss

Table 1. Oxidative and antioxidative status of the kidney and protective and therapeutic effects of dexpanthenol against isoproteronol-induced renal damage.

Groups n=10	MDA nmol/g tissue	SOD U/mg protein	CAT K/g protein	GPX U/mg protein
Control	11.19±1.23	2.44±0.04	35.06±3.49	231.55±14.43
ISO	22.28±2.17*	1.14±0.9*	11.76±2.10*	132.71±10.47*
ISO+DEX	10.54±2.30**	2.18±0.01**	35.42±3.17**	210.88±9.78**
DEX+ISO	11.47±1.15**	2.12±0.16**	33.12±2.25**	218.47±6.49**
	GSH µmol/g tissue	TAC mmol Trolox equivalent/L	TOS mmol H_2O_2 equivalent/g protein	OSI Arbitrary Unit
Control	6.45±0.12	0.28±0.01	4.01±0.12	14.20±1.26
ISO	2.62±0.22*	0.13±0.01**	9.64±0.01*	68.99±6.78*
ISO+DEX	6.39±0.45**	0.20±0.02**	4.35±0.15**	21.28±2.20**
DEX+ISO	6.49±0.10**	0.23±0.01*	4.39±0.10**	19.07±1.18**

**p<0.05 vs Control; **p<0.05 vs ISO. Results are presented as mean±SD. ISO: Isoproterenol; DEX: Dexpanthenol; MDA: Malondialdehyde; SOD: Superoxide dismutase; CAT: Catalase; GPX: Glutathione peroxidase; GSH: Reduced glutathione; TAC: Total antioxidative capacity; TOS: Total oxidative stresss; OSI: Oxidative stress index.*

P086: Retrospective evaluation of pregnant women consulted because of drug exposure during pregnancy

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Drug use may be necessary for diseases diagnosed during or before pregnancy. Most of such drug use is in early stages of pregnancy while mothers are often not aware of their pregnancy. The pregnant cases prescribed drugs in the primary or secondary health care units have been consulted by Karadeniz Technical University Teratogenicity Information Service(KTU-TIS) between 1999-2015. After the review of the data available in the literature, obstetricians, other physicians and the families were informed about the risk levels proposed due to the drugs and obstetric history. Overall data were retrospectively reviewed and presented in this study. Age, habituations, consanguineous marriage, Rh incompatibility, abortion history, X-ray, names of drugs, drug dosing and exposure periods were recorded and a risk assessment was performed. Gestation weeks were established with ultrasonography. The health status and development of newborns were followed up for a year. 2645 pregnant women (16-49 age) were consulted and followed up since 1999. 608 kinds of drugs were recorded. The most common drug groups were chemotherapeutics(95), drugs affecting central nervous system(94), drugs affecting gastrointestinal system(60), cardiovascular system drugs(58), analgesic/anti-inflammatory drugs(50), hormones(15), steroids(30). Pregnancies were resulted in 169 therapeutic abortions, 113 spontaneous abortions. 41 congenital abnormalities were observed among 1742 alive deliveries birth have been observed. The pregnancy status or planning must be considered while prescribing especially to women of reproductive age. In case of prescribing to women who are not aware of pregnancy mainly in early stages, information and consultation of such cases by TIS has a critical and an important role especially for preventing curettage intervention clinically not needed. Better performance for TIS units is possible when such units are organized at a national level.

Keywords: drug exposure, pregnancy, teratogenicity

P087: Medroxyprogesterone acetate use in pregnancy

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Medroxyprogesterone acetate(MPA) is a synthetic derivative of progesterone often used in treatments of secondary amenorrhea, endometriosis, contraception, etc. In some animal studies, genital (hypospadias, masculinization in females, feminization in males) and non-genital (limb defects, chromosomal abnormalities, spina bifida) anomalies have been reported. In humans, it is suggested that MPA use in early pregnancy was not associated with non-genital malformations. However, conflicting data with regard to hypospadias are also available. In this study, we evaluated the outcomes of pregnant women exposed to MPA in terms of teratogenicity. 69 pregnant women with an age range of 17-41 years who have been treated with MPA because of several gynecologic problems were consulted by Karadeniz Technical University Teratogenicity Information Service(TIS) between 2000-2015. We performed a risk assessment for the pregnant women by considering the additional risk factors. Postnatally, we have received delivery information of the infants and followed up their development. All women used MPA during first trimester of pregnancy, 66 by oral route(5-10 mg/day) and 3 by intramuscular route(150 mg). We could not contact 17 of these pregnant women. The outcomes of 52 pregnant women contacted is: 3 spontaneous abortions, 8 therapeutic abortions, one intrauterin exitus at 22th gestation week due to predisposition of thrombosis and one intrauterin exitus at 28th gestation week due to unknown reasons. Although 37 of the infants were healthy, one of them had hypothyroidism and another one had minimal atrial septal defect(ASD)-ventricular septal defect(VSD). In humans, it is reported that MPA increases the risk of perinatal mortality and low birth weight. In this study, five prenatal deaths are remarkable while compared to 37 healthy infants. One of the known reasons of prenatal deaths is the predisposition to coagulation. Therefore, it should be noted that MPA may contribute to coagulation.

Keywords: drug exposure, medroxyprogesterone acetate, pregnancy, teratogenicity

P088: The influence of risperidone and olanzapine on vas deferens in mice

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Several classes of prescription drugs contribute to sexual dysfunction in men that has been found especially with and antipsychotic drugs. Variety of mechanisms are likely to contribute to antipsychotic-related sexual dysfunction including hyperprolactinemia and antagonism of some of neurotransmitter receptors (alfa-adrenergic, dopaminergic, histaminic and muscarinic).

Male inbred mice were randomly divided into experimental groups (n=7-8) as follows: saline; risperidone 0.25 mg/kg; risperidone 0.5 mg/kg; olanzapine 1 mg/kg; olanzapine 2 mg/kg. Mice were treated by ip injection of drugs during 21 days. Mice receiving only the vehicle (0.9% saline, i.p.) during 21 days served as control group (n=7). Then, the effects of drugs were investigated on noradrenaline and KCl-induced contractile responses in isolated vas deferens strips.

Noradrenaline-induced contractile responses were significantly inhibited in the epididymal and prostatic portion of the vas deferens obtained from the risperidone and olanzapine-treatedgroup. However, risperidone and olanzapine treatment had no effect on KCl responses in both epipdidymal and prostatic portions of mice vas deferens. There were no significant differences in KCl-induced contractile responses among the groups.

Noradrenergic receptors may contribute to changes in vas deferens contractions in mice with chronic treatment of risperidone and olanzapine. These results may partly explain risperidone and olanzapine-related sexual dysfunction.

Keywords: antipsychotic drugs, risperidone, olanzapine, sexual dysfunction

P089: The effects of dehydroepiandrosterone and resveratrol on ovarian and endometrial morphology, serum hormone, oxidant and antioxidant levels in primary ovarian failure-induced rats

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Dehydroepiandrosterone (DHEA) is widely used in the treatment of infertility in patients with reduced ovarian reserve. Resveratrol(RES) is a phytoalexin with antioxidant features. We aimed to investigate the effects of DHEA and RES on the number of ovarian follicles and apoptosis in ovary and pinopod formation and epithelial thickness in endometrium in rats with 4-vinylcyclohexene diepoxide (VCD)-induced primary ovarian failure (POF). The effects of DHEA and RES on serum antimullerian hormone (AMH), estradiol(E2), follicle stimulating hormone (FSH), oxidant and antioxidant levels were also studied.

28 female Spraque Dawley rats were randomly divided into 4 groups (n=7 in each). Group1: unilateral oophorechtomy as control group; Group2: VCD-induced POF+unilateral oophorechtomy; Group3: VCD-induced POF+DHEA treatment after unilateral oophorechtomy; Group4: VCD-induced POF+RES treatment after unilateral oophorechtomy. POF was induced with the administration of VCD (160mg/kg,i.p.). DHEA (60mg/kg,i.p.) and RES (10mg/kg,i.p.) were injected for 15 days. After the drug treatmets, rats were sacrificed, intracardiac blood samples were taken, uterus and ovaries were removed out. TAS and TOS tests were performed for oxidant and antioxidant levels.

VCD reduced the number of follicles and endometrial epithelial thickness while it induced apoptosis. DHEA and RES partially reversed the toxic morphologic effects of VCD, however this couldn't reach to the levels of control group. Serum hormone, oxidant and antioxidant levels were also in accordance with the morphological changes.

DHEA and RES may be useful to increase the ovarian reserve in the treatment of women with ovarian failure. The increase in the follicles in ovarian follicle pool after the treatments suggest that the hipothesis of the 'fixed follicle number at birth' should be discussed.

This study is supported by Scientific Research Projects Unit of Ahi Evran University, Project No: TIP. E2.16.006
Keywords: DHEA, resveratrol, primary ovarian failure, TEM, immunhistochemistry

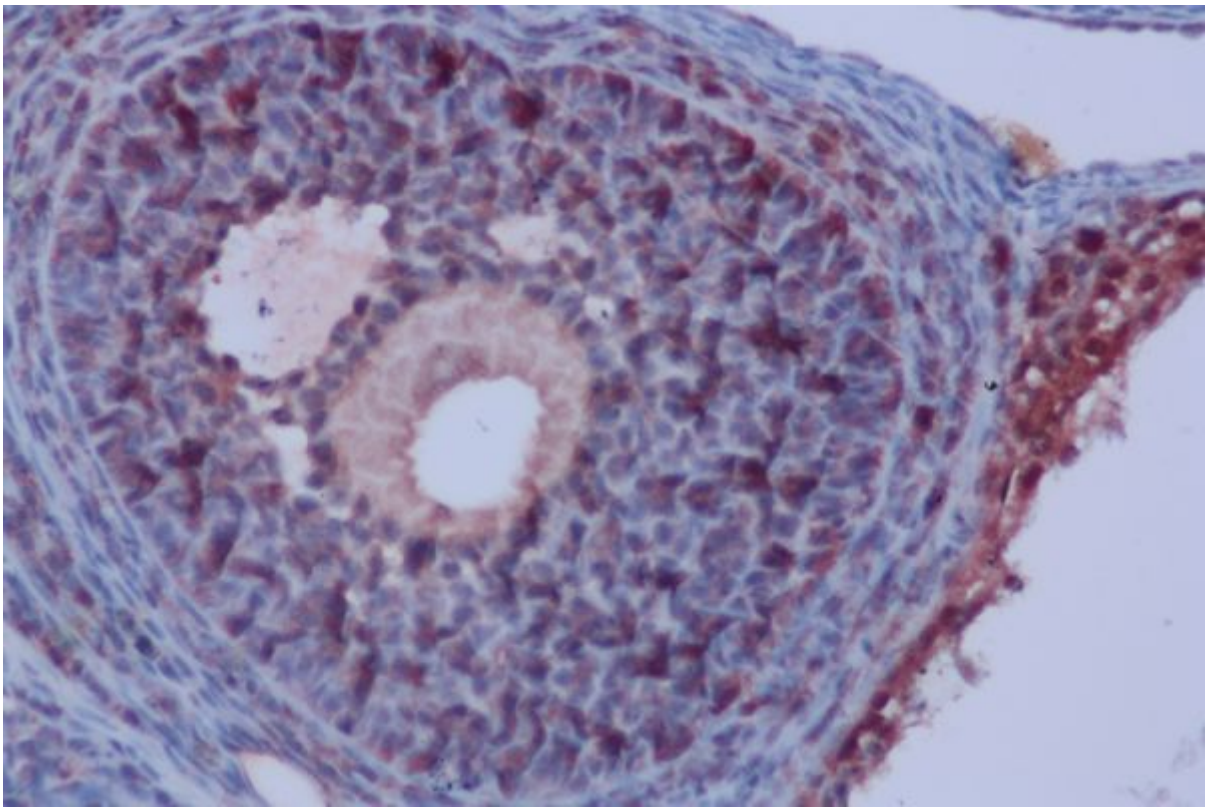

Figure 1. Group 3 ovary section tunel stain X30.

P090: Effect of a fructose rich diet on vas deferens contractility of rats

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We aimed to investigate the effects of a fructose-rich diet on vas deferens contractility and histology.

Twenty mature male rats were randomized into two groups. The control group received regular food and water and the study group received regular food and a fructose-rich liquid diet mixture of 37–40% fructose, 30–36% sucrose and 27–30% glucose instead of water, which was then diluted with 50% water. Animals were weighed and sacrificed after six weeks and the vas deferens were removed and placed in organ baths.

The mean body weights were 247.1±4.8 g in the control group and 318.8±9.3 g in the study group ($p<0.001$). The cumulative addition of ATP (10^{-7}–10^{-4}) elicited rapid, transient, concentration-dependent contractions in both groups. However, no significant differences were seen between the control and study groups ($p>0.05$). The cumulative addition of NA (10-8–10-5 M) induced repetitive phasic, concentration-dependent contractions in the vas deferens of both the control and study groups. There were no significant differences between the control and study groups ($p>0.05$). Electrical stimulation of the vas deferens strips of the normal fed rats were recorded in frequencies of 2–64 Hz. The contractile responses at the same frequencies of electrical field stimulation (EFS) were similar in both groups ($p>0.05$). Histological examinations showed no abnormalities in either group.

A fructose rich diet showed no increase in fibrotic activity, even though it produced marked obesity. The fructose-rich diet caused obesity over a short time period, while there were no changes in contractile response and histological structure of vas deferens.

Keywords: obesity, fructose diet, vas deferens, contractility

Veterinary Pharmacology

P091: Cardiac safety of gamithromycin in ewes

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Gamithromycin is used in treatment of respiratory disease of sheep as extra-label. It is well known that macrolide antibiotics have cardiotoxic effect; however, it cannot be found the cardiac safety of gamithromycin in sheep in the literature. The first aim of this research was to determine the cardiac safety of gamithromycin in sheep. In addition, hepatic, renal and bone marrow safety were investigated.

Gamithromycin (6 mg/kg, SC) was administered as a single dose to 10 sheep. Blood samples were taken before (0. day, control) and after treatments at 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 9. days. Serum creatine kinease-MB mass and troponin I levels which are specific cardiac damage markers, liver (Alkaline phosphatase, total bilirubin, alanine aminotransferase, aspartate aminotransferase, gamma glutamyltransferase, total protein), kidney (Blood urea nitrogen, creatinine) damage markers and hemogram values (White blood cell, red blood cell, platelet, hematocrit, hemoglobin) were measured.

Increased troponin I levels were determined at first day but it was not statistically significant, and there was no change determined in the creatine kinase-MB mass levels. Statistically significant ($p<0.05$) fluctuations were determined in the total bilirubin, total protein, creatinine and white blood cell counts, but these results were within the normal ranges.

It may be stated that single dose (6 mg/kg bw, SC) gamithromycin administration has no distinctive side effects on the heart, liver and kidney functions and hemogram values in sheep.

Keywords: sheep, gamithromycin, safety

P092: Effect of dexamethasone on blood thiobarbituric acid reactive substances and 13,14-dihydro-15-keto-prostaglandin-F2α levels

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The first aim of this research was to determine the effects of dexamethasone on serum thiobarbituric acid reactive substances (TBARS) and plasma 13,14-dihydro-15-keto-prostaglandin-F2α (PGM) levels in healthy rams. In addition, effects of dexamethasone on the hemogram and biochemical parameters were evaluated. Single dose dexamethasone (0.5 mg/kg, SC) was administered to 10 rams. Blood samples were collected before (0. hour, Control group) and after drug treatment at 0.25, 0.5, 1, 2, 4, 8, 12 and 24 hours. Serum TBARS and plasma PGM levels were measured with ELISA reader. Hemogram values were determined with hemacell counter, whereas biochemical values were measured with auto-analyzer. Dexamethasone caused statistically not significant fluctuations in the serum TBARS levels, while it did no effect plasma PGM levels. In addition, dexamethasone increased ($p<0.05$) white blood cell and serum glucose levels. In conclusion, it may be stated that dexamethasone has no distinctive antioxidant and antiprostaglandinF2α effects in healthy rams; however, different effects of dexamethasone may be observed in the sick situation.

Keywords: ram, dexamethasone, TBARS, PGM

Pain and Inflammation

P093: The role of opioidergic mechanisms on the anti-allodynic and antihyperalgesic effect of valnoctamide in rats

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The purpose of the present study is to assess the anti-allodynic and antihyperalgesic effect of valnoctamide, second generation derivative of antiepileptic valproic acid by using dynamic plantar and Hargreaves tests in a rat model of neuropathic pain induced by the chronic constriction injury (CCI) and to investigate the role of opioidergic mechanisms on these effects.

The dynamic plantar and Hargreaves tests are used to measure the paw withdrawal latency response to mechanical and infrared heat stimulus applied to the plantar surface, respectively. Valnoctamide was administered at the doses of 40, 70 and 100 mg/kg (i.p., single dose at 8th day after nerve injury). The involvement of opioidergic mechanisms on the effect of 100 mg/kg valnoctamide was investigated by pre-treatment with 5 mg/kg naloxone (i.p.), a non-specific opioid antagonist. 32 mg/kg (i.p.) carbamazepine was used as positive control.

The allodynia and hyperalgesia developed 7 days after CCI ($P<0.05$). Valnoctamide at the doses of 70 and 100 mg/kg significantly enhanced ($P<0.05$ and $P<0.001$) the thresholds response to mechanical and thermal stimulus in dynamic plantar and Hargreaves tests, respectively, as carbamazepine ($P<0.05$). Naloxone pre-treatment is not significantly but relatively reversed the anti-allodynic and antihyperalgesic effect of valnoctamide. These results indicate that valnoctamide has antihyperalgesic effect as well as anti-allodynic effect.

It is thought that the opioidergic mechanisms involve, in part, in its anti-allodynic and antihyperalgesic effects since the effectiveness of 100 mg/kg valnoctamide disappeared after the naloxone pre-treatment. Thereby, it is most probably that other mechanisms involve in anti-allodynia and antihyperalgesia induced by valnoctamide. The investigations related to these possible mechanisms are still going on.

This study is supported by Anadolu University Scientific Research Projects Unit (Project No: 1404S125).

Keywords: valnoctamide, neuropathic pain, opioidergic mechanisms

P094: Assessment of the antinociceptive effect of levetiracetam in a rat craniotomy pain model

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Levetiracetam, a novel antiepileptic drug, has recently been shown to produce antihyperalgesia in various animal pain models. Aim of the study was to investigate the impact of levetirecetam on stress response elicited by an experimental craniotomy pain model, as assessed by neurobehavioral testing.

Forty-two, male, Wistar rats were subjected to administration of levetirecetam (400mg/kg/d intraperitoneally), one day before and upon the day of a planned craniotomy. Spatial anxiety and cognitive behavior due to postoperative pain, and "social memory", which is similar to factual memory in humans, were assessed by elevated plus-maze and olfactory social memory test, respectively. Both tests were applied on first post-craniotomy day. Seven groups of animals (6 rats/group) were identified: 1) no intervention (control), 2) only normal saline was injected (placebo), 3) only levetirecetam was administered, 4) sham-operation was performed in placebo animals, 5) craniotomy was performed in placebo animals, 6) sham-operation was applied in levetirecetam animals and 7) craniotomy was performed in levetirecetam animals. Data were analyzed using Oneway-ANOVA and Kruskal-Wallis test.

The craniotomy rats spent less time in investigating the open arms in maze test than control ($p<0.001$) and sham-operated animals ($p=0.05$). In the olfactory social memory test craniotomy rats spent less time to investigate the same juvenile than during the first contact indicating anxiety disorder ($p=0.003$). Craniotomy rats receiving levetirecetam spent more time investigating the open arms in maze test than sham-operated and craniotomized placebo rats ($p=0.033$), while no statistical differences in olfactory social memory occurred. The ratio of entries reflected the decrease of anxiety-like avoidance behavior caused probably from postoperative pain ($p=0.003$).

It seems that preemptive use of levetirecetam attenuates stress response elicited by a craniotomy pain model. Our results suggest that levetirecetam improves anxiety-like behavior without significant impairment of social memory in rats suffering from postoperative craniotomy pain.

Keywords: levetiracetam, craniotomy pain

P095: Serum calcium levels correlate with serum cartilage oligomeric matrix protein levels in knee osteoarthritis patients

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Cartilage oligomeric matrix protein (COMP) is a osteoarthritis biomarker. COMP is a calcium-binding protein. COMP levels can be determined by enzyme-linked immunosorbent assay (ELISA), but ELISA is not popular in Indonesia, because it is expensive and requires high skills. This study aims to determine the correlation between serum calcium levels to serum COMP levels in knee osteoarthritis patients. The subjects who Participated in this study are patients who visit the orthopaedic clinic at one private hospital in Bandung, Indonesia. Serum calcium levels were determined by colorimetry and serum COMP levels were determined by ELISA. The level of serum calcium and COMP of knee osteoarthritis patients was higher than normal individuals. Elevated serum calcium levels were proportional to serum COMP levels.

Keywords: osteoarthritis, COMP serum, calcium serum, ELISA, colorimetric

P096: Investigation of local and systemic injected bone-marrow derived mesenchymal stem cells on cold allodynia and electrophysiologic parameters in mononeuropathic mice

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In this study, we evaluated possible effect of mesenchymal stem cells (MSC) on motor coordination, pain, sciatic nerve action potential and soleus muscle membrane potential in an experimental mononeuropathy model in mice.

Swiss Albino mice were used in the experiments after approval of Cukurova University Medical Science Research Center. Neuropathy was developed by ligation of right sciatic nerve. Neuropathic pain was evaluated by cold-plate (CP) and motor coordination by rota–rod (RR) tests. MSCs obtained from healthy mice injected into the muscle around the ligated nerve (local) and tail vein (systemic) 24 h after ligation. Mice were divided into control, sham, mononeuropathy (MNP), MNP+MSC injected (local, peripheric and local+peripheric) groups. CP and RR tests were performed on 4th, 6th and 10th week after ligation. Sciatic nerves and soleus muscle of mice were excised 10 weeks after ligation. Peak to peak action potential amplitude, depolarization and repolarization durations of action potentials were determined. One-way analysis of variance followed by Bonferroni test was used for statistical analysis

CP and RR latencies were decreased on 4th and 10th week. Local and peripheric MSC application enhanced these values on 4th week. Local application was more effective then peripheric application on 6th and 10th week. No difference was seen when local and pheripheric injection of MCS at the same time.

Membran potential of right soleus muscle was decreased significantly in MNP group and local MSC injection reversed it. Amplitude of sciatic nerve was decreased where depolarization and repolarization values were enhanced. Local MSC application was more effective then peripheric application in these groups.

The results show that persistent neuropathy was developed in sciatic nerve ligated mice. Local and peripheric MSC injection ameliorates painful behaviors, motor functions, electrophysiologic parameters and local application of MSC is more effective then peripheric route.

Keywords: peripheral neuropathy, pain, mesenchymal stem cell, electrophysiologic parameters, mice

P097: Contribution of platelet $P2Y_{12}$ receptors to chronic inflammatory pain

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Platelet $P2Y_{12}$ receptor inhibition is widely used in clinical practice to prevent stroke and myocardial infarction. In this study, we explored the contribution of platelet $P2Y_{12}$ receptors to chronic inflammatory pain.

We investigated the role of $P2Y_{12}$ receptors in complete Freund's adjuvant (CFA)-induced chronic inflammatory pain using P2ry12 gene-deficient mice (P2ry12-/-), and the potent, direct-acting and reversible $P2Y_{12}$ receptor antagonists, PSB-0739, and cangrelor. Mechanical sensitivity of the hind paws was measured using a dynamic plantar aesthesiometer. Myeloperoxidase activity was visualized and traced with in vivo imaging techniqe. Samples from the hind paws were collected to measure the levels of the following inflammatory mediators: IL-1α, IL-1β, IL-6, IL-10, TNF-alpha and KC (CXCL1) by flow cytometry. To investigate the contribution of platelet $P2Y_{12}$ receptors in this chronic inflammatory pain model, a specific antibody raised against CD41 was used to deplete platelets.

CFA-induced mechanical hyperalgesia was significantly decreased in P2ry12-/- mice for up to 14 days, and increased neutrophil myeloperoxidase activity as well as TNF-alpha and KC levels in the hind paws were also alleviated in the acute inflammation phase. At day 14, elevated IL-1β, IL-6, TNF-alpha and KC levels were attenuated in P2ry12-/- mice. PSB-0739 and cangrelor reversed hyperalgesia in the wild-type but had no effect in the P2ry12-/- mice, and PSB-0739 was also effective when applied locally. Platelet depletion by anti-mouse CD41 antibody alleviated hyperalgesia and attenuated the pro-inflammatory cytokine response in wild-type but not in P2ry12-/- mice on the 14th day.

In conclusion, $P2Y_{12}$ receptors regulate CFA-induced hyperalgesia and local inflammatory response, and platelet $P2Y_{12}$ receptors contribute to these effects in the chronic inflammation phase.

Keywords: $P2Y_{12}$, platelet, pain, inflammation, cytokines

P098: Anti-inflammatory activity of neurotensin-peptide hybrid in murine model of contact sensitivity response

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The objective of the study was to investigate the possibility of modulation of skin inflammation by topical treatment with novel compound: opioid-neurotensin hybrid peptide-PK20. Its peptide scaffold has been modified by the presence of additional and unnatural amino acids in order to obtain a better enzymatic stability. It was hypothesized that performed hybridization of neurotensin and endomorphine-2 pharmacophores would positively influence the inhibition or reduction of several indications of inflammation such as local infiltration of inflammatory cells, tissue edema, and production of pro-inflammatory cytokines.

Contact sensitivity response was induced in mice by skin sensitization with dinitrofluorobenzene (DNFB) followed by topical hapten application on the ears. Mice were treated locally 2 hours after the hapten challenge. 2 and 24 h after the challenge, ear thickness was determined. Ears were collected and homogenated. The supernatants were used for measurement of contents of cytokines and lipid peroxidation products.

Treatment with PK20 reduced significantly the late phase of contact sensitivity response, which was confirmed by 22% ear thickness diminution. The average concentrations of IL-1α, MCP-1, TNF-alpha and thiobarbituric acid-reactive substances were significantly decreased in the ears treated with the chimera in comparison to the control cream treated ears. In the same time control/vehicle treated animals showed vestigial levels of cytokines and no tissue swelling in both: cream and PK20 smeared ears. The latter indicates that chimeric peptide did not produce any adverse effects in healthy tissue.

We found that PK20 topical treatment alleviates hypersensitivity response triggered by DNFB challenge but the mechanism remains unclear and needs further investigation.

The study is supported by a grant from National Science Centre, Poland, No. 014/13/B/NZ7/02247.

Keywords: contact sensitivity, ear swelling, cytokines, hybrid peptide

P099: Effects of nociceptin receptor antagonism on experimentally-induced scratching behavior in mice

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Itch and pain are two distressing sensations sharing a lot in common. In addition to periphery, central nervous system is proposed as a therapeutic target for the development of antipruritic drugs. The contribution of the most recently discovered opioid peptide, nociceptin/orphanin FQ (N/OFQ), and its receptor (NOP) in pain transmission is controversial. It seems to be pronociceptive when given supraspinally, but elicit antinociceptive action when injected spinally.

Considering the discrepancies in the effects of N/OFQ and NOP receptors in modulation of pain and the similarities between pain and itch, we aimed to observe whether local and systemic NOP receptor antagonism influence serotonin- and/or nociceptin-induced scratching behavior in mice.

Scratching behavior was induced by intradermal injection of 50 µg/50 µL of serotonin or 30 nmol/50 µl of nociceptin into the pre-shaved rostral part of the back of the female Balb/c mice. Immediately after intradermal serotonin or nociceptin administration, scratching of the injected site by the hind-paws were videotaped and counted for 30 min under quiet circumstances. To evaluate the effect of NOP receptor antagonism on serotonin- and nociceptin-induced scratches, different doses of JTC-801 (1, 3, 10 mg/kg, i.p.), a NOP receptor antagonist, was given systemically. JTC-801 (100 nmol/50 µl) was also administered intradermally to determine its peripheral effect.

JTC-801 (1, 3, 10 mg/kg, i.p.), attenuated both serotonin- and nociceptin-induced scratches. When given intradermally, JTC-801 (100 nmol) reduced serotonin-induced but not nociceptin-induced scratches. We propose that antagonizing NOP receptors either systemically or locally may be a novel target for the development of antipruritic agents.

This work is supported by a grant from Trakya University Research Council (TUBAP-2013/17).
Keywords: nociceptin, NOP receptors, JTC-801, pruritus

P100: Contribution of nociceptin/orphanin FQ receptors to the antinociceptive and hypothermic effects of dipyrone

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Dipyrone (metamizole) is one of the most commonly used non-opioid analgesic and antipyretic drugs. Its antinociceptive and hypothermic effects have long been suspected to be centrally mediated. The involvement of the most recently discovered opioid peptide, nociceptin/orphanin FQ (N/OFQ), and its receptor (NOP) in pain transmission is controversial. It appears to be pronociceptive when administered supraspinally, but exerts antinociceptive effects when injected spinally or systemically. Like dipyrone, evidence also suggests a central component to the mechanism of action of paracetamol. N/OFQ administration has been shown to prevent the antinociceptive action of paracetamol on the rat hot-plate test.

Taking into account the similarities between dipyrone and paracetamol, the purpose of this work was to determine whether NOP receptors are involved in the antinociceptive and hypothermic effect of dipyrone.

Adult male Balb/c albino mice weighing 20-30 g were used. Hot-plate and tail-flick tests were used to assess nociception, and a rectal thermometer was used to measure rectal temperature in mice. Test latencies were converted to the percentage of the maximal possible effect (%MPE) according to the following equation: %MPE =

[(post-drug latency – baseline latency)/cut-off time – baseline latency] x 100

Mice injected with dipyrone (150, 300, 600 mg/kg, i.p.) displayed dose-related anti-nociception and hypothermia. The NOP receptor antagonist JTC-801 (3 mg/kg, i.p.), at a dose that exerted no effect when used alone, alleviated dipyrone-induced anti-nociception but did not reverse dipyrone-induced hypothermia.

We conclude that NOP receptors participate in the anti-nociceptive, but not in the hypothermic, effects of dipyrone.

This work is supported by a grant from Trakya University Research Council (TUBAP-2013/32).

Keywords: dipyrone, N/OFQ, JTC-801, antinociception, hypothermia

P101: Involvement of descending serotonergic and noradrenergic systems and their spinal receptor subtypes in the antinociceptive effect of dipyrone

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The antinociceptive effect of dipyrone (metamizole) is partly due to its action upon pain-related central nervous system structures. Despite intensive research, the precise mechanisms mediating its analgesic effects remain unclear.

We aimed to determine whether neurotoxic destruction of descending inhibitory pathways affect dipyrone-induced antinociception and whether various spinal serotonergic and adrenergic receptors are involved in this antinociception

Male Balb/c mice (Center of the Laboratory Animals, Trakya University) weighing about 20-30 g were used throughout the study. The nociceptive response was assessed by the tail-flick test. Test latencies were converted to the percentage of the maximal possible effect (%MPE) according to the following formula: %MPE =

[(post-drug latency – baseline latency)/cut-off time – baseline latency] x 100. Mice injected with dipyrone (150, 300, 600 mg/kg, i.p.) elicited dose-related antinociception. The neurotoxins 5,7-dihydroxytryptamine (50 µg/mouse) and 6-hydroxydopamine (20 µg/mouse) are applied intrathecally to deplete serotonin and noradrenaline in the spinal cord.

Three days after neurotoxin injections, a significant reduction in the antinociceptive effect of dipyrone was observed. Intrathecal administration of monoaminergic antagonists (10 µg/mouse), the 5-HT2a antagonist ketanserin, the 5-HT3 antagonist ondansetron, the 5-HT7 antagonist SB-258719, α1-adrenoceptor antagonist prazosin, α2-adrenoceptor antagonist yohimbine, and the β-adrenoceptor antagonist propranolol also attenuated dipyrone antinociception.

Conclusion: We propose that descending serotonergic and noradrenergic pathways play pivotal role in dipyrone-induced antinociception and spinal 5-HT2a, 5-HT3, and 5-HT7-serotonergic and α1, α2, and β-adrenergic receptors mediate this effect.

This work is supported by a grant from Trakya University Research Council (TUBAP-2012/145).

Keywords: antinociception, descending inhibition, dipyrone, noradrenergic system, serotonergic system

P102: Histopathological characteristics of sciatic nerve and soleus muscle tissues after local and systemic administration of mesenchymal stem cells in mononeuropathic mice

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Our aim was to investigate the effect of local and systemic administration of mesenchymal stem cells (MSCs) on endogenous repair processes in the sciatic nerve and soleus muscle histology.

Swiss Albino mice were used after receiving approval from Cukurova University Medical Science Research Center. MSCs were injected into the muscle around the ligated right sciatic nerve (local) and tail vein (systemic) 24 hours after ligation. Mice were divided into control, sham, mononeuropathy (MNP), MNP+MSC injected (local, peripheric and local+peripheric) groups. Sciatic nerve and soleus muscle isolated from mice were examined histologically ten weeks after injections.

Normal muscle view and vascularization was seen in the control group microscopy. The distribution of small myelinated axons and normal endoneurium in the nerve preparations was determined. In MNP group extensive muscle fiber atrophy, compensatory hypertrophy in neighboring muscle fibers and increased internal nucleus was observed. Loss of large myelinated axons, large groups of regenerating axon and endoneurium fibrosis was observed in the nerve fibers of same groups. Rare scattered angular muscle fiber atrophy and occasional small groups of atrophic muscle fibers were detected in local and systemically injected MSCs group. In the nerve fibers large and small myelinated axons were found that were conserved in a wide area. Schwann cell proliferation, increased regenerate axon sites and light connective tissue was determined in endoneurium.

The results show that local and systemic administration of MSCs results partial regeneration of Nerve muscle tissues in MNP groups. However there were no significant difference between groups.

Keywords: peripherial neuropathy, mesenchymal stem cell, histopathological characteristic, mice

P103: The antinociceptive effect of intravenous pregabalin in colorectal distension-induced visceral pain in rats

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Pregabalin, a gamma-aminobutyric acid analog, is known to inhibit the α2δ subunit of the voltage-gated calcium channel. In this study, we investigated the effect of pregabalin on colorectal distension induced visceral pain in rats.

Male Sprague Dawley rats (250–300 g) were implanted with venous catheters for drug administration and implanted with enamelled nichrome electrodes for electromyography of the external oblique muscles. The rats were fully awake and placed inside the Bollman cages during testing. The descending colon and rectum were distended to 80 mm Hg for 20 s, which is noxious in the rat, by air inflation of a 6–7 cm long, flexible latex balloon tied around a flexible plastic tube. The visceromotor response to noxious colorectal distension (CRD), quantified electromyographically, was recorded before and at every 10 min throughout 90 min after the administration of saline or pregabalin.

Administration of saline or pregabalin (25 mg/kg) had no effect on the visceromotor response induced by CRD. However, pregabalin at doses of 50, 100, and 200 mg/kg elicited a dose-dependent antinociceptive effect on CRD-induced visceral pain ($p<0.05$-0.001). Our data indicate that, in the CRD-induced rat visceral pain model, intravenous pregabalin appeared to have antinociceptive potential on visceral pain in rats. The mechanism of this visceral antinociceptive effect needs to be elucidated by further studies.

Keywords: pregabalin, visceral pain, rat

P104: Effect of ceftiofur on hyperalgesia and allodynia in rat neuropathic pain model and the role of immune processes on this effect

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Inflammatory and immune mechanisms and nitric oxide (NO) play a role in the pathogenesis of neuropathic pain (NP). Ceftiofur, a third-generation cephalosporine, has antiinflammatory effects; it inhibits the signal of tumor necrosis factor-alpha (TNF-alpha), interleukin-1β (IL-1β), nuclear factor-kappa-B (NF-κB) and mitogen activated protein kinase (MAPK). This study aimed to investigate the effect of repeated application of ceftiofur on hyperalgesia and allodynia in rat NP and to define the possible contribution of immune mechanisms and NO formation in this effect.

NP model was performed by ligating the right sciatic nerves of rats. Mechanic hyperalgesia and allodynia was measured by analgesimeter and dynamic plantar esthesiometer respectively. Following sciatic nerve ligation, ceftiofur was administered intraperitoneally (i.p.) at 10 and 20 mg/kg/day doses for 14 days. Control group was injected with 1 ml/kg i.p. saline. Pain thresholds were recorded preoperatively and 3rd, 7th, 10th, and 14th postoperative days in all groups. 3rd and 4th lumbar section of the spinal cord was removed under anesthesia on day 14 and protein expressions of TNF-alpha, IL-1β, p65NF-κB, p38MAPK and iNOS were evaluated by Western Blotting technique in the homogenised spinal cord tissue.

Rats with NP showed decreased pain thresholds in analgesiometer and estheziometer. Ceftiofur at 20 mg/kg/day, but not at 10 mg/kg/day, increased hyperalgesia threshold significantly in the right and left paw comparing to the control group. Allodynia thresholds did not change significantly. Ceftiofur attenuated the increased iNOS and IL-1β tissue levels in both doses and p38MAPK levels only at 20 mg/kg/day. TNF-alpha and p65NF-κB expressions remained unchanged.

Ceftiofur application to rats with NP at repeated doses for 14 days showed antihyperalgesic effect. Decrease in lumbar spinal cord iNOS, IL-1β and p38MAPK levels may play a role in this effect.

This study is supported by Ege University Research Fund (2010-TIP-082).
Keywords: ceftiofur, immune mechanisms, neuropathic pain

P105: The effects of silver nanoparticles and graphene induced with electro-magnetic fields on human and bacterial cells

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For the first time, the correlation between shape and concentration of silver nanoparticles (AgNPs) was studied. We assessed their cytotoxicity, antimicrobial ability, and formation of reactive oxygen species (ROS) in the presence of electromagnetic fields (EMF). In addition, we researched the bio-effects caused by the combination of EMF and graphene.

The AgNPs of three shapes (triangular, spherical and colloidal) and graphene were added in four concentrations to the culture of human fibroblasts, and exposed to EMF of three different frequencies: 900, 2400 and 7500 MHz. For antimicrobial tests, AgNPs and graphene were incubated with *Staphylococcus aureus* after exposure to EMF frequencies of 900 and 7500 MHz for different time periods, up to 1 hour.

The acquired data demonstrated the dependence of cytotoxic effect on the shape and concentration of AgNPs. Moreover, the results indicated the direct correlation between cytotoxicity and frequency of EMF, where maximal cell killing effect was observed at 900 MHz frequency for all particles' shapes and concentrations. These data conform to the results of temperature monitoring, where frequency-dependent rise of temperature was detected with maximal peak at 900 MHz for all particles. The highest temperature elevation was observed for graphene solutions irradiated by 900 MHz frequency. The exposure to EMF led to significant increase of ROS formation in triangular and colloidal AgNPs solutions. However, we did not observe any impact of EMF on ROS production for spherical AgNPs. The lowest ROS production rate was observed for 900 MHz, while the highest one was in the groups irradiated with frequency 7500 MHz. Notably, graphene nanoparticles demonstrated ROS-protective potential, which has had concentration-dependent character. This could be potentially extended for the protection of cells against ischemia and oxidative stresss. Antimicrobial properties of AgNPs were only slightly affected by EMF and only in triangular shaped particles.

Keywords: silver nanoparticles, graphene, electro-magnetic field

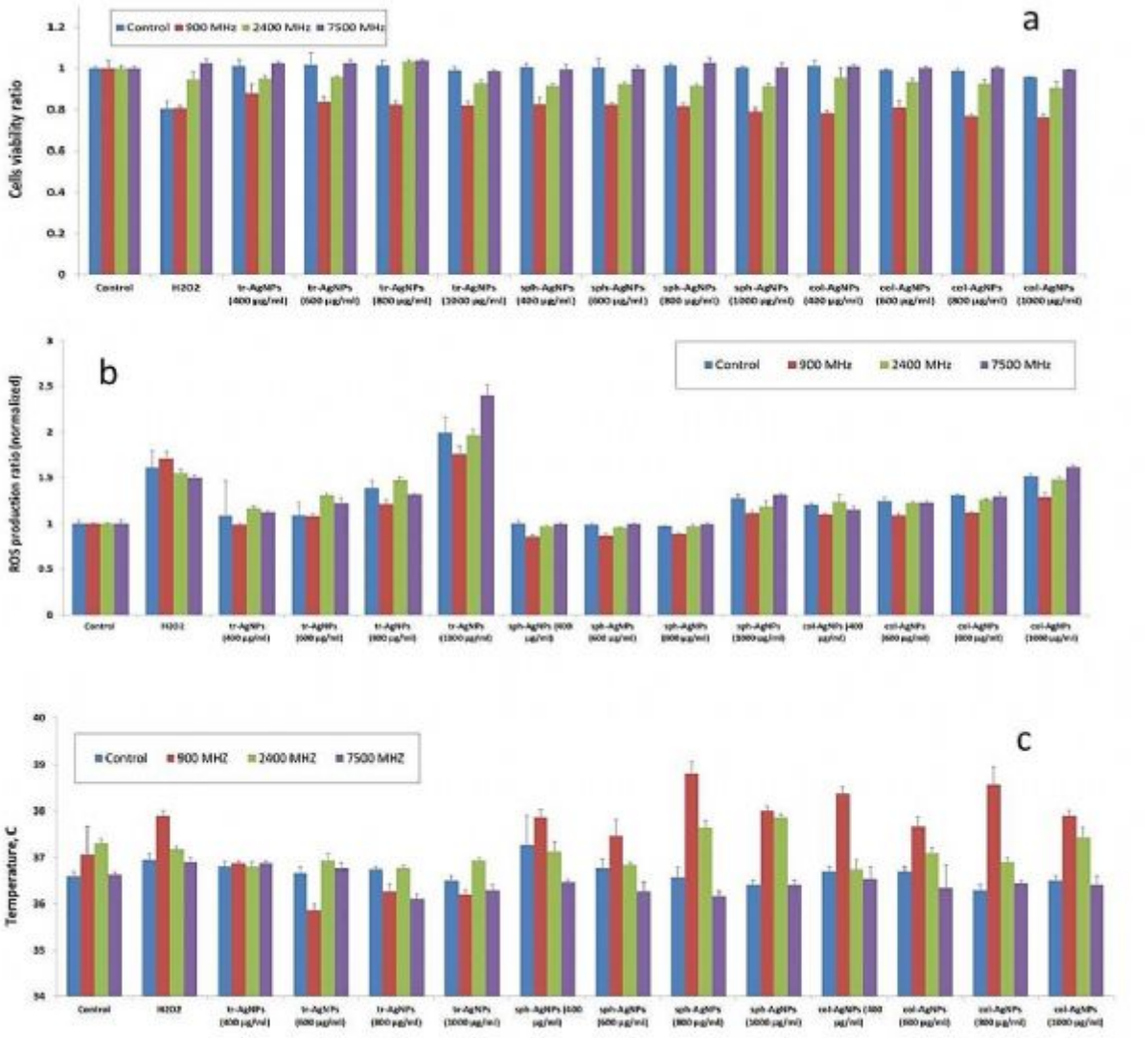

Figure 1. The bio-effects of combination of AgNPs and EMF.
The bio-effects of combination of AgNPs and EMF. AgNPs are presented in four concentrations (400, 600, 800 and 1000 µg/ml). Hydrogen peroxide (H202) is a positive control. Three EM frequencies were applied: 900, 2400 and 7500 MHz. a) cell viability normalized ratio; b) ROS production (normalized ratio); 3) data of temperature monitoring (C0). Bars represent means (n = 3).

P106: The specific activity of vaginal suppositories based probiotic consortium

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We investigated the specific activity of the vaginal suppositories with probiotic consortium on the model of bacterial vaginosis in animals. Disorders of vaginal microecology - bacterial vaginosis - the most widely common in women of reproductive age.

Object of research - vaginal probiotic suppositories (test- complex) with consortium lactobacilli (L): L. salivarius, L. fermentum and gelatin-glycerin base. Number of viable cells of lactic acid bacteria after the lyophilised drying reaches 5,2x1011 CFU/ml.

We studied a model of bacterial vaginosis on the white laboratory rats of both sexes (3 groups of 12 animals) weighing 200-220 g. Model of bacterial vaginosis were obtained by co-administration of intragastric within 7 days of antibiotics: ampicillin at the rate of 15 mg per animal and metronidazole 10 mg per animal. Model of vaginosis were confirmed by microbiologically and histomorphologically. In the experiment: placebo were suppositories with a gelatin-glycerin; comparative preparation - suppositories "Acilact" with acidophilic lactobacilli. Intravaginal suppositories were administered to animals for 10 days, one time per day.

Microbiological analysis showed: the test-complex provides the formation of normal vaginal microbiota and bacterial vaginosis picture eliminates. The study found a significant improvement in the structure of the epithelial sheet, reducing the inflammatory response after 10 days administration. Application "Acilact" are also associated with improvement in histologic picture of vaginal tissue, but with the preservation of inflammatory infiltration in the vaginal mucosa and severe disorders of blood circulation. In the placebo and in the control groups were noted the stable worsening of the morphological picture of the vagina during the whole period, which was characterized by severe pathological changes.

We determined the specific efficacy of vaginal suppository with probiotic consortium on the model of bacterial vaginosis in animals which exceed efficiency of comparative preparation - vaginal suppositories "Acilact."

Keywords: probiotic consortium, bacterial vaginosis, inflammatory infiltration

P107: Effects of agmatine on cisplatin-induced neuropathy and neurotoxicity

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Cisplatin is a commonly used antineoplastic agent for the treatment of several solid tumors. Peripheral neuropathy is one of its major side effects. Agmatine has been shown to relieve neuropathic pain in different animal models. The aim of this study was to investigate the in vivo and in vitro effects of agmatine on cisplatin-induced neuropathy and neurotoxicity respectively.

Neuropathy was induced in male Wistar rats (250-300 g, n=6) by intraperitoneal injections of cisplatin 2 mg/kg twice a week for five consecutive weeks with the total of nine injections. 2 ml of saline was also injected to prevent nephrotoxicity. Single dose of agmatine (100 mg/kg) was given intraperitoneally. The development of neuropathy was evaluated using tests for both mechanical allodynia and heat-hypo/hyperalgesia. After 5 weeks of treatments, cardiac perfusion with paraformaldehyde was performed and dorsal root ganglion (DRG) neurons were collected for further electron microscopic evaluation. Moreover, primary cultures of DRG were prepared from 1-day old rats. The neurotoxic effects of cisplatin were evaluated by incubating the cells with cisplatin (50-500 µM). 200 µM concentration of cisplatin which showed submaximal neurotoxicity was used alone and with 10-500 µM concentration of agmatine for determining its possible neuroprotective activity. MTT assay was used to detect the toxicity on DRG cells. Results were evaluated by using ELISA test system at a wavelength of 450 nm.

Cisplatin treatment induced mechanical allodynia but there was no alteration in thermal sensitivity. Single dose of agmatine significantly attenuated cisplatin-induced mechanical allodynia. Cisplatin had dose-dependent neurotoxic effects on DRG in vitro and agmatine did not alter this effect.

It seems that agmatine may have antinociceptive activity on cisplatin-induced neuropathy but is not sufficient to prevent neurotoxicity of cisplatin in vitro. Other mechanisms may involve in antinociceptive effect of agmatine on cisplatin-induced neuropathy.

Keywords: agmatine, neuropathic pain, neurotoxicity, dorsal root ganglion

P108: Effects of anandamide on cisplatin-induced neuropathy and neurotoxicity

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Cisplatin is a widely used antineoplastic drug in the treatment of malignancies. However, peripheral neuropathy is one of its major side effects which reduces the life quality of the patients. Cannabinoids have shown analgesic features in neuropathic pain models. The aim of this study was to investigate the in vivo and in vitro effects of anandamide, a cannabinoid receptor agonist, on cisplatin-induced neuropathy and neurotoxicity respectively.

Neuropathy was induced in male Wistar rats (250-300 g, n=6) by intraperitoneal injections of cisplatin 3 mg/kg once a week for five consecutive weeks. Single dose of anandamide (1 mg/kg) was given intraperitoneally. The development of neuropathy was evaluated using tests for both mechanical allodynia and heat-hypo/hyperalgesia. Spontaneous locomotor activity, rectal temperature, antinociception and catalepsy were evaluated for cannabinoid tetrad. After 5 weeks of treatments, cardiac perfusion with paraformaldehyde was performed and dorsal root ganglion (DRG) neurons were collected for further electron microscopic evaluation. Primary cultures of DRG were also prepared from 1-day old rats. The toxic effects of cisplatin were evaluated by incubating the cells with cisplatin (50-500 µM) alone and with cisplatin 200 µM, the concentration which showed submaximal neurotoxicity, in combination with anandamide (10-1000 µM). MTT assay was used to detect the toxicity of DRG cells. Results were evaluated by using ELISA test system at a wavelength of 450 nm.

Cisplatin treatment induced thermal hyperalgesia. However, single dose of anadamide did not alleviate cisplatin-induced thermal hyperalgesia. Cisplatin had dose-dependent neurotoxic effects on DRG in vitro and high concentration of anandamide attenuated cisplatin neurotoxicity.

We suggest that exogenous cannabinoid may represent a promising new protective strategy against cisplatin neurotoxicity.

Keywords: anandamide, neuropathic pain, neurotoxicity, dorsal root ganglion

P109: Laboratory data and mannose-binding lectin characteristics in Turkish children with a history of acute rheumatic fever related carditis

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Acute rheumatic fever (ARF) is the leading cause of acquired heart diseases in children and adolescents worldwide. The present study aims to determine the mannose-binding lectin (MBL) protein levels and molecular features of the Turkish children who are diagnosed with ARF related carditis.

This is a retrospective review of 100 children who were hospitalized due to the diagnosis of ARF related acute stage carditis and 100 healthy children who were matched with respect to age and body mass index. MBL levels were measured with ELISA kits. Genotyping of exon 1 and of the X/Y promoter region was carried out by sequence-specific primed polymerase chain reaction (PCR) using specific primers for the mutations in the MBL.

The carditis and control groups were statistically similar in aspect of the laboratory findings including hemoglobin, red cell distribution width, leukocyte, neutrophil, lymphocyte and platelet count. The carditis group had significantly higher serum C-reactive protein levels than those of the control group ($p<0.001$). There is no significant change in MBL protein levels and genetic characteristics between control and carditis groups.

C-reactive protein can be used to assess the cardiac functions in pediatric ARF patients but MBL is not a definitive criterion for diagnosis.

Keywords: acute rheumatic fever, carditis, child; mannan binding lectin, C-reactive protein

P110: Antiinflamatory effect of carvacrol on cotton pellet granuloma test

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Essential oils containing carvacrol are used for varios diseases including inflammation since antiquity in East Meditterenaan countries.

Carvacrol is a volatile monoterpene widely found in nature including essential oils of the Labiatae family and it is shown to interact by transient receptor potential (TRP) ion channels. Thus carvacrol has recently become an important agent for investigating the TRP related mechanisms.

The aim of the present study is to investigate the effect of carvacrol on proliferative phase using cotton pellet method on rats.

Three different doses (10, 50 and 100 mg.kg-1) of commercially purchased carvacrol diluted in DMSO was used as test substance and ruthenium red as TRP bloker, acetylsalicylic acid and indomethacine was used as standard antiinflammatory drugs. Adult Sprague Dawley rats of either sex were anesthetized by propofol prior to the surgicallly implantation of sterilized and dried cotton pellets into the subcutaneous parts of scapular regions.

Following the application of the test subtances and standard drugs for 7 days intraperitoneally, pellets were removed, dried and weighed.

ANOVA and Tukey HSD was used for statistical evaluation using packages of R programming language was used for statistical evaluation.

Carvacrol was observed to inhibit inflammation at 50 and 100 mg.kg-1 but not at 10 mg.kg-1.

Ruthenium red was observed not to abolish the antiinflammatory action of carvacrol. Since there are 27 TRP members in mammalian systems, our results suggest a complex interaction of the carvacrol molecule on TRP ion channels.

Keywords: carvacrol, inflammation, granuloma, TRP channel

P111: Efficacy of pulsed radiofrequency therapy to dorsal root ganglion adding to transcutaneous electrical nerve stimulation and exercise for persistent pain after total knee arthroplasty

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The majority of patients achieve substantial pain relief and improved function after total knee arthroplasty (TKA), but a proportion continues to experience life-disturbing persistent postsurgical pain (PPSP) in the months and years after surgery. This study aimed to assess the efficacy of transcutaneous electrical nerve stimulation (TENS), exercise and pulsed radiofrequency (PRF) treatment on pain severity, neuropathic pain, knee flexion range of motion (ROM), functional status and patient satisfaction in patients with PPSP after TKA. This is a retrospective study of prospectively collected data. Patients who were identified retrospectively from hospital charts were divided into two groups: Group 1 (n = 17) received TENS and exercise treatment and Group 2 (n = 22) received TENS, exercise and PRF application to the dorsal root ganglion (DRG). The following procedure-related parameters were collected from the special registry form: visual analogue scale (VAS), Douleur Neuropathique 4 (DN4) questionnaire, knee flexion ROM, the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), patient satisfaction scale scores. The mean follow-up was 253.8±109 days. When the two groups were compared, a significant difference of at least a 50% improvement in the VAS (activity) and a significant reduction in the DN4 scores following the last control examination were found in Group 2. There was a significant reduction in total WOMAC scores in Group 1 compared to Group 2 for the four study periods. Higher scores for the patient satisfaction scale were found in Group 1 compared to Group 2 following the last control examination.

Adding PRF to TENS and exercise therapy is useful in reducing the degree of pain and the neuropathic component of PPSP in patients with PPSP.

Keywords: persistent postsurgical pain, total knee arthroplasty, transcutaneous electrical nerve stimulation, pulsed radiofrequency

P112: Pulsed radiofrequency applied to the dorsal root ganglia for treatment of post-stroke complex regional pain syndrome: A case series

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Complex regional pain syndrome (CRPS) is a painful and disabling syndrome where the patient presents with neuropathic pain, edema, or vasomotor or pseudomotor abnormalities that are often refractory to treatment. CRPS type 1 may occurs in stroke patients. Pulsed radiofrequency (PRF) is a therapeutic modality that has been used for years for diseases associated with neuropathic pain. We present two cases that suffered from CRPS type 1 after stroke and were successfully treated with PRF current application to the cervical dorsal root ganglia (DRG).

We present two cases of a 69-year-old and a 48-year-old women who suffered CRPS type 1 after stroke and who were resistant to medical therapy, physical therapy, and neurological rehabilitation. PRF current application to the cervical 5 (C5) and cervical 6 (C6) DRG was administered to patients. A marked improvement in the severity of pain, increased range of motion of the affected joints and muscle strength, and a favorable response to the rehabilitation program were observed after the application of PRF compared to pretreatment levels.

To our best knowledge, this is the first case report in the literature on the application of PRF to DRG for treatment of CRPS type 1 after stroke. This case suggests that PRF applied to C5 and C6 DRGs might play a significant role in multi-modal approach of CRPS type 1 management after stroke. Further randomized, controlled studies are needed to support this argument.

Keywords: stroke, complex regional pain syndrome, pulsed radiofrequency, neuropathic pain

P113: Ethanol extract of Cumin (*Cuminum cyminum L.*) seeds inhibited lipopolysaccharide-induced inflammatory responses in Raw 264.7 cells

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Inflammation is a process that involves multiple factors that act in concert. The ingress of leukocytes into sites of inflammation is an important aspect of the pathogenesis of inflammatory conditions. Macrophages are recruited to inflammatory sites, and are activated by various signals that stimulate many intracellular cascades of cytokines and chemokines. In macrophages, lipopolysaccharide (LPS), a well-known endotoxin, induces the productions of inflammatory cytokines, such as, tumor necrosis factor-α (TNF-alpha) and interleukin-1β (IL-1 β), and inflammatory mediators, such as, nitric oxide (NO) and prostaglandin E2 (PGE_2), which are synthesized by inducible NO synthase (iNOS) and cyclooxygenase-2 (COX-2), respectively. Part of the ongoing research is focused on searching for anti-inflammatory compounds from natural sources, so we investigated the effects of Cumin (Cuminum cyminum L.) seeds on inflammation induced by lipopolysaccharide (LPS) in Raw 264.7 cells to test the hypothesis that anti-inflammatory effects of Cumin seeds.

In this study, concentration of 0, 10, 20, 30, 40 and 50 µg/mL ethanol extract of Cumin seeds were treated to LPS stimulated Raw 264.7 cells.

Although 10, 20 and 30 µg/mL Cumin seed extract didn't alter the inflammatory responce, 40 and 50 µg/mL Cumin seed extract lowered nitric oxide production and iNOS gene expression in LPS-stimulated Raw 264.7 cells by 40%. However, the level of PGE_2 and expression of COX-2 were not altered in any Cumin seed extract concentration. On the other hand, treatment of cells with 30, 40 and 50 µg/mL Cumin seed extract before LPS stimulation also reduced the mRNA expression of proinflammatory cytokines including TNF-alpha and IL-1β.

These results suggested that ethanol extract of Cuminum cyminum seeds exerted anti-inflammatory effects in LPS-stimulated RAW 264.7 cells; the extract could be used as a source of anti-inflammatory agents as well as dietary complement for health promotion.

Keywords: anti-inflammatory, Raw 264.7, LPS, Cuminum cyminum

Neuropharmacology / Psychopharmacology

P114: Effects of carbamazepine (CBZ) and/or phenytoin (PHE) on liver enzymes and urea in Wistar Rats

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Epilepsy encompasses a group of syndromes with varying pathology and seizure type. Seizure is characterized by episodic high frequency discharge of impulses by a group of neurones. CBZ, an anticonvulsant administered alone or combined with other medications reduces sustained repetitive firing in neurones by blocking voltage-gated sodium channels. PHE, a classical antiepileptic drug acts by blocking sodium channels and inhibiting persistent sodium currents in neurones thus, inhibiting neuronal firing in the brain.

Forty adult male Wistar rats were used for the experiment. They were divided into 4 groups of 10 animals each. Rats in groups II, III and IV were given CBZ (20mg/kg), PHE (100mg/kg) and CBZ+PHE (20 and 100mg/kg separately), respectively. Rats in group I were given distilled water at 2ml/kg, as control. Administration was oral, by gavage daily for eight weeks, rats were sacrificed and serum samples obtained for analysis of urea and activities of liver enzymes. Values were expressed as mean ±SEM, subjected to statistical analysis using repeated- measures ANOVA with Tukey's post-hoc test. Values of $P<0.05$ were considered significant.

The research was conducted in accordance with the University guidelines on Animal Research and National Institutes of Health Guide for Care and Use of Laboratory Animals (1985).

There was a decrease in urea ($P<0.05$) in the CBZ and PHE groups; this may result from impaired protein metabolism due to persistent assault on the liver by the drugs or their metabolites. The activities of ALT in the CBZ and CBZ+PHE groups and that of AST in the PHE group increased ($P<0.05$) due to hepatocellular damage; this has been implicated in long-term phenytoin therapy in rats.

Administration of CBZ and/or PHE induced transient changes in urea and liver enzymes in Wistar rats and can be reversed when the drugs are discontinued.

Keywords: carbamazepine (CBZ) , phenytoin (PHE), liver enzymes, electrolytes, serum proteins

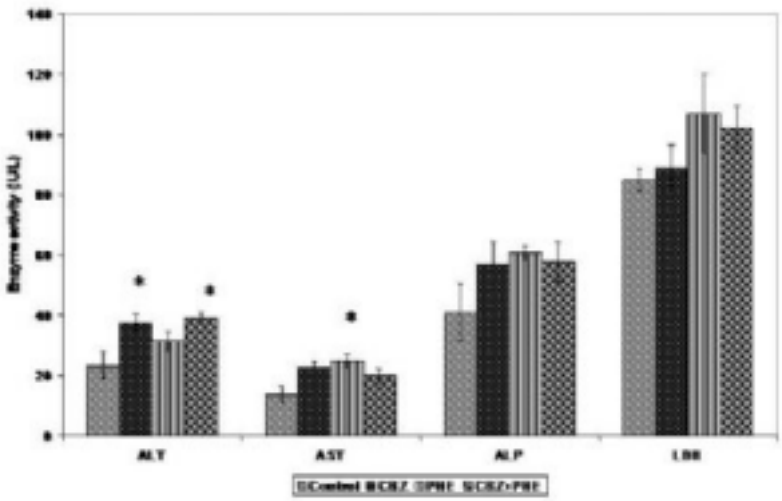

Figure. 1. Effect of administration of carbamazepine (CBZ) and/or phenytoin (PHE) on serum liver enzyme activities in Wistar rats (n = 10)

P115: Alterations in brain energy metabolism following acute administration of L-glutamate

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Glutamate is a major excitatory neurotransmitter in the CNS. Nevertheless, high extracellular levels of this amino acid have been shown to be toxic to several neuronal populations. Glutamate has been implicated in several acute as well as chronic neurological disorders., To study how glutamate homeostasis is altered in response to local infusion of glutamate, 1µM of glutamate was stereotactically injected into cerebral cortex, striatum, and hippocampus of adult rat brain, and the activities of key metabolic enzymes, lactate dehydrogenase (LDH), glutamate dehydrogenase(GDH), aspartate amino transferase(AST), and alanine amino transferase(ALT) were evaluated by post mortem analysis in tissue homogenates. Glutamate administration significantly enhanced LDH activity in the cortex ($P<0.01$), striatum ($P<0.05$), and hippocampus ($P<0.05$). Glutamate increased GDH activity in the cortex ($P<0.01$) and striatum ($P<0.05$) but not in hippocampus. Glutamate also significantly lowered AST activity in cortex ($P< 0.001$), striatum ($P<0.05$), and hippocampus ($P<0.001$). Local infusion of glutamate has no influence on the ALT activity in cortex. In the glutamate + dizocilpine group also, the activity of ALT is not significantly different from that in the glutamate group, implying that dizocilpine has no effect on cortical ALT activity. However, there is a significant decrease ($P<0.001$) in the ALT activity of striatum and hippocampus on administration of glutamate. The results show that glutamate bolus, induced significant alterations in in vivo glutamate and energy metabolism, as evidenced by marked alterations in these enzyme activities, whereas peripheral administration (1µg/kg) of dizocilpine, a glutamate receptor antagonist, inhibited many of the effects induced by high glutamate. However, the degree of involvement of these observations in glutamate-induced neurotoxicity remains to be ascertained.

Keywords: excitotoxicity, LDH, GDH, AST, ALT

P116: Nitric oxide synthesis (NOS) inhibitors decrease global DNA methylation in the ventral hippocampus of rats submitted to learned helplessness

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Our first aim was assess the effect of NOS inhibitors on behavioral changes and global DNA methylation induced by learned helplessness (LH). Rats were submitted to the LH test and treated with inhibitors of nNOS (7-nitroindazole, 60 mg/kg, i.p.); iNOS (aminoguanidine, 30 mg/kg, i.p.) or vehicle for 7 days. Ventral (vHPC) and dorsal (dHP) hippocampus and prefrontal cortex (PFC) were collected and for analysis of global DNA methylation (ELISA). The animals previously submitted to pretest with inescapable footshocks showed a significant increase in escape failures in the test (condition=$F_{1,45}=31,85$; $p<0.01$; interaction=$F_{1,45}=8,04$; habituated group versus stressed group; $p<0.01$; $t=5,6$, $df=22$; $p<0.01$). Treatment with iNOS and nNOS inhibitors was able to attenuate stress-induced escape failures in the test ($t=5,5$, $df=24$; $p<0.01$ and $t=3,8$, $df=23$; $p<0.01$; respectively, stressed vehicle versus stressed treated). Stress exposure increased global DNA methylation in the vHP (ANOVA, condition= $F_{1,28}=26,24$; $p < 0.01$; interaction= $F_{1,28}=9,59$; $p<0.01$; habituated versus stressed; $t=5,5$, $df=14$; $p<0.01$), but not in the dHP or the PFC. Treatment with NOS inhibitors attenuated stress-induced global DNA methylation only in the vHP ($t=2,6$, $df=14$; $p<0.05$ and $t=2,6$, $df=14$; $p<0.05$; respectively, stressed vehicle versus stressed treated). Treatment with both NOS inhibitors induced antidepressant-like effects in rats exposed to LH and decreased stress-induced DNA methylation in the vHP. This is the first evidence suggesting that NO synthesis during stress could modulate DNA methylation. Additional experiments are being conducted to demonstrate the role of NO in modulating DNA methylation in candidate genes.

This study is supported by IBRO international travel grants program, FAPESP (2015/06271-1 and 2015/25067-6) and CNPq.

Keywords: major depression, learned helplessness, DNA methylation, nitric oxide, behavior stress

P117: Pregabalin has an atypical profile of anxiolytic-like activity in rodents

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Pregabalin is occasionally used in the treatment of anxiety disorders. However, the anxiolytic-like activity of pregabalin is less extensively described in the literature as compared with its reported anticonvulsant or analgesic activity. The present study aimed to characterize the effects of pregabalin in standard tests of anxiolytic-like activity in rodents.

Pregabalin was first evaluated in the Irwin test in order to identify intraperitoneal doses devoid of behavioral effects susceptible to affect the interpretation of data in the subsequent tests which included the marble burying (MB) and elevated plus-maze (EPM) tests in the mouse and the EPM, Vogel conflict (VC) and fear-potentiated startle (FPS) tests in the rat.

In the Irwin test, pregabalin was devoid of effects in the mouse up to 60 mg/kg whereas it displayed weak sedative effects in the rat at 30 mg/kg starting 90 minutes after administration and clear effects at 60 mg/kg. Based on these data, the doses of 10, 30 and 60 mg/kg and of 3, 10 and 30 mg/kg were chosen for the evaluation of anxiolytic-like activity in the mouse and in the rat, respectively.

In the mouse, pregabalin was devoid of effects in the MB test at 10, 30 and 60 mg/kg whereas it displayed significant effects in the EPM at 30 and 60 mg/kg. In the rat pregabalin displayed weak anxiolytic-like activity at 30 mg/kg in the EPM. In the VC test, pregabalin displayed clear activity at 3, 10 and 30 mg/kg when administered 60 minutes before the test and its efficacy at 30 mg/kg increased with longer pre-treatment times.

These data confirm dose-dependent anxiolytic-like effects of pregabalin. Nevertheless, the absence of activity in the MB test at doses observed to be actives in other tests in the mouse or in the rat, suggests an atypical anxiolytic profile when compared with benzodiazepines.

Keywords: anxiolytic-like activity, pregabalin, rodents, behavioral effects

P118: Effects of asenapine and paliperidon on analgesy and locomotion: Altered gen expression levels of FGF2, synapsin and NGF in the hippocampus of mice

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Neutrophins are a family of structurally related proteins that regulate the survival, differentiation, and maintance of function of different neuronpopulations. Fibroblast growth factor-2 (FGF2) has an important role in many aspects of neuronal functioning. Synapsin, another key marker of synaptic activity plays an important role in hippocampally based behaviors. There is evidence that nerve growth factor (NGF) also mediates multiple biological phenomena. Asenapine and paliperidon are new generated atypical antipsychotic drugs used in clinics. This study aimed to investigate the effects of these drugs on analgesy and locomotion in naive mice, using hot plate (HP) and open field tests. Since the genes involved in neuritere modeling are among the primary targets of regu¬lation, the effects of chronic administration of drugs on FGF2, synapsin and NGF levels in the hippocampus of mice were determined using quantitative real-time polymerase chain reaction (RT-PCR). Mice were treated chronically with asenapine (0.05 and 0.075 mg/kg) and paliperidon (0.25 and 0.5 mg/kg) for 15 days and drugs were also administered intraperitoneally 60 min. Before the tests.

Our study revealed that (1) In open field test, both asenapine (0.075 mg/kg) and paliperidon (0.5 mg/kg) did not alter significantly total distance moved and speed of the animals ($p>0.05$) (2). In the hot plate test, paliperidon (0.25 and 0.5 mg/kg; $p<0.001$) significantly increased the latency for licking the hindpaws while asenapine had a partial effect but it was not significant (3). Chronic administration of asenapine and paliperidon significantly increased the expression of FGF2, synapsin and NGF. Our results suggest that paliperidon had a superior analgesic effect compared to asenapine and administration of the new generated antipsychotic asenapine and paliperidone increased the expression of FGF2, synapsin and NGF in the mice hippocampus. Thus chronic administration of asenapine and paliperidon may promote neuroplasticity via the up-regulation of neutropic factors.

Keywords: asenapine, paliperidone, analgesy, locomotion, gene expression

Table 1. Target genes

Groups	target gene 1 : NGF	target gene 2 : SYNAPSİN	target gene 3 : FGF2
Cont.+ Ase.	3.813↑	5.337↑	80.896↑
Cont.+ Pal.	3.523↑	6.936↑	62.121↑

P119: Effects of selective PDE-2 Inhibitor BAY60-7550, PDE-5 inhibitor sildenafil and PDE-9 inhibitor PF-04447943 on learning and memory in the Morris water maze test in naive mice

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Phosphodiesterases are enzymes that hydrolyze cAMP and/or cGMP throughout the body, including the brain. PDE inhibitors present a novel therapeutic approach with which to arrest cognitive decline or possibly reverse the decline with cognition enhancement. Aim of this study was to investigate effects of BAY 60-7550, a PDE2 inhibitor, Sildenafil, a PDE5 inhibitor and PF-04447943, a PDE9 inhibitor, on learning and memory in the Morris water maze test in naive mice. Male Balb-c mice were treated subchronically with BAY 60-7550 (1-3 mg/kg), Sildenafil (3-10 mg/kg) and PF-04447943 (1-3 mg/kg) for 6 days in the MWM test. We performed an intraperitoneal injection (i.p.) One way ANOVA post hoc Tukey's test was used for the statistical analysis of the data.

BAY 60-7550 1mg/kg and PF-04447943 1 mg/kg have no significant effect but BAY 60-7550 3mg/kg, PF-04447943 3 mg/kg, sildenafil 3 mg/kg and sildenafil 10 mg/kg significantly increased time spent in escape platform's quadrant in probe trial of MWM test. BAY 60-7550 3mg/kg, PF-04447943 1 mg/kg, PF-04447943 3 mg/kg, sildenafil 3 mg/kg and sildenafil 10 mg/kg significantly decreased mean distance to platform in the probe trial of MWM test, while BAY 60-7550 1mg/kg had no significant effect on mean distance to platform.

Our results confirm that BAY 60-7550, sildenafil and PF-04447943 have a positive effect on the spatial memory in the morris water maze test in naive mice.

Keywords: phosphodiesterase inhibitors, learning memory, Morris water maze test

P120: Effects of hippocampal histone acetylation and HDAC inhibition on spatial learning and memory in the Morris water maze in rats

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In recent years, it has been pointed out that epigenetic changes affect learning and memory formation. Particularly, it has been shown that histone acetylation and DNA methylation work in concert to regulate learning and memory formation.

We aimed to examine whether acetylation of H2B within rat hippocampus is altered by trainings in the Morris water maze test and a histone deacetylase inhibitor, sodium buytrate, has effects on learning and memory performance.

In this study, 2-3 months old male Sprague Dawley rats were used. Groups A-G were swum in Morris water maze for an in increasing number of days (4 trials per day), Groups H-K were swum 4 trials per day for 5 days. Sodium buytrate (1.2 gr/kg, i.p.) and vehicle (NaCl 0.9%) were injected Groups H-K. After memory tests, the rats (Group A-K) were euthanized and their brain tissues were removed. Histon 2B and Acetylated histon 2B levels were analyzed by immunohistochemistry. Scopolamine (0.5 mg/kg) was injected to Groups L-O to induce memory impairment. Experiment plan is shown in Figure 1 in detail.

In the immunohistochemical examination, it was found that H2B acetylation in area CA1 of hippocampus increased during the early stage of swimming trials (first and second days, $p<0,001$). Sodium butyrate did not affect learning and memory performance of normal rats; however, it partially ameliorated learning and memory disruption induced by scopolamine ($p<0.05$).

Our results show that acetylation of H2B plays role in the early stage of learning and inhibition of deacetylation partially ameliorates learning and memory performance.

This study is supported by Trakya University Scientific Research Projects Unit (TUBAP-2012/210).
Keywords: histone acetylation, HDAC inhibition, spatial memory, scopolamine, rat

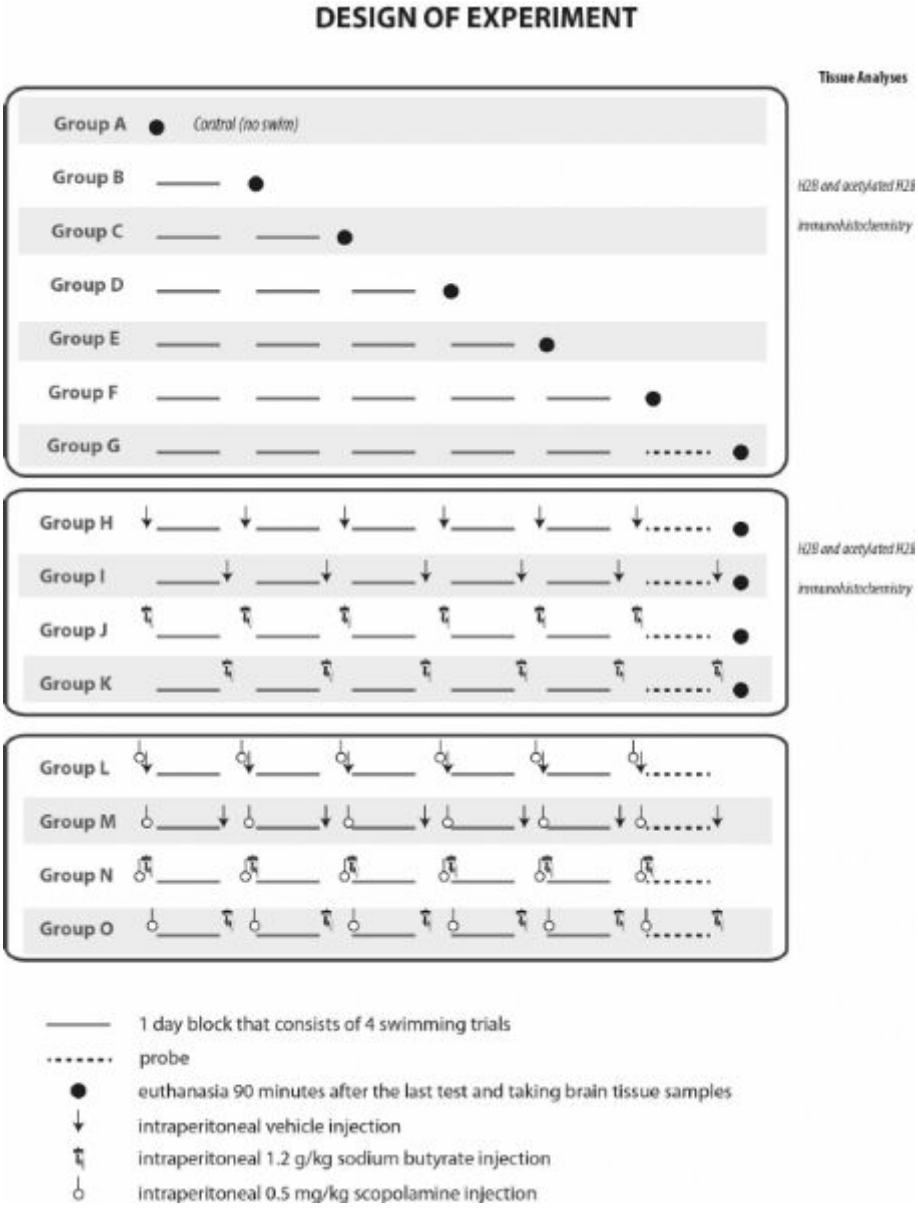

Figure 1. Design of the experiment.

P121: Effects of rufinamide on spatial and avoidance memory in Morris water maze, elevated plus maze and passive avoidance tests in mice

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Antiepileptic drugs are a major treatment consideration for epileptic patients. The main concern in choosing an appropriate antiepileptic drug is efficient control of seizures. Rufinamide is a third generation antiepileptic drug and can modulate the gating of voltage-gated sodium channels.

This study was designed to investigate the effects of rufinamide on spatial memory retrieval in Morris water maze test (MWM) and on acquisition and memory formation in modified elevated plus maze (mEPM) and passive avoidance (PA) tests. Male, BALB/c mice were treated acutely with rufinamide (2 and 4 mg/kg). Rufinamide was administered intraperitoneally just before the acquisition sessions of EPM and PA tests; and before the probe trial of MWM. One-way analysis of variance post-hoc Tukey test was used to analyse PA and MWM tests data. Wilcoxon t-test, Kruskal–Wallis ANOVA followed by Dunns were preferred to analyse mEPM test. Data are expressed as the mean values ±SEM. $p < 0.05$ accepted as statistically significant.

Rufinamide (2 and 4 mg/kg) did not change the spontaneous locomotor activity (total distance moved (cm)) of mice ($p>0.05$). In the EPM test, rufinamide (2 and 4 mg/kg) decreased the TL2 (TL:transfer latency) of mice compared to TL1 ($p<0.05$). In the PA test, the TL1 and TL2 did not affected via drug administration ($p>0.05$). In the MWM test, rufinamide (2 and 4 mg/kg) decreased the time spent in escape platforms quadrant ($p<0.05$). Rufinamide (2 and 4 mg/kg) increased the distance to platform ($p<0.05$) compared to control group. Rufinamide (2 and 4 mg/kg) did not affect the swim speed of mice.

Hence, according to our results, rufinamide showed an ameliorative effect on spatial memory in the EPM. Rufinamide did not make a change in avoidance memory in PA test. In MWM test, rufinamide impaired spatial memory retrieval without affecting the spontaneous locomotor activity of mice.

Keywords: rufinamide, learning, memory, mice

P122: Effects of retigabine and lacosamide on acquisition and retrieval of memory in naive mice

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Impaired learning and memory is among the most common complaints of patients with epilepsy. Therefore novel antiepileptic drugs and antiepileptic treatment gain more importance. Retigabine and lacosamide are the third generation antiepileptic drugs.
This study was designed to investigate the effects of retigabine and lacosamide on spatial memory retrieval in Morris water maze test (MWM) and on acquisition and memory formation in modified elevated plus maze (mEPM) and passive avoidance (PA) tests. Male, BALB/c mice were used. Retigabine (1 and 3 mg/kg) and lacosamide (2 and 4 mg/kg) were administered acute, intraperitoneally just before the acquisition sessions of EPM and PA; and before the probe trial of MWM test. One-way analysis of variance post-hoc Tukey test was used to analyse PA and MWM tests. Wilcoxon t-test, Kruskal–Wallis ANOVA followed by Dunns were preferred to analyse EPM. Data are expressed as the mean values ±SEM. $p<0.05$ accepted as statistically significant.

Lacosamide (2 and 4 mg/kg) and retigabine (1 and 3 mg/kg) did not change the total distance moved ($p>0.05$). In the EPM test, lacosamide (2 and 4 mg/kg) and retigabine (1 and 3 mg/kg) decreased the TL2 (TL: transfer latency) compared to TL1 ($p<0.05$). In the PA, TL1 and TL2 did not affected via drug administration. In MWM test, lacosamide (4 mg/kg) decreased the time spent in escape platforms quadrant ($p<0.05$). Lacosamide (2 and 4 mg/kg) and retigabine (3 mg/kg) increased the distance to platform ($p<0.05$) compared to control. The swim speed of mice did not changed by antiepileptic treatment.

According to results, retigabine and lacosamide showed a positive effect on spatial memory in EPM. The drugs did not show any negative impact on avoidance memory in PA. In MWM, the drugs were shown to exert slight negative effect on spatial memory retrieval without affecting the locomotor activity.

Keywords: retigabine, lacosamide, learning, memory, mice

P123: The Effects of Kai Xin San on the cognitive deficits and pathological changes of hippocampus in APP/PS1 double transgenic mice

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The deposition of neurotoxic amyloid-β peptide (Aβ) within the brain is the major pathological hallmark of Alzheimer's disease (AD) and related with the cognitive dysfunction. Kai Xin San (KXS), a traditional prescription, was used to improve the intelligence in ancient. This study is to investigate the effects of KXS on the cognitive deficits and pathological alterations of the hippocampus in APP/PS1 transgenic mice.

3-month-old male APP/PS1 mice were randomly divided into the model control group and KXS groups. C57/BL6J mice were used as the normal control group. The APP/PS1 mice were orally administered KXS (15g/kg, 7.5g/kg), respectively, once daily for 4 months. The vehicle was given to the normal control group mice and model control group mice at the equal amount (10ml/kg). Learning and memory ability was detected with Morris Water Maze and step-down test. The pathological changes in the hippocampus were observed by HE staining and transmission electron microscope.

Compared with APP/PS1 model group mice, the Morris water maze results showed the mice treated with KXS reduced the escape latency ($P<0.05$) in day 5 in training trails, shortened the escape latency and increased the platform-crossing times ($P<0.05$) in the probe trial. KXS treatment significantly prolonged the step-down latency and decreased the number of step-down errors ($P<0.05$) in the step-down test. The pathological alterations of neurons, mitochondria, endoplasmic reticulum, and Golgi's complex of hippocampal neuron were alleviated in the APP/PS1 mice treated with KXS. KXS also increased the number of synapses and the curvature of synaptic interface, decreased the synaptic gap width and increased the thickness of postsynaptic dense zone($P<0.01$) in hippocampus of APP/PS1 mice.

KXS can significantly improve learning and memory impairments and delay the pathological injury of hippocampal neuron in APP/PS1transgenic mice.

Keywords: APP/PS1 transgenic mice, Kai Xin San, learning and memory, synapse, hippocampus

P124: Comparison of the effects of varenicline and bupropion on the conditioned place preference test which is induced by morphine in rats

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In the present study we investigated the effect of varenicline and bupropion on morphine with drawal symptoms, development of morphine dependence, expression, extinction and addiction relapse on rats.

The first experiment was conducted to evaluate the morphine physical dependence, for that increasing doses of morphine twice daily (1st day 10, 2nd day 20, 3rd day 30, 4th day 40, 5th day 50 mg/kg, i.p) was administered for 5 days to Wistar rats. To elicit the with drawal symptoms, naloxone (2 mg/kg, s.c) was given after 4 hours of morphine single dose (50 mg/kg) administered. In second experiment, psychological dependence of morphine was evaluated by conditioned place preference (CPP) test. To assess the development of dependence, 15 minutes prior to morphine administration, bupropion (5, 10, 20 mg /kg, i.p) or varenicline (0.5, 1, 2 mg/kg, s.c) was administered to the rats given morphine (10 mg/kg, ip) and saline solution for the duration eight days whereas, for the evaluation of expression the test was conducted on 9 th day in same manner. To assess the elimination of rewarding effects (Extinction), bupropion and varenicline was administered daily and tested on 14th, 18th, and 22nd day. After the administration of morphine (10 mg/kg) on 23rd day the relapse related with drug addiction,on was evaluated by conditioning. Bupropion and varenicline reduce morphine physical dependence. CPP test showed increase in elimination of morphine psychologicald ependence, expression, addiction relapse and extinction. Bupropion high dose (20 mg/kg) showed rewarding properties and also increased the locomotor activity.

Varenicline with out changing the locomotor activity and lack of extinction at high doses found to be more effective than bupropion. It can be concluded from the data of the present study that bupropion and varenicline can be useful in the treatment of morphine and other opioid dependence.

Keywords: bupropion, varenicline, morphine, addiction, conditioned place preference (CPP)

P125: Effects of Kai Xin San on hippocampal synaptic plasticity in APP/PS1 double transgenic mice

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Alzheimer disease (AD) is an age-dependent and neurodegenerative disorder characterized by learning, memory, and communication deficits. Kaixinsan (KXS) is an ancient Chinese herbal decoction which shows a good effect to improve learning and memory, but its mechanism is not clear. The long-term potentiation (LTP) is a potential neurophysiological mechanism for learning and memory and a widely used in the study of synaptic plasticity. This study was to investigate the effects of KXS on hippocampal LTP in APP/PS1 transgenic mice which shows the impairment of learning and memory, in order to explore the mechanism of KXS about cognition improvement.

3-month- old male APP/PS1 mice were randomly divided into the model control group and KXS group. C57/BL6J mice were used as the normal control group. The APP/PS1 mice were orally administered KXS (15g/kg, 7.5g/kg), respectively, once daily for 3 months. The vehicle was given to the normal control group mice and model control group mice at the equal amount (10ml/kg). The hippocampal synaptic plasticity was assessed by measuring the long-term potentiation (LTP) in the performant pathway (PP) to dentate gyrus (DG) in vivo.

Slopes of LTP during the time course of the last 20 mins were calculated and compared for each other. It was found that the fEPSPs slopes were significantly decreased in APP/PS1mice compared with control group mice ($P < 0.01$). But there was a significant enhancement in the APP/PS1 mice treated with KXS ($P < 0.01$). The effect of the high-dose KXS is better than that of the low-dose of KXS.

KXS can promote the LTP formation of hippocampal PP-DG pathway, suggesting that enhancement of synaptic plasticity maybe related with its improvement on the cognitive deficits of APP/PS1 transgenic mice.

Keywords: Kai Xin San, LTP, APP/PS1 transgenic mice

P126: Effect of learned helplessness on spine synapse density in the dentate gyrus. Suggested role of P2X7 receptor

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Emerging major depression is one of the prevailing central nervous system disorders with high burden on the society. Previous studies revealed mutations in the gene encoding the P2X7 purinergic receptor (P2X7R) associated with the development of major depression.

Since among many other known symptoms, the loss of hippocampal spine synapses is a revealing feature of the disorder, firstly we measured the density of spine synapses in the molecular layer of the dentate gyrus in the learned helplessness paradigm and later see if inhibition of P2X7R could have influence on this condition.

C57Bl/6 male mice were exposed to inescapable footshocks in shuttle boxes during 2 consecutive training days. On the third day the aversive stimuli could be avoided, but helpless animals failed to escape. Control animals did not receive footshocks until the test day. Number of escape failures and the latency to escape was measured automatically to determine helpless behaviour. Electron microscopy analysis was performed for synapse counting and the difference in spine synapse density was calculated between the treated and control groups with a Student's t-test.

Both average escape latency and the number of failed escapes were significantly higher in the inescapable footshock treated group. Electron microscopy analysis demonstrated a decrease in spine synapse density in WT animals compared to the naïve values, while P2rx7 KO animals showed similar synaptic densities as the naïve KO ones.

Alterations in the mRNA transcript level of the P2X7 receptor in the learned helplessness model was measured using real-time RT-PCR. Western blot experiments proved the decrease of synaptopodin, an actin-associated protein, which occurs in the postsynaptic densities and part of spine apparatus, in the treated P2X7 WT mice.

Our present results may underline the importance of P2X7R in depression and together with further experiments may lead us to a better understanding of the disorder.

Keywords: major depression, learned helplessness paradigm, dentate gyrus

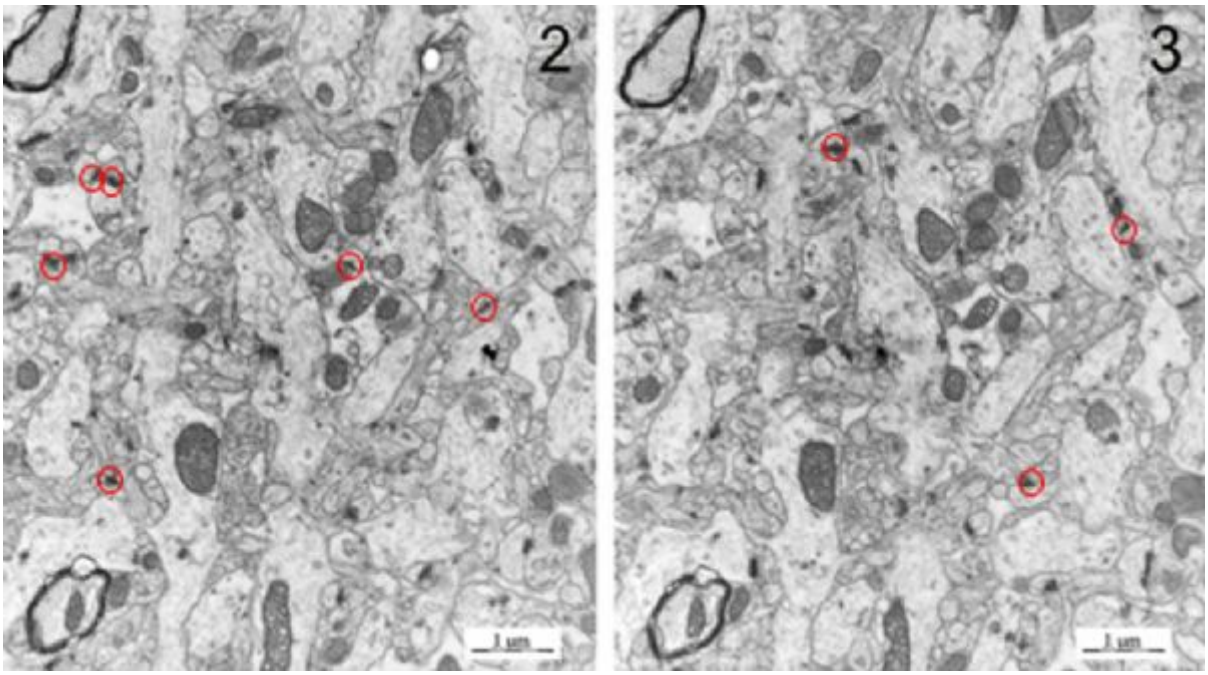

Figure 1. Counting spine synapses on consecutive snapshot pairs. We prepared samples from the molecular layer of the dentate gyrus for electron microscopy analysis. From the ultrathin serial sections two consecutive snapshots were investigated at once, counting only the newly appearing spine synapses.

P127: The effect of GLT-1 transporter activation on learning and memory impairment in Morris water maze induced by scopolamine in rats

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In the central nervous system, glutamate appears to be the principal excitatory neurotransmitter. It is known that GLT-1 transporters mediate nearly 90% of glutamate uptake. In our study it has been aimed to investigate the effects of ceftriaxone in rats on learning and memory impaired by scopolamine.

The groups of animals (n=9) were given 4 trials per day for 5 consecutive days to locate a hidden platform. On the sixth day the platform is removed and the animals were swum. Learning and memory functions of the animals were evaluated based on their performances in these tests. After memory tests, brain tissues of the animals were removed and GLT-1 levels in the tissue samples taken from hippocampus were determined.

Our results show that chronic ceftriaxone (50, 100, and 200 mg/kg, i.p.) has no effect on memory impairment induced by chronic scopolamine. Although ceftriaxone produced an increase in GLT-1 level in hippocampus, this increase was statistically significant only in the group given 100 mg/kg dose ($p<0.001$).

Our results suggest that ceftriaxone exerts an effect to increase GLT-1 level in hippocampus, but has no effect on spatial learning and memory functions.

This study is supported by Trakya University Scientific Research Projects Unit (TUBAP-2013/45).
Keywords: ceftriaxone, GLT-1, glutamate, scopolamine, spatial memory

P128: Determination of the changes in PP1, BDNF and Reelin gene expressions in hippocampus during spatial learning and memory in morris water maze in rats

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In this study, it is aimed to investigate changes of *PP1*, *BDNF*, *reelin* gene expressions in hippocampus during learning and memory formation after successive trainings in rats.

Data were obtained from 8 week-old, male Sprague Dawley rats weighing between 300 and 350 g. 7 groups (n=5) were formed. The groups of animals were given 4 trials per day for 5 consecutive days to locate a hidden platform. On the sixth day the platform was removed and the animals were swum. Learning and memory functions of the animals were evaluated based on their performances in these tests. After memory tests, the rats that completed training were euthanized and their brain tissues were removed. Tissue samples were taken at hippocampal CA1 and CA3 zones with punch methods. *PP1, BDNF* and *Reelin* gene expressions in the taken tissue samples were analyzed by polymerase chain reaction.

It was seen that the rats learned the loctation of the platform in Morris water maze, especially starting from the third day of the trials. Our results show that in the CA1 region, *BDNF* gene expression increased in a statistically significant degree ($p<0.01$) on the fourth day. In tissue samples taken from both regions, there were no statistically significant differences in *reelin* and *PP1* gene expressions.

Our results suggest that *BDNF* has a role in spatial learning and memory processes starting from the first day.

This study is supported by Trakya University Scientific Research Projects Unit (TUBAP-2014/51).

Keywords: *BDNF*, *reelin*, *PP1*, spatial learning, memory

P129: Investigation of antidepressant-like effect of dipyrone in forced swimming test

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In most cases pain and depression are comorbid; depression is known to increase pain, pain is known to increase depression. Recently some antidepressant drugs are used for some type of pain and some analgesic agents were an found to have antidepressant-like effect. We aimed to investigate if an effective analgesic-antipyretic dipyrone has antidepressant-like effect and/or increase the effect of antidepressant drugs in this study.

Swiss albino mice were used in the experiments. Depression-like effect was evaluated by forced swimming (Porsolt Test) test. In the experiments, 150, 300 and 450 mg/kg ip doses of dipyrone were used. Fluoxetine 20 mg/kg and tryptophan hydroxylase inhibitor para-chlorophenylalanine 250 mg/kg were administered ip. The results were analyzed by the one way ANOVA (post hoc Bonferroni) test. $P<0.05$ value was significant.

Three doses of dipyrone significantly reduced the immobility time of mice in water when compared with control group. Fluoxetine 20 mg/kg and dipyrone 150 mg/kg showed the similar effect. The effect was not changed when two drugs used in combination. Para-chlorophenylalanine administered with dypirone did not change the effect of dipyrone.

According to our study, dipyrone showed similar antidepressant-like effect as fluoxetine, evaluated by forced swimming test and administration of dipyrone and fluoxetine together did not changed the effect of fluoxetine. Serotonin synthesis inhibitor, para-chlorophenylalanine did not change the antidepressant-like effect of dipyrone. All these findings suggest that dipyrone has antidepressant-like effects and serotonergic system does not play a role on this effect. Results of our study suggest that dipyrone's antidepressant-like effect should be investigated by other tests and finding out the mechanism of action will contribute to the future treatment protocols of comorbid patients of depression and progressive pain.

Keywords: depression, dipyrone, forced swimming test

P130: The Effects of a Ginkgo biloba extract on extinction and relapse of ethanol-induced conditioned place preference in mice

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Alcohol addiction is a chronic recrudescent brain disease characterized by compulsive drug-seeking behaviors, relapse and adverse outcomes. It has been reported Ginkgo biloba extract (GBE) indicated the modulatory effects on N-Methyl-D-aspartic acid. The aim of this study was to investigate the effects of GBE on psychic dependence on the ethanol-incuded conditioned place preference (CPP).

The mice were administered ethanol injections (2 g/kg, i.p.) on days 1, 3, 5, and 7, and saline (10 ml/kg, i.p.) injections on days 2, 4, 6, and 8 of the conditioning phase (the duration of each conditioning was 30 min). To determine whether GBE influenced the extinction time of ethanol-induced CPP, the mice were subjected to the extinction testing daily for a period of 13 days following the establishment of CPP. During this time, the mice were divided equally into five groups which were applied daily injections of GBE (25, 50 and 100 mg/kg, gavage) or saline, 60 min before the daily extinction test. For the relapse trial, GBE (25, 50 and 100 mg/kg) injections were applied to the mice, 60 min before the injection of ethanol (2 g/kg, i.p) or saline.

Extinction (ext) time was completed, 25 mg/kg GBE (ext 9th day, $p < 0.05$), 50 and 100 mg/kg GBE (ext 7th day, $p < 0.01$), saline group (ext 14th day, $p > 0.05$) when compared to the ethanol. GBE was found to be effective in reducing the relapse time at the doses of 50 mg/kg and 100 mg/kg GBE, respectively ($p < 0.01$ and $p < 0.05$).

In conclusion, the present research indicates that GBE reduced extinction and relapse on the ethanol induced-CPP. Further studies are necessary in the clinical use of GBE in alcohol addiction.

Keywords: ethanol, Ginkgo biloba extract (GBE), mice, addiction, conditioned place preference (CPP)

P131: Standardized methanolic extract of *Cuscuta reflexa* caused phenelzine like actions in animal models of depression

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Depression is a neuropsychiatric disorder associated with symptoms such a feeling of despair,low self esteem and feeling of derisiveness. Despite of the fact that lot of studies have been done on this topic but neither the exact etiology nor the treatment has been yet discovered of this ailment. Keeping this need of the hour in mind this study was conducted to assess the antidepressant potential of folklorically used plants. The *Cuscuta reflexa*extract is traditionally used for the treatment of depression. The current study was aimed at exploring the potential of *Cuscuta reflexa* against depression. The standardized methanolic extract of the vine of C.reflexa was assessed for antidepressant action using various behavioural paradigms such as tail suspension test (TST), forced swim test (FST) and locomotor activity test. The serotonergic and noradrenergic changes were evaluated using 5-hydroxytryptophan (5-HTP) induced head twitches and yohimbine potentiation tests, respectively. The fluoxetine and phenelzine was used as positive control in the study. The C.reflexa extractsignificantly declined the immobility time in TST (IC50 ~ 50mg/kg)and FST while no significant increase in locomotor count was observed. The extract also significantly increased the 5-HTP induced head twitches and yohimbine induced lethality. The aforesaid results are similar to that caused by standard phenelzine. The C. reflexa extract demonstrated antidepressant activity that can be attributed to rise in serotonin and noradrenaline levels in the brain via MAO inhibition.

Keywords: depression, antidepressant activity, behaviour

P132: Famotidine reverses social withdrawal but not fear conditioning deficits induced by acute ketamine model in rats

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Ketamine, a noncompetitive N-methyl-D-aspartate receptor antagonist, induces cognitive deficits and social withdrawal in rodents of relevance to cognitive and negative symptoms domains of schizophrenia which are unresponsive to current medications (1). Earlier clinical reports suggested that famotidine, a histamine-2 receptor antagonist, might serve as an adjunctive therapy in the treatment of schizophrenia (2,3). Herein, we examined the effect of famotidine on cognitive deficits and social withdrawal domain induced by acute ketamine model in fear conditioning (FC) and social interaction (SI) tests.

Female Sprague-Dawley rats (150-210 g) were assigned to; control (Saline), ketamine (15 mg/kg; s.c.) and ketamine+famotidine (15 mg/kg; s.c.+ 4.4 mg/kg; i.p.) groups (n=8/group). Famotidine was administered 30 min prior to ketamine followed by SI and FC tests 30 min later. In SI, two conspesific rats unfamiliar with each other, were placed in an open arena and behaviors were video-recorded for 10 min. In FC training, rats were individually placed in the FC box. After 150 sec, 4000Hz tone was presented for 20 sec coupled with 0.4mA shock in the last 2 sec. After 30 sec, this trial was repeated 6 times. 24 h later, rats underwent the same procedure except footshock. The freezing duration was automatically measured by the computer software.

In SI, ketamine decreased total social interaction time ($p<0.05$), sniffing ($p<0.001$) and following ($p<0.05$), increased avoiding time compared to control. However, pre-treatment with famotidine reversed ketamine-evoked all social withdrawal parameters nearly to control levels. In FC, ketamine decreased freezing duration compared to control group ($p<0.001$). Famotidine did not show any differences compared to ketamine group.

According to our results, acute ketamine administration induced cognitive deficits and social withdrawal in FC and SI paradigms, respectively. Further, ketamine-induced social impairments but not cognitive deficits abolished by famotidine pre-treatment which require future investigations.

Keywords: famotidine, ketamine, fear conditioning, social interaction

P133: Preclinical research of focal muscle hyperactivity disorders by clostridial neurotoxins

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Focal muscular hyperactivity resulting from impaired inhibitory neurotransmission at different CNS levels, including ventral horn and brainstem motor nuclei, is a common symptom of different movement disorders such as dystonias, post stroke spasticity, and spinal cord injuries. We hypothesize that selective impairment of ventral horn inhibitory neurotransmission by employing low intramuscular doses of tetanus toxin (TeNT) derived from Clostridium tetani, might be employed to study the focal muscular activity and preclinical investigation of common antispastic drugs.

Focal unilateral muscle spasm will be induced by low dose-TeNT injection into rat gastrocnemius muscle. Resulting lasting movement impairment, and therapeutic response to known antispastic drugs such as baclofen and botulinum neurotoxin type A (BoNT/A) will be assessed by different motor parameters and motor tests in conscious animals (dorsiflexion resistance, digit abduction score, tibioplantar angle, fatigue, balance and swimming tests). Expression of ventral horn inhibitory and excitatory neurotransmitters and synaptic markers will be analyzed by ELISA, Western blot and immunohistochemistry.

Our preliminary data suggests that low local intramuscular doses of TeNT induce easily quantifiable, lasting, and reproducible focal muscle impairments, which can be validated by BoNT/A and other commonly employed therapeutics. TeNT-induced spasiticity as a potential non-lesional and lasting reversible model of focal muscular hyperactivity might be employed to study patophysiology of focal hyperkinetic movement disorders, and potential novel drug screening.

This study is supported by European Social Fund and Croatian Ministry of Science, Education and Sport (grant no. HR3.2.01-0178, awarded to I.M.).

Keywords: focal muscle hyperactivity, movement disorders, tetanus neurotoxin, botulinum neurotoxin type A

P134: Optimizing effects of botulinum toxin treatment by post injection activity

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Currently, the best treatment option for idiopathic cervical dystonia (CD) is application of botulinum toxin type-A (BoNT-A) into the affected muscles. However, there is striking difference between the injection intervals given in everyday practice. The beneficial effects of BoNT-A in CD lasts for 3-4 months and repeated injections are needed. Increased motor activity increases presentation of synaptic vesicle protein (SV2), specific receptor for BoNT-A, that might results in higher efficacy of BoNT-A. The aim of present study was to investigate whether post injection activation program may increase the duration of clinical benefit.

A total of 43 patients (23 female and 20 male) who have been improved significantly to BoNT-A injections during first year of treatment were enrolled into this single-centre, single-blind study. Each patient was injected with previously used effective dose (100-200 U of ona and/or incobotulinumtoxinA, BoNT-A). CD was assessed using validated scales for dystonia (TWSTRS, ADL, pain score) every 2 weeks. Only after second injection of BoNT-A (second study period: 4 months after first injection), activation and physical therapy, with help of physiotherapist, was performed immediately after BoNT-A administration and 30 minutes daily during 2 weeks post injections. Physical activity protocol included active stretching to increase the muscle activity.

BoNT-A was effective in improving CD symptoms during both treatment periods. However, the duration of clinical improvement was significantly longer ($p<0.05$) during second study period when the same dose of BoNT-A was administered in combination with physiotherapy.

Our results indicate that post injection activation could increase the duration of BoNT-A effect, and that physiotherapy could prolong intervals between injections.

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Keywords: cervical dystonia, botulinum toxin type-A, post-injection activation

P135: Comparison of glutamic acid decarboxylase immunoreactivity in substantia nigra pars reticulate between genetic absence epileptic rats and nonepileptic control rats

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Substantia nigra pars reticulata (SNR) is critically involved in cognition and coordination of motor functions and associated within the control of epileptic seizures. SNRanterior and SNRposterior are two funtionally discrete subregions which have been demonstrated to mediate distinct effects on epileptic seizures. The inbred Genetic Absence Epilepsy Rats from Strasbourg (GAERS) is one of the well-validated genetic models of absence epilepsy. The GAERS rats show a resistance to secondary generalization of focal limbic seizures evoked by kindling, as a model of temporal lobe epilepsy. This study aimed to compare the GABAergic activity with glutamic acid decarboxylase (GAD) immunoreactivity in SNRanterior and SNRposterior neurons between GAERS and nonepileptic control (NEC) Wistar rats.

Adult male GAERS and Wistar rats (4–6 month-old, 250–350 g) were transcardially perfused with neutral buffered formalin solution and brains were removed. Brains were cut in a cryostat to obtain 40 µm sagittal sections containing the SNR. The sections were treated with anti-GAD67 antibody and were analyzed with a computer based programme.

GAD67-ir was determined semiquantitatively with immunohistochemistry in the both SNR subregions. There was no significant difference in GAD67-ir in the SNRanterior and SNRposterior subregions between the control NEC rats and GAERS. In conclusion these results indicate that the strain differences in kindling resistance of GAERS are not associated with preexisting differences for GAD67-ir in the SNR subregions of these animals.

This study is supported by TUBITAK (SBAG: 111S209).

Keywords: substantia nigra, absence epilepsy, glutamic acid decarboxylase

P136: Effects of diphenhydramine on long-term potentiation in healthy and REM sleep deprived rats

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REM sleep deprivation (REMD) was shown to impair learning and memory and inhibits long term potentiation (LTP). Diphenhydramine and doxylamine, OTC antihistamines, widely used for sleep induction without addictive effects. However, there is still no data about the effects of these drugs on learning and memory neither in healthy nor in sleep deprived individuals. Here, we have examined the effects of 3 day diphenhydramine (DPH) treatment on neuronal plasticity and learning via in vivo electrophysiological measurements in normal and sleep deprived rats.

Rats were divided into four groups with n=6 for each: vehicle (VHC), REMD, DPH and REMD+DPH. I.p. injections of vehicle or 20 mg/kg DPH were administered at 0, 24 and 48 hours. REMD was induced using columns-in-water method (Alkadhi et al., 2013) for 72 hours. At the end 72 hours, animals were anethesized with 1.2 mg/kg urethane and in vivo hippocampal field potentials were recorded from stratum radiatum and pyramidale layers of CA1 region by stimulating Schaffer collaterals.

Three days of DPH injections increased LTP in control rats but this increase was not statistically significant. REMD decreased LTP while DPH treatment neither improved nor worsened this impairment in rats. DPH treatment shifted the input/output curves of neuron firing thresholds and fEPSPs to the right compared to that of controls. DPH treatment decreased paired-pulse inhibition in control rats.

These findings suggest DPH treatment alone does not alter LTP, which is the electrophysiological correlate of learning and memory. DPH also does not ameliorate deleterious effects of REMD on LTP. Furthermore, DPH administration in non-REMD rats decreased the neuronal excitability of pyramidal cells.

Keywords: diphenhydramine, LTP, sleep deprivation, learning and memory

P137: Effects of modafinil on MDMA-induced spatial memory impairment

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Modafinil is a drug with complex profile of neurochemical effects and FDA approval for treatment of narcolepsy, shift work sleep disorder as well as obstructive sleep apnea/hypopnea syndrome. Because of many similarities, its mechanism of action may be comparable to classical psychostimulants, but the exact mechanisms of modafinil's actions in wakefulness and cognitive enhancement are unknown. Modafinil has also been suggested as a non-aphetamine-type stimulant in agonist pharmacotherapy of psychostimulant dependence. The aim of this study was to examine the effects of modafinil as a cognitive enhancer on hippocampus-dependent learning and memory in ecstasy-treated rats in. Male Wistar rats were intraperitoneally injected with MDMA (10 mg/kg), Modafinil (15, 30 and 60 mg/kg) or combination of them before the first training trial in four training days of Morris water maze (MWM). Interestingly, only high modafinil dose (60 mg/kg) significantly decreased the destructive effects of MDMA on spatial memory as evidenced by increased time spent in target quadrant in probe test of MWM. The results of this study showed that modafinil can improve spatial memory in MDMA-treated rats. Our results validate clinical and preclinical findings that modafinil may be an appropriate treatment option for meth addiction.

Keywords: modafinil, MDMA, memory, Morris water maze test

P138: The effects of doxycycline against chronic stress-induced anxiety in rats

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Chronic stress(CS) triggers various central nervous system disorders including depression and anxiety. Stress-induced increases in blood glucose levels and free radical formation are likely to play role in these disorders.

In addition to known antimicrobial activity, tetracyclines inhibit matrix metalloproteinase enzymes, decrease oxidative stresss, prevent non-enzymatic glycation of elevated blood glucose. There are some studies on doxycycline's role to protect the organism against the deleterious effects of stress, further studies are needed.

In present study, we aimed to determine the dose-dependent protective effect of doxycycline against CS-induced anxiety in rats.

48 male Sprague Dawley rats (394,8±14,80g) were randomly assigned into 6 groups; Control(C), Stress(S), Doxycycline15mg/kg+Stress(D15S), Doxycycline30mg/kg+Stress(D30S), Doxycycline15mg/kg (D15), Doxycycline30mg/kg. Restraint stress for 2 hours at different times for 15 days was used. Doxycycline was administered through an orogastric cannula.

Body weight and blood glucose level of rats were measured before and after the protocol. Open field (OF) and elevated plus maze (EPM) tests were performed to examine the anxiety status of rats. Results were expressed as mean±SEM.

Stress application resulted in weight loss (C;-3,9±1,37g vs S;16,6±5,88g, D15;-0,6±0,22g vs D15S;-18,4±6,50g, D30;5,1±1,81g vs D30S;-20,8±7,34g $p<0,001$). Acute stress caused an increase in blood glucose levels (139,0±2,52mg/dl vs 113,2±1,47mg/dl, $p<0.05$) however, elevated levels of blood glucose returned to normal (107,2mg/dL±1,81) at the end of CS application, which might be due to an adaptive process. Increased tendency of S group to choose closed arm (C;71,5±9,80 vs S;105,3±4,43s $p<0.05$) and increased number of defecation (C;0,75±0,490 vs S;4,12±1,273 $p<0.05$) supported increased anxiety. İncreased declining trend in the number of passes through the crossroad in D15S group (S;1,5±0,26 vs D15S;3,5±0,70 $p<0.05$) supported the protective effect of Doxycycline15mg/kg against anxiety. Our findings revealed the protective effect of doxycycline, at the dose of 15 but not 30mg/kg/day, against chronic restraint stress-induced increase in anxiety-related behaviors in rats.

Keywords: anxiety, blood glucose, chronic stress, doxycycline, restraint stress

P139: Protective effects of melatonin against 2,3,7,8-Tetrachlorodibenzo-p-dioxin-induced brain injury in rats

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), is the prototype of a group of highly toxic environmental chemicals. It has been well established that TCDD induces oxidative stresss in humans and experimental animal models. TCDD exposure has been shown to induce oxidative stress in brain. Although there are some suggestions regarding TCDD-induced brain injury, the exact mechanisms underlying of this process are not fully solved. One mechanism of this toxicity is believed to be the generation of reactive oxygen species (ROS).

Melatonin is known to be a strong antioxidant and has free radical scavenging ability. Therefore, the goal of this study was to investigate the TCDD-induced brain injury and the protective effect of melatonin in rats.

Rats were randomly divided into 4 equal groups (n=7 in each group). Group 1 served as control; group 2 was TCDD group (2 µg/kg/week, p.o); group 3 was melatonin group (5 mg/kg/day, i.p.) and group 4 was TCDD and melatonin treatment group. All agents were continued to 45th days. At the end of the experiment protocol, motor coordination of the rats was measured by using rotarod (from 5, 10, 20, 30, to 40 rpm) and accelerod (from 1 to 79 rpm within 4 and 10 min) tests. Also biochemical analysis of brain tissue was carried out.

TCDD exposure caused significant decrease of reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) levels whereas thiobarbituric acid reactive substances (TBARS) production was evaluated as increased. Melatonin treated rats showed an increased length of time when compared to the TCDD group during the both rotarod and accelerod tests. Also, melatonin treatment reduced TBARS production and increased antioxidant contents (Table 1).

According to our results treatment of melatonin, a powerful antioxidant and free radical scavenger, significantly protected of TCDD-induced brain injury.

Keywords: TCDD, melatonin, brain, motor coordination

Table 1. The levels of SOD, GSH-Px, CAT, GSH and TBARS in rat brain tissue.

Groups	TBARS (nmol/g tissue)	GSH nmol/ml	SOD (U/mg protein)	CAT kU/mg protein	GPx (U/mg protein)
Control	7.09±0.91a	130.92±9.54a	63.84±5.49a	0.064±0.0051a	391.01±69.8a
TCDD	9.87±0.76b	98.17±7.80b	43.37±3.89b	0.037±0.0059b	244.9±62.9b
MEL	5.69±0.52c	138.78±6.39a	68.94±3.61c	0.072±0.0057a	451.6±65.28a
TCDD+MEL	7.46±0.89a	108.45±8.61c	49.80±4.27d	0.052±0.0081c	293.4±50.9b

TCDD: 2,3,7,8-Tetrachlorodibenzo-p-dioxin; MEL: Melatonin Means bearing different superscripts within same line were significantly different (P<0.01).

P140: The effect of orphenadrine on morphine induced conditioned place preference

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Anticholinergic drugs are potentially used in clinical practice, but from last few years they are subjected to drug abuse and dependence. The present experiment was designed to study the effect of Orphenadrine on Morphine dependent rats and its potential as drug abuse by using conditioned place preference (CPP) test.

In the following study, all groups were evaluated for rewarding effects by using conditioned place preference test. Male Wister Albino rats weighing 250-300g were used in the experiment. The animals were divided into six groups as 1. Control (Serum Physiological, SF), 2. Morphine (10mg/kg), 3. 20mg/kg Orphenadrine, 4. 30mg/kg Orphenadrine, 5. 20mg/kg Orphenadrine + 10mg/kg Morphine, 6. 30mg/kg Orphenadrine + 10 mg/kg Morphine. The experiment followed the total period of 12 days with 4 different stages; habituation, pretest, conditioning and postest. Conditioning phase was followed for 10 days, 4 days conditioning and 2 days rest and again 4 days conditioning. In the postest phase the rewarding effects were evaluated. Statistical analysis was done by using one way analysis of variance (one way ANOVA) followed by Newman-Keuls.

Morphine significantly enhanced the preference scores in the drug paried side ($p<0,001$) while, both the doses of Orphenadrine (20mg/kg ve 30mg/kg) did not exhibit any preference than that of control. When compared to Morphine combined group; Orphenadrine 30 mg/kg + Morphine showed no preference in rats whereas Orphenadrine 20 mg/kg + Morphine group significantly decreased the CPP of Morphine ($p<0.05$).

Orphenadrine alone did not show any reward effects, combined used of Orphenadrine 20 mg/kg + Morphine reduced the preference, while Orphenadrine 30 mg/kg + Morphine did not exhibit acquisition of CPP in rats. The acquisition of CPP is thought to be due to NMDA receptor antagonistic effects of Orphenadrine at low dose.

Keywords: conditioned place preference (CPP), morphine, orphenadrine

P141: Diabetic conditions alters insulin degrading enzyme levels

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Insulin degrading enzyme (IDE) cleaves small polypeptides including insulin, IGFs, amylin and amyloid beta. However up to date little is known about how diabetes affects IDE.

We performed *in vitro* experiments using rat primary neuronal cultures incubated with high glucose, zero insulin or both conditions. *In vivo* experiments were performed using diabetic rats by injecting streptozotocin. IDE protein and mRNA levels were examined by western blot and RT-PCR respectively.

In neurons from rat primary culture, IDE protein levels decreased in (-) insulin group (15.67 %, $p<0.05$) and (-) insulin/high glucose group (19.41 %, $p<0.01$) compared to control group after 5 days constant treatment. mRNA levels of IDE in (-) Insulin/high glucose group displayed a 26.4 % decrease compared to control ($p<0.05$).

Glucose fluctuations similar to those observed in poorly controlled diabetic patients have been considered to be more deleterious than being exposed to continuous levels of high glucose. Glucose fluctuations accompanying to insulin fluctuations reduced IDE protein expression by 36.79 % ($p<0.01$) but not mRNA levels after 5 days treatment. Glucose or insulin fluctuations alone did not change IDE protein expression or mRNA levels.

In cortex tissues of 12-week diabetic rats, IDE protein levels decreased by 29.2 % ($p<0.001$) compared to control group.

Several studies have shown that Diabetes Mellitus increases the risk of Alzheimer's Disease (AD). In AD, both IDE protein levels and activity is known to be reduced. Our results suggest that constant and intermittent diabetic conditions lower the levels of IDE. Thus this could be one of the mechanisms by which Diabetes Mellitus confers high risk of AD development.

Keywords: diabetes mellitus, insulin degrading enzyme (IDE), insulin, Alzheimer's disease

P142: Plasma corticosterone levels in behavioural model-induced stress in rats

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It is important to use test-naive animals for the reliability of obtained results in behavioural models. But this unfamiliarity to the environment causes stress in animals. The objective of the present study was to examine stress levels of rats in various behavioural models commonly used worldwide. Male Wistar albino rats divided into five different experimental groups were used in the experiments. Experimental groups were formed as control group (rats were not exposed to behavioural models), three behavioural model groups and the food-restricted group (rats were not exposed to behavioural models) to compare food-restricted memory task group. Rats placed on a food restriction schedule, maintained 85-90 % of their initial body weights. Plasma corticosterone levels of rats were measured fluorometrically. Blood samples were collected just after the tests completed by the animals. The behavioural models used were elevated plus-maze test, forced swim test and three-panel runway test. The data were analysed using Student's t test. Behavioural test results obtained were within the related test normal values. The highest corticosterone level was determined in forced swim test group (49.2±4.1 mcg/100 mL), food-restricted and three-panel runway groups followed it (41.5±4.7 mcg/100 mL and 37.3±2.8 mcg/100 mL, respectively) and the lowest corticosterone level was in the elevated plus-maze group (25.1±2.7 mcg/100 mL). However, corticosterone levels were higher in all experimental groups than the control group (8.6±0.6 mcg/100 mL). The results indicate that various behavioural models induce stress with different intensities in animals. In conclusion, behavioural model-induced stress levels of animals should be taken into account in order to obtain better animal welfare and more reliable results when designing a research project.

Keywords: behavioural models, stress, plasma corticosterone, rats

P143: Scopolamine-induced convulsions in fasted mice after food intake: Involvement of nitrergic system

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Fasting mice administered scopolomine, develop convulsions soon after re-fed. It has been shown that various drugs that affect certain neurotransmitter systems supress these convulsions. On the other hand accumulating data indicate that nitric oxide may mediate the release of neurotransmitters. The aim of the present study was to clarify the role of nitrergic system in the mechanisms underlying these convulsions. For this purpose, 10 minutes before the scopolamine injections (3 mg/kg, i.p.), Balb/C male mice fasted for 18 hours were administered with nitric oxide precursor L-arginine (1000 mg/kg, i.p.) or biologically inactive enantiomer of L-arginine, D-arginine (1000 mg/kg, i.p.) and selective neuronal nitric oxide synthase inhibitor 7-nitroindazole (100 mg/kg, i.p). Control groups were pre-treated either saline (0.9 % NaCl) or the solvent of 7-nitroindazole, pea-nut oil in equal volumes (10 ml/kg, i.p). Mice re-fed in the 20th minute following the scopolamine injection were observed for 30 minutes in the cages individually to assess convulsions. Second groups of mice in L-arginine+7-nitroindazole+scopolamine, D-arginine+7-nitroindazole+scopolamine and saline+pea-nut oil+ scopolamine groups were not re-fed. Data were analysed by Fischer's exact test or Student's t-test whenever appropriate. There was no significant difference between control groups regarding incidence, stage and onset of convulsions. Pre-treatment of 7-nitroindazole suppressed the convulsions while L-arginine and D-arginine pre-treatments did not cause significant change comparing to the control group. Supression of the convulsions with the pre-treatment of 7-nitroindazole was reversed by L-arginine but not by D-arginine. In all groups which mice were not re-fed convulsions did not occur with the exception of L-arginine+7-nitroindazole+scopolamine group. Mice in this group exhibited later convulsion onset than in the other groups. The results suggest that nitrergic system may have a major role in the mechanism underlying these convulsions.

Keywords: scopolamine-induced convulsions, fasted mice, food intake, nitrergic system

P144: GABAA receptor agonist muscimol at super low doses improves spatial memory and prevents neuroinflammation in a non-transgenic rat AD-type model

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Recent findings focused the role of the GABAergic system to compensate neurotransmitter imbalance and synaptic dysfunction in Alzheimer's disease (AD). This dysfunction leads to neuroinflammation, deficits in synaptogenesis and memory. Although muscimol in vivo at doses above 1 mg/kg is known to impair memory, we suggest that at 50-100-fold lower doses (probably via non-specific mechanisms) it might prevent memory impairment and neuroinflammation and stimulate synaptic plasticity in AD model animals.

Non-transgenic AD-type model was obtained in male Wistar rats (280±20 g) by icv injection of neurotoxin streptozocin (STZ, 100 µg/ml). Muscimol at 0.01 and 0.05 mg/kg was injected ip for 7 days and tested in water maze. Artificial cerebrospinal fluid (aCSF) served as control. Spatial learning/memory parameters: time to find hidden platform (escape latency), number of platform zone crossings and time in platform quadrant were recorded. Macroglial marker glial fibrillary acidic protein (GFAP) and microglial IBA-1 and growth-associated protein-43 (GAP-43) were assessed immunohistochemically in cortical and hippocampal structures. Data calculated using ANOVA and Bonferroni post-test.

STZ rats showed memory/learning impairment vs. control group, which demonstrated improvements in measured memory parameters on days 3 and 4. Muscimol at both doses significantly protected against all STZ effects. Ex vivo, STZ markedly increased GFAP (in the cortex and hippocampus) and IBA-1 (in the cortex) density, decreased GAP-43 expression in the cortex. Muscimol normalized all these values.

Muscimol at very low doses (0.01 and 0.05 mg/kg) was capable to act as anti-neuroinflammatory agent by regulating expression of proteins participating in macro- and micro-gliosis (GFAP and IBA-1, resp.) and synaptic growth (GAP-43) and enhanced learning and memory. One may suggest that muscimol at super low doses provides indirect (apart from typical inhibitory GABA effect) regulatory influence on neuronal signalling processes.

This study is supported by NFI/R/2014/023.
Keywords: muscimol, Alzheimer's disease, non-transgenic model, memory

P145: Comparison of the effects of three different PDE inhibitors, sildenafil, BAY 60-7550 and PF-04447943 on fear memory in the fear conditioning test in mice

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PDE inhibitors increase intracellular levels of cAMP and cGMP. There are number of studies showing that PDE inhibitors affect different kinds of memory. Fear memory is formed in the hippocampus, in the basolateral amygdala, and in the lateral amygdala. Aim of this study was to investigate effects of BAY 60-7550, a PDE2 inhibitor, Sildenafil, a PDE5 inhibitor and PF-04447943, a PDE9 inhibitor, on fear memory by using fear conditioning test in naive mice.
Fear conditioning procedure was carried out in a FCS 21200-M fear conditioning system that allows recording and analysis of the signal generated by the animal's movement through a high sensitivity weight transducer system. The analogue signal is transmitted to the freezing and startle software modules through the load cell unit for recording purposes and later analysis, in terms of activity/immobility.
Male Balb-c mice were treated with BAY 60-7550 (1-3 mg/kg, ip), Sildenafil (3-10 mg/kg, ip) and PF-04447943 (1-3 mg/kg, ip) acutely before the contexual trial of fear conditioning test and also chronically for 21 days in fear conditioning test. One-way ANOVA post hoc Kruskal Wallis tests were used for the statistical analysis of the data.
BAY 60-7550 1mg/kg has no significant effect but BAY 60-7550 3mg/kg caused significant increase of % freezing in fear conditioning test. PF-04447943 1 mg/kg, PF-04447943 3 mg/kg, sildenafil 3 mg/kg and sildenafil 10 mg/kg have also a significant increase of % freezing in fear conditioning test. Chronic treatment with BAY 60-7550 (1, 3 mg/kg), PF-04447943 (1, 3 mg/kg) and sildenafil (3, 10 mg/kg) have no significant effect in fear conditioning test.
Our results confirm that BAY 60-7550, sildenafil and PF-04447943 have a positive effect on the fear memory in naive mice.
Keywords: fear conditioning test, PDE inhibitors

P146: Investigation of some central activities of fresh leaf essential oil of Lantana camara in mice

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Lantana camara L. (family: Verbenaceae) is a well-known medicinal plant found in several regions of the world including West Africa. In south-west Nigeria, it is used in the treatment of various diseases including tetanus, rheumatism and malaria. The plant is known to be poisonous to grazing animals in some countries including South Africa and India. The acute toxicity profile of the essential oil of the plant has not been documented nor its central activities in laboratory animals, hence this study.

Fresh leaf L. camara was collected and hydro-distilled to obtain its essential oil (EOLC) which was analyzed with GC/MS. The EOLC was evaluated for acute toxicity (LD50) through the oral (p.o.) and intraperitoneal (i.p.) routes in mice. The EOLC (100-400 mg/kg, i.p., or p.o.) was further evaluated for its central effect on novelty-induced behaviour, anxiolytic, sedative and anticonvulsant properties using appropriate standard models. The results obtained were expressed as mean±SEM and analyzed with appropriate statistical procedures.

Six compounds were detected in the oil with the major ones being thujene (29.3%), pyridine (25.7%) and ethanone (22.0%). The results obtained indicate that the LD50 values of the essential oil were ≥4472 mg/kg, p.o., and 1789 mg/kg, i.p. The oil in both routes caused significant ($p<0.05$-0.01) reduction in novelty-induced behaviours of rearing, grooming and locomotion signifying CNS depressant activity. The oil also caused decrease in head dips compared to vehicle indicating sedative or anxiogenic effect, significantly ($p<0.05$-0.01) shortened sleep latency and increased total sleeping time suggesting sedative effect. Finally, the oil prolonged latency to convulsion and prolonged death time caused by pentylenetetrazol-induced convulsions signifying anticonvulsant activities.

It is hereby concluded that the essential oil of L. camara was slightly toxic orally but moderately toxic intraperitoneally, displayed significant CNS depression; and possessed sedative and anticonvulsant activities in mice.

Keywords: Verbenaceae, volatiles, chemical composition, sedative, anticonvulsant

P147: Antipsychotics decreased the uptake of dietary antioxidant quercetin into neonatal rat astrocytes

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Antipsychotics drugs are associated with significant side effects due to their non selective and/or pleiotropic mechanism of actions. Here we try to address the drug flavonoid interaction on the level of membrane transporters in central nervous system, namely astrocytes. Quercetin is a flavonoid widely distributed in fruits and vegetables, and is a potent antioxidant with neuroprotective activity. The aim was to study the interaction between antipsychotics drugs and quercetin by measuring the quercetin uptake into astrocytes.
We isolated astrocytes form the cerebral cortex of the neonatal rats, and grown them into monolayer cultures. We determined the time dependence and concentration dependence of [3H]-quercetin uptake into the cultured astrocytes at 37°C (total uptake) and at 4°C (non-specific uptake). We tested the quercetin uptake by concomitant incubation with 5 typical antipsychotics (chlorpromazine, fluphenazine, thioridazine, promazine, triflupromazine) and 1 atypical antipsychotics (clozapine) in the physiologically relevant concentration range (0.1 nM – 10 microM).
We have found that the uptake of quercetin is mediated by the facilitated diffusion by comparing the uptake at 37° C and at 4° C, where we have obtained no kinetic differences (K_m=4.5 µM; V_{max}= 94 pmol/mg proteins/min). All studied antipsychotics inhibited the quercetin uptake into astrocytes with IC_{50} values in nM range.
Uptake of quercetin is mediated by the facilitated diffusion involving several membrane transporter systems. Importantly antipsychotics inhibited the uptake of quercetin into astrocytes in the concentration range normally found in plasma of patients on medication therapy for managing psychosis. Our study opens the perspective that antipsychotics can reduce the health protective activity of flavonoid-enriched diet.
Keywords: antipsychotics, quercetin, astrocytes, rat

P148: The evaluation of the neurotoxic effects of levosimendan on neuroblastoma cells

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The disturbance of the brain cognitive functions is common after cardiac surgery although it is temporary. Levosimendan is a positive inotropic agent used to increase the pumping power of the heart and neurotoxic effects are not known during cardiac operations. The inhibition of cell proliferation is one of the markers of neurotoxicity. The purpose of this study was to investigate the effects of different concentrations of Levosimendan on the proliferation of the NB2a mouse neuroblastoma cells.

NB2a cells were seed in the DMEM culture media used for neurite extension. NB2a cells were exposed to different concentrations of Levosimendan (0.1, 0.3, 1,3,10, 30,100µM) and neurotoxic effects of the drug were examined using MTT assay, inverted microscopy imaging and TUNEL staining for apoptosis.

Inverted microscopy images of NB2a cells showed that the cells were confluent and in the shape of round in the control group. In the experimental groups, cells showed similar cell proliferation and had similar morphology as the control group. The toxic effect of the Levosimendan was not shown in the NB2a cells at different concentrations. With MTT analysis, there was not significant difference between control and experimental groups. Moreover, there was no difference in staining of apoptotic cell by TUNEL, which shows moderate neurotoxicity.

It was established that high doses of the Levosimendan did not inhibit the proliferation of the NB2a cells. However these results are not sufficient to reveal the neurotoxic effects of these drugs. The inhibition of the cell proliferation occurs in the presence of severe toxicity. Apoptotic cell death did not occur even at highest doses showing that drug is safe for moderate toxic effect. It is concluded that of Levosimendan does not have severe neurotoxic effects in-vitro though the neural toxicity of these substances should be evaluated with the indicators of moderate and chronic toxicity.

Keywords: levosimendan, NB2a, MTT, TUNEL, neurotoxicity

Cancer Chemotherapy

P149: The effect of metformin alone and in combination with 5-fluorouracil and oxaliplatin on human HT-29 colon cancer cell line

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The biguanide metformin is the most prescribed drug for the treatment of type 2 diabetes worldwide. Its mechanism of action is explained by regulation of the AMPK/mTOR pathway which is implicated in the control of protein synthesis and cell proliferation. This enzyme is controlled by the tumor suppressor gene liver kinase B1. Furthermore metformin has been reported to inhibit the growth of various cancers including colon cancer. This study aimed to investigate antiproliferative and apoptotic effects of metformin alone and in combination with chemotherapeutic agents on colon cancer cell line.

HT-29 human colon cancer cells were maintained in RPMI medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic/antimycotic in a humidified atmosphere with 5% CO2 at 37 °C. Cells were treated with metformin alone at 1, 5, 10, 20 and 40 mM concentration for 24, 48 and 72 h and in combination with 5 FU (50 µM) and oxaliplatin (10µM) for 48 h. Cell viability was measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and apoptosis was monitored by measuring caspase-3 levels, as well as by deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) method.

Metformin decreased cell viability and induced apoptosis on HT-29 colon cancer cell line as a single agent and had an additive effect when combined with combination chemotherapeutic agents in the experiment. Metformin may provide an important contribution to the treatment of colon cancer and enhance the activity of chemotherapeutic agents.

Keywords: 5-fluorouracil, cell culture, colon cancer, metformin, oxaliplatin

P150: Comparison of anticancer activity of hydroalcoholic extracts of Curcuma longa L, Peganum harmala L and Boswellia serrata on HeLa cell line

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Cervix cancer is the second most common cancer in women. There are several Iranian native herb used in traditional medicine which is proved to have cytotoxic effect. The present study was designed to compare the anticancer effects of three medicinal herb; Peganum harmala L., Curcuma longa L, Boswellia serrata on cervix cancer cell lines (HeLa).

Hela cells exposed to different doses of hydroalcoholic extracts of these three plants (12.5, 25, 50, 100 and 200 µg/ml) for 24, 48, 72 and 96 hours. After the incubation period, modified colorimetric MTT method was used to determine cytotoxicity. After collecting data statistical analysis was performed using analysis of variance (ANOVA), and P values less than 0.05 was considered significant.

The highest percentage of cell death for harmala was observed after 72 h incubation and its 50 % growth inhibitory concentration (IC50) in 24 h was 12.5 µg/ml. In case of Boswellia serrata the highest percentage of cell death was observed after 72 h incubation and IC50 in 24 and 48 h were 50 and 12.5µg/ml, respectively. Curcuma longa L had IC50 =12.5 after 72 h of incubation.

The results of MTT assay showed that Boswellia serrata and Peganum harmala L extract had time and dose dependent cytotoxic effect however, Curcuma longa L extracts induced apoptosis in a time-dependent manner.

Keywords: Peganum harmala L, Boswellia serrata, Curcuma longa L, HeLa cell line, MTT

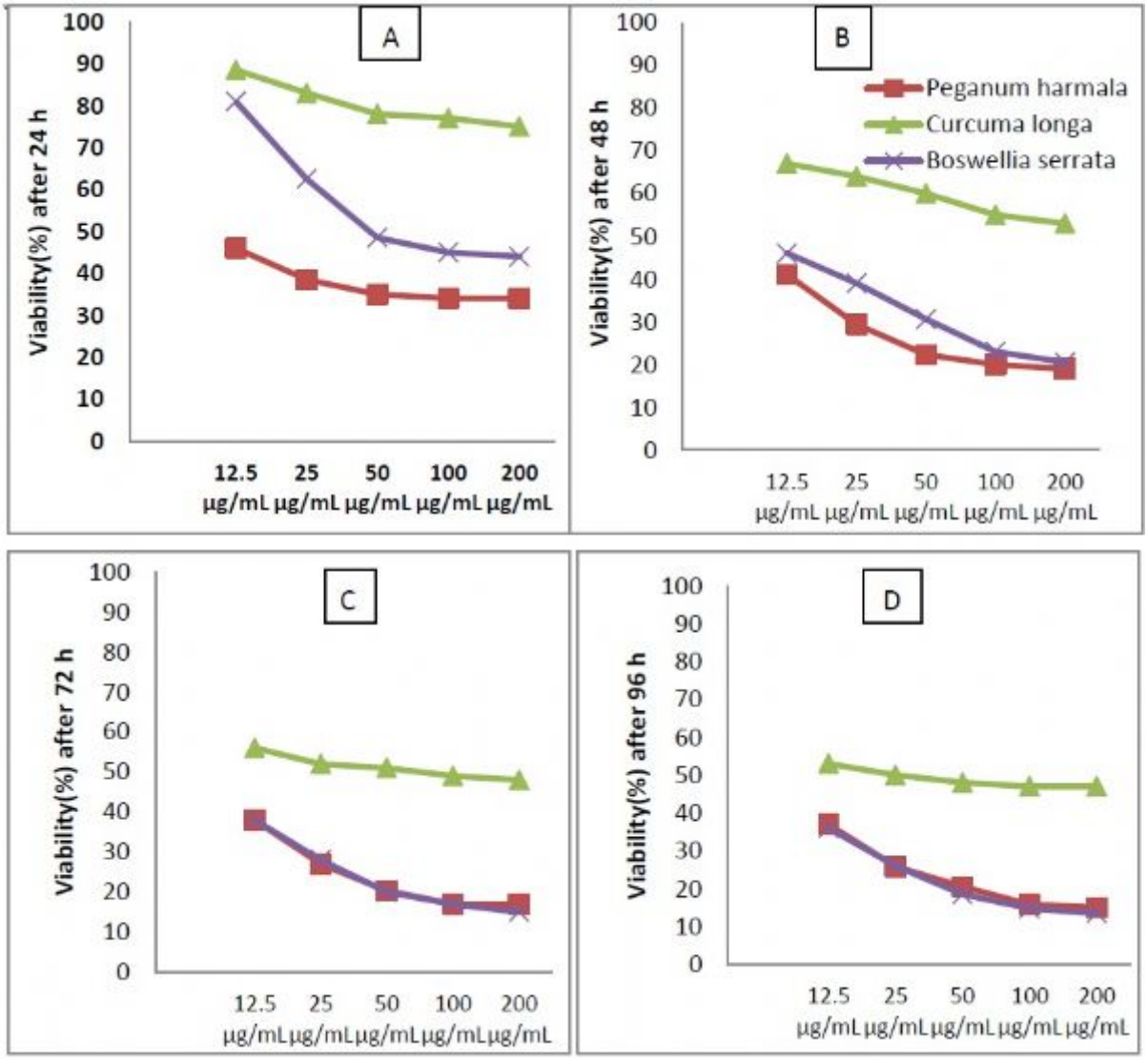

Figure 1. This figure shows percentage of Hela cells viability, after 24 (A), 48(B), 72(C), and 96(D) hours incubation with hydroalcoholic extracts of Curcuma longa L, Peganum harmala L and Boswellia serrate.

P151: Cytotoxic activity of the alkaloids from the stem of Xylopia laevigata

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Xylopia laevigata (Annonaceae), popularly known as “meiú” or “pindaíba”, is widely used in folk medicine on the Northeast of Brazil; however, only some chemical and pharmacological studies have been reported for this species. The aim of this study was to investigate the chemical analyses and cytotoxic properties of the stem of X. laevigata. The phytochemical analyses of the stem of X. laevigata allowed the isolation of 19 alkaloids, roemerine, anonaine, lanuginosine, glaucine, xylopine, oxoglaucine, norglaucine, xylopinine, norpurpureine, N-methyllaurotetanine, norpredicentrine, discretine, calycinine, laurotetanine, reticuline, corytenchine, discretamine and flavinantine. The cytotoxic activity was determined on cultured tumor cells. All compounds were identified by a series of spectrometric data, such as MS, NMR, as well as comparison with data reported in the literature. The in vitro cytotoxic activity was performed towards the tumor cell lines B16-F10 (mouse melanoma), HepG2 (human hepatocellular carcinoma), K562 (human chronic myelocytic leukemia), and HL-60 (human promyelocytic leukemia) and non-tumor peripheral blood mononuclear cells (PBMC), using the alamar blue assay. Lanuginosine, xylopine and norglaucine presented the highest cytotoxic activities. Additionally, the pro-apoptotic effects of lanuginosine and xylopine were investigated in HepG2 cells using light and fluorescence microscopy and flow cytometry-based assays. Cell morphology consistent with apoptosis and a remarkable phosphatidylserine externalization were observed in lanuginosine- and xylopine-treated cells, suggesting induction of apoptotic cell death. In conclusion, these data suggest that X. laevigata is a potential resource of cytotoxic alkaloids.

Keywords: Xylopia laevigata, Annonaceae, alkaloids, cytotoxicity, apoptosis

P152: Ehrlich Ascites Carcinoma: One of the most lucid cell lines to carry out in-vitro anticancer research- a review

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Cancer has been one of the major causes of mortality in all parts of the world. With time increasing interest in anticancer research has been observed and eventually its importance have been realised. Thus scientists in search for a better technique and also keeping in mind the raised concern regarding the ban on using living animals in biological experiments, have led the path of in-vitro cell line research. So the search for an alternative which could easily resemble the desirable features of the tumors, such as easily transplantable, with rapid proliferation, shorter life-span and 100% malignancy gave birth to Ehrlich Ascites Carcinoma cell lines. These are easily maintained and can be inoculated in the peritoneal cavity of the Swiss albino mice. These cells play a vital role in carrying out preliminary anticancer drug screening which minimises the need for sacrificing the animals in order to confirm the claimed pharmacological response of a certain plant extract. Thus this present review work extensively focuses on the different characteristics that these cell lines posses, which are how to culture them in the mouse peritoneal cavity as well as how assay them using the experimental protocols. So through this work I have tried to make use of all the available literature and tried make the information as lucid as possible so that all young researchers can make use of these cell lines and make them as one of the basic tools for anticancer drug screening.

Keywords: cancer, Ehrlich Ascites Carcinoma (EAC), tumor, ascites

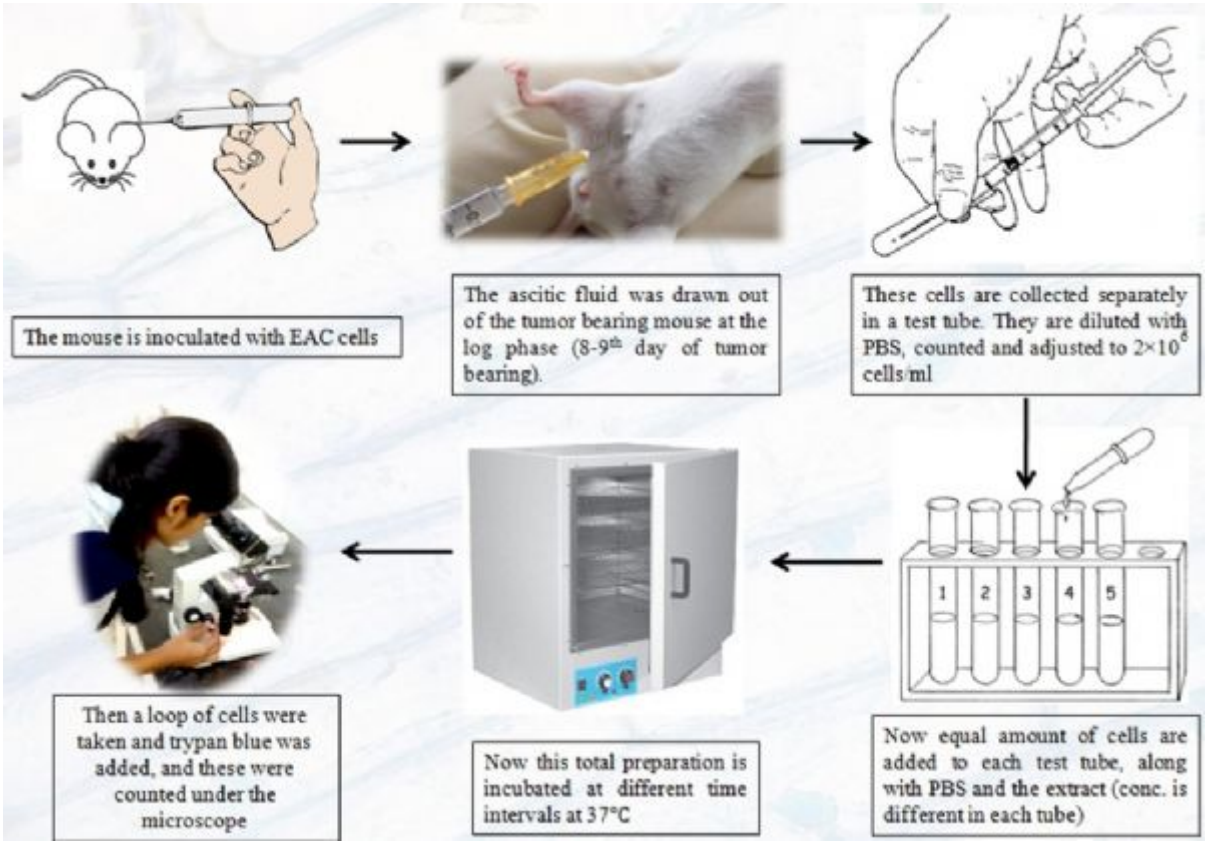

Figure 1. Process of carrying out the whole assay procedure. A pictorial description of the whole experimental procedure.

P153: Apoptotic and cytotoxic effects of Pistacia vera nut extract on human hepatocellular carcinoma cell line SNU-423

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Hepatocellular carcinoma (HCC) is the most common primary malignant cancer affecting the liver. In spite of the use of chemotherapy and tyrosine kinase inhibitors in treating HCC, patients with HCC still face poor prognosis. The aim of this study was to investigate the anticancer activity of water extract of the pistachio (*Pistacia vera*) nut on the human HCC cell line SNU-423.

SNU-423 cells were cultured in DMEM medium supplemented with 10% fetal bovine serum. When cells reached 70% of confluence, they were treated with water extract (150 µg/ml) or saline during 24 h. Apoptotic cells were quantified by Annexin-V/7AAD-positive staining, using an Annexin-V-FITC/7AAD kit from Beckman Coulter. For the cell cycle assay, BD Cycletest™ Plus DNA Reagent kit (Biosciences, Germany) was used. Apoptosis and cell cycle analysis were performed by using flow cytometer (NAVIOS Beckman Coulter, USA). For gene expression study, mRNA was isolated from cells by using miRNeasy Mini Kit (Qiagen GmbH, Germany). Then cDNA was produced with the Ipsogen RT Kit (Qiagen GmbH). qRT-PCR was performed by Rotor Gene 6000 (Qiagen GmbH, Hilden, Germany).

MTT assay results demonstrated that extract decreased cell viability by 21.6%. NO levels, measured by NO chemiluminescence method, diminished by 23.3%. Apoptosis of the SNU-423 cells was stimulated with water extract (1.5%) when compared to saline (0.8%). Cell cycle progression was not modified with extract in cell cycle assay (water extract: G0/G1 75.6%, G2 2.8%, and S 21.6%; saline: G0/G1 79.3%, G2 0.2%, and S 20.5%, p=0.2148). p53 (2.6 fold) gene expression was significantly increased with water extract treatment.

Our results showed that water extract of the pistachio nut exhibited its anti-tumor activity against SNU-423 cells through decreasing cell viability, diminishing NO levels, promoting apoptosis, and elevated p53 gene expression.

This study is supported by Gaziantep University (Project: TF.13.10).

Keywords: apoptosis, cell cycle, flow cytometer, cancer, gene expression

P154: Anticancer effects of cannabinoid agonists ACEA and L-759656 on MCF-7 breast cancer cell line and HUVEC

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Cannabinoids, are a group of chemicals derived from a plant named "Cannabis sativa linnaeus" also known as marijuana. It has been shown that cannabinoids have two receptors CB1 and CB2, which endogenous ligands bind. Cannabinoids have been used for treatment with the discovery of the mechanism of action of agonists and antagonists. In this study we aimed to evaluate the anticancer effects of ACEA (CB1) and L-759656 (CB2) agonists on MCF-7 breast cancer cell line and human umbilical vein endothelial cells (HUVECs) we also aimed to observe the changes that these agonists will create on proliferation and angiogenesis stages.

To determine the effects of cannabinoid agonists on cell proliferation and angiogenesis, cytotoxicity of ACEA and L-759656 were tested on MCF-7 breast cancer cell line and HUVEC by using XTT assays. 0.1, 0.5, 2.5, 7.5, 15 and 30 µM concentrations of ACEA and L-759656 were administered. Results were evaluated as absorbance at 450 nM and IC50 values were calculated according to the absorbance data.

Both ACEA and L-759656 produced concentration dependent cytotoxic effect on MCF-7 and HUVEC. IC50 values for ACEA were 5.2 and 16 µM, respectively. IC50 values for L-759656 were 7.6 and 17 µM, respectively. Significant difference was observed between MCF-7 cells and HUVECs in terms of cytotoxic effects of both cannabinoid agonists ($p<0.05$).

Cytotoxic drugs remain the backbone of cancer treatment, but they are limited by a narrow therapeutic index, significant toxicities and frequently acquired resistance. This requires the development of new agents. These results clearly show that cannabinoid agonists ACEA (CB1) and L-759656 (CB2) have potential cytotoxic and antiangiogenic effects and these agonists may be an alternative treatment option in breast cancer with further studies underlying the mechanisms behind the positive effect.

Keywords: cannabinoid agonist, ACEA, L-759656, breast cancer, cytotoxicity

P155: Anticancer effects of Rho kinase inhibitors AS 1892802 and Fasudil hydrochloride on MCF-7 breast cancer cell line and HUVEC

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Rho kinases (ROCKs) which play central roles in the organization of the actin cytoskeleton, are observed to be involved in the pathogenesis of variety of diseases. The inhibition of ROCKs has shown beneficial effects in human and animal disease modals. This study was conducted to explore the antitumor effect of Rho kinase Inhibitors, AS 1892802 and Fasudil hydrochloride by investigating their toxicity on MCF-7 breast cancer cell line and human umbilical vein endothelial cells (HUVECs). In this study we also aimed to observe the antiangiogenic potential of these inhibitors.

In order to determine the effects of Rho kinase inhibitors on cell proliferation and angiogenesis, cytotoxicity of the AS 1892802 and Fasudil hydrochloride was tested in MCF-7 breast cancer cells and HUVECs by using XTT assays. 0.1, 0.5, 2.5, 7.5, 15 and 30 µM concentrations of AS 1892802 and Fasudil hydrochloride were administered. Results were evaluated as absorbance at 450 nM and IC50 values were calculated according to the absorbance data.

Both AS 1892802 and Fasudil hydrochloride produced concentration dependent cytotoxic effect on both MCF-7 cell line and HUVECs. IC50 values for AS 1892802 were 8.6 and 15 µM, respectively. IC50 values for Fasudil hydrochloride were 3.25 and 13.5 µM, respectively. It has been observed that there was significant difference between MCF-7 cell line and HUVECs in terms of cytotoxic effects of both Rho kinase inhibitors ($p<0.05$).

Although Rho kinase inhibitors are well known to have effect on many biological systems, there are not many studies aiming anticancer effects of Rho kinase inhibitors. The present study shows that Rho kinase inhibitors may be drug candidate for treating breast cancer with their cytotoxic and antiangiogenic effects.

Keywords: AS 1892802, fasudil hydrochloride, rho kinase, angiogenesis, cytotoxicity

P156: Protective and therapeutic effects of molsidomine on retinopathy and oxidative stresss induced by radiotheraphy in rat eyes

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We aimed to determine the role in preventing radiation-induced retinopathy after head and neck region irradiation of rats with a single radiation dose of 15 Gy.
Wistar albino rats were randomly grouped into 5 as follows: (1) control group rats which were applied intraperitoneal (i.p.) vehicle without radiotherapy (RT); (2) RT group rats received a single dose of 15 Gy irradiation and after daily 0.1 ml vehicle i.p. for 5 consecutive days; (3) molsidomine (MOL) group rats were treated for 5 consecutive days by i.p. with 4 mg/kg/day MOL; (4) irradiation plus MOL group rats (RT+MOL) received irradiation and after 10 days single daily i.p. dose of MOL for 5 consecutive days; (5) MOL+RT group rats were treated for 5 consecutive days by i.p. with MOL before RT. At the end of the work the rats were sacrificed under high-dose anesthesia on the 16th day and then eye tissues were taken for analyses.
RT significantly decreased both the content of GSH and the activity of SOD, and significantly increased the production of MDA level in the rat eyes. MOL treatment significantly increased SOD and GSH levels and significantly decreased the MDA production ($p<0.0001$).
We suggest that MOL is a powerful antioxidant and free radical scavenger that prevents the rat eyes from radiation-induced retinopathy and oxidative stresss.
Keywords: antioxidant, apoptosis, ionizing radiation, molsidomine, oxidative stresss

Figure 1. Irradiation field (A), and simulation radiograph (B)

Table 1. The comparison of the eye-tissue lipid peroxidation parameter and antioxidant contents among the study groups.

Parameters	Control	RT	MOL	MOL+RT	RT+MOL
MDA (nmol/g tissue)	14.38±4.28	27.47±3.49a	14.86±4.06b	14.89±1.66b	16.18±3.12b
SOD (U/mg protein)	0.60±0.05	0.42±0.01c	0.58±0.05d	0.57±0.03d	0.59±0.06d
GSH (µmol/g tissue)	29.63±2.47	15.49±2.99c	27.20±3.49d	28.86±3.82d	26.93±2.24d

Data were presented as mean±SD. aSignificant increase vs. control group ($p<0.0001$). bSignificant decrease vs. RT group ($p<0.0001$). cSignificant decrease vs. control group ($p<0.0001$). dSignificant increase vs. RT group ($p<0.0001$).

P157: A novel HSP90 inhibitor, PU-H71 decreases tumor growth and alters anti-tumoral immune response in metastatic breast carcinoma model

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PU-H71, a novel HSP90 inhibitor, is under evaluation for treatment of advanced cancer in Phase I trials. It is not known whether PU-H71 alters inflammatory and anti-tumoral immune response in metastatic breast carcinoma. The purpose of the present study was to evaluate possible anti-tumoral and pro-inflammatory effects of PU-H71 on orthotopic metastatic murine breast carcinoma.
4TBM cells were injected orthotopically into mammary gland of 8–10-week-old Balb-c mice. PU-H71 was formulated 10% DMSO in PBS and were administered i.p. 75 mg/kg three days a week for four weeks. Animals were sacrificed 26 days after injection of tumor cells and primary tumors, lung and liver tissues were collected for macroscopic metastasis analysis. Spleen and draining lymph nodes of animals were removed and single cell suspensions were prepared for mixed leukocyte cultures (MLC). Measurements of TNF-alpha, IFN-γ, IL-10 and IL-17 were performed in MLC supernatants by ELISA.
PU-H71 significantly delayed the growth of tumor and decreased macroscopic lung metastasis. TNF-alpha, IFN-γ and IL-17 secretion was higher in tumor challenged MLC supernatants prepared from PU-H71 treated animals compared to untreated mice. IL-10 secretion on the other hand was lower in PU-H71 treated group.
These results demonstrate that PU-H71 can be effective in decreasing metastatic growth in breast carcinoma. PU-H71 also seems to increase anti-tumoral immunity and could be considered as adjuvant in immunotherapy.
Keywords: PU-H71, HSP90, anti-tumoral immunity, breast cancer

P158: PU-H71 and radiotherapy in combination decreases tumor growth and lung metastasis in metastatic breast carcinoma model

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HSP90 (Heat shock protein 90) inhibitors are considered as a new radiosensitizing agents. PU-H71, a novel HSP90 inhibitor, inhibits breast cancer growth but it is effects in combination with radiation therapy (RT) in metastatic breast cancer in vivo are not known. The goal of the present study was to evaluate possible anti-tumoral, anti-metastatic effects of PU-H71 and RT co-treatment on orthotopic metastatic murine breast carcinoma.

4TBM cells were injected orthotopically into mammary gland of 8–10-week-old Balb-c mice. PU-H71 was administered i.p. 75 mg/kg three days a week for four weeks. Single dose RT of 18 Gy was applied 6 days after injection of tumor cells. Animals were sacrificed 26 days after injection of tumor cells and primary tumors, lung and liver tissues were collected for macroscopic metastasis analysis. Spleen and draining lymph nodes of animals were removed and single cell suspensions were prepared for mixed leukocyte cultures (MLC). Measurements of TNF-alpha, IFN-γ, IL-10 and IL-17 were performed in MLC supernatants by ELISA.

PU-H71 and RT in combination treatment was more effective than RT alone. Combination treatment markedly decreased the tumor growth and lung metastasis. Combined treatment increased IFN-γ, and IL-17 secretion in MLC challenged with irradiated tumor cells demonstrating that combination therapy may enhance anti-tumoral immunity. On the other hand we observed an increase TNF-alpha levels which may indicate increase inflammatory response to co-treatment.

These results demonstrate for the first time that PU-H71 may enhance therapeutic effects of radiotherapy in highly metastatic breast carcinoma murine model. Furthermore, PU-H71 and RT in combination may increase anti-tumor and pro-inflammatory immune response.

Keywords: PU-H71, HSP90, radiation therapy, breast cancer, immune response

P159: Effects of alfacalcidol and calcitriol on cell proliferation in HEC1A endometrial adenocarcinoma cells

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Endometrial adenocarcinoma is the most common gynecologic cancer in women. The presence of the vitamin D receptor in endometrial tissue has been shown. Furthermore the active vitamin D calcitriol regulates numerous cellular pathways that could have a role in determining cancer risk and prognosis. Proliferation is an important parameter in the development and spread of cancer cells. Therefore we aimed to study the effects of alfacalcidol and calcitriol on proliferation, in endometrial adenocarcinoma HEC1A cells.

HEC1A human endometrial cancer cell line was used in cell culture experiments. McCoy 5A was used as medium and cell were grown in an incubator at 37^{o}C under 5% CO2 + 95% air. Cells were grown to confluency at the day 10-14. Proliferations were measured by the xCELLigence device which can analyze cellular events in real time without marking the cells with chemical substances (5). Alfacalcidol and calcitriol were dissolved in ethanol (Final concentration were 0.05 %). Experimental groups were as below.

I.Group: saline, II.Group: ethanol, III.Group: alfacalcidol 10-6M, IV.Group: alfacalcidol 10-7M, V.Group: alfacalcidol 10-8M VI.Group: calcitriol 10-6M, VII.Group: calcitriol 10-7M, VIII.Group: calcitriol 10-8M. For statistical analysis, one-way analysis of variance followed by the Bonferroni post hoc test was used. $P<0.05$ were considered significant.

There was no significant difference between saline and ethanol groups. Alfacalcidol (10-8M-10-6M) and calcitriol (10-8M-10-6M) significantly increased proliferation ($P<0.05$). Proliferative effect of alfacalcidol became significant 30 minutes after application and sustained for 56 hours whereas for calcitriol started after 16 hours and sustained 46 hours.

In the present study, alfacalcidol and calcitriol increased proliferation in HEC1A cells. Proliferative effects of alfacalcidol start more rapidly than calcitriol. For the elucidation of the role of vitamin D in pathogenesis of endometrial cancer, further studies are needed.

Keywords: HEC1A, endometrium, adenocarcinom, alfacalcidol, calcitriol

P160: A meta analysis for comparison efficacy and safety of epidermal growth factor receptor inhibitors cetuximab and panitumumab

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The aim of this study were meta analyzed of cetuximab and panitumumab by comparing with their efficacy and safety properties. Both of these drugs are epidermal growth factor receptor (EGFR) inhibitors that are approved and can be used at the first line therapy or these condline therapy by following similar therapy protocols.Although both active substances are used metastatic colorectal cancer treatment for the same patient population, who have the same therapy background and performance characteristics, these agents can not be used interchangeably and/or must be continued to the treatment in which the agent was used in the initial treatment. Therefore the question of which agent will be selected comes up.

Different literature searchings were achieved for cetuximab+FOLFIRI (5-Flourouracil+Leucovorine+İrinotecan) and panitumumab+FOLFIRI combinations separately. And the clinical trials which had inclusion criterions, are involved to the meta analysis. “Personalized therapy” concept is so important, especially for oncologic patients, a demonstrative and develop able machine learning software was developed with decision tree algorithm and c45 learning for this purpose. By making data enterance to the software, as patient demographic properties, treatment step, different chemotherapy regimens which are used with drugs (cetuximab and panitumumab), the propable overall survival time, progression free survival time and side event incidence are calculated by the programme.

According to the results, KRAS wild typ patients have more benefit than others, compatible with the earlier study results. And the patients (KRAS wildtype) who used cetuximab, had longer progression free survival and overall survival times, statistically significant. The percentage of side events diarrhea, erythema and dermatitis acneiform observed at cetuximab group is significantly higher than the panitumumab group.

Keywords: cetuximab, panitumumab, colorectal cancer, meta analysis, machine learning

Immunopharmacology

P161: Erythrocyte ghosts' influence on the phenotypic plasticity of macrophage

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The phenotype of macrophages adjusts the reprogramming of the inflammatory response for an actual problem of modern medicine.

Purpose of the study is to evaluate the influence of erythrocyte ghosts with rifampicin on the macrophages phenotype.

Encapsulation of antibiotic rifampicin into the erythrocyte ghosts was performed by the method of hypotonic preswelling. It is conventionally called pharmacocytes. Human monocytes, red blood cells were prepared of the peripheral blood of healthy volunteers.

Suspension of monocytes was isolated by centrifugation on Histopaque gradient density 1.077 g/cm3 and it was obtained by the magnetic cell sorting using a monocyte isolation kit Dynabeads® (Invitrogen).

100 ng/ml gamma interferon (INF γ) was added for activation of pro-inflammatory phenotype in the incubation medium of monocytes, and 10 ng/ml interleukin 4 (IL4) was added for stimulation of anti-inflammatory phenotype. Incubation was carried out during 3 and 5 days. The concentration of the cytokines was determined using the ELISA (enzyme-linked immunosorbent assay) kits in the culture medium.

Pharmacocytes reduce the secretion of pro-inflammatory cytokines interleukin-1β (IL1β), tumour necrosis factor α (TNF-alpha) and increase the production of anti-inflammatory cytokines by almost 6 times interleukin-1 receptor antagonist (IL1ra) (up to 0,584±0,15, $p<0.01$) and pulmonary and activation-regulated chemokine (PARC/CCL18) (up to 646,49±35,76, $p<0,001$) when monocytes incubated with different phenotypes for 3 days. Level PARC/CCL18 remains high (585.65 pg / mL) in these populations of cells on the 5th day of cultivation, but cytokine (6,082 pg / ml) secretion reduced when incubated without pharmacocytes.

Pharmacocytes with rifampicin led to an increase in anti-inflammatory cytokine production PARC/CCL18. PARC/CCL18 is a known marker M2 macrophage phenotype, so, probably; pharmacocytes stimulate the formation of anti-inflammatory phenotype.

Keywords: pharmacocytes, phenotypic plasticity of macrophages

P162: Outer Membrane Vesicles (OMVs) secreted by probiotic *Escherichia coli* Nissle 1917: Effects on chronic experimental colitis induced by dextran sulfate sodium (DSS) in mice

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Escherichia coli Nissle 1917 (EcN) is a probiotic strain widely used for the treatment of intestinal disorders, including inflammatory bowel disease (IBD). This Gram-negative probiotic releases Outer Membrane Vesicles (OMVs) as a secretion pathway of proteins and other mediators that interfere with the immune system. Moreover theses vesicles have an important role in bacteria-host interactions, being a way of communication that avoids direct intercellular contact.

The aim of the present study was to evaluate whether the secretion of OMVs by the EcN strain may contribute to its reported beneficial effects on the intestinal immune response in a murine model of colitis.

We used a 16 days preventive treatment protocol in male C57BL/6J mice, which included 10 days of pre-treatment with 5 µg or 20 µg of OMVs per mouse followed by 6 days of colitis-induction using a 3% DSS solution in drinking water. The inflammatory status was evaluated daily by a Disease Activity Index (DAI) and the colonic damage was assessed by evaluating mRNA relative expression of different mediators by qPCR.

Both doses of OMVs resulted in a reduction of DAI, which was higher for the lowest dose, compared with the DSS-control. Moreover, only 5 µg-OMVs were capable of increase the expression of IL-10 and TFF-3, but at the same time, reduce the expression of different inflammatory markers, including IL-1β, TNF-alpha, ICAM-1, MMP-2, MMP-14, COX-2 and iNOS.

The administration of OMVs from EcN to mice ameliorated DSS-induced colitis, being this beneficial effect associated with an improvement of the altered immune response that occurs in intestinal inflammation.

Keywords: OMVs, EcN, inflammation, cytokines, dextran sulfate sodium (DSS)

P163: Intestinal anti-inflammatory effect of *Satureja obovata* extract in the TNBS model of rat colitis

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Satureja obovata whole plant is used in mediterranean traditional medicine as an anti-inflammatory remedy. It contains polyphenols, which display antioxidant properties, being of potential interest for the treatment of inflammatory conditions associated with oxidative stresss in humans, including inflammatory bowel disease (IBD).The aim was to evaluate the intestinal anti-inflammatory properties of a hydroalcoholic extract of S.obovata, in the trinitrobenzenesulphonic acid (TNBS) model of rat colitis, a well characterized model with some resemblance to human IBD.

Female Wistar rats were assigned to five groups: non-colitic, control colitic and colitic treated groups with S. obovata extract (10 and 25 mg/kg/day), or with dexamethasone (1.2 mg/kg/day). Colonic damage was assessed macroscopically and biochemically. Several pro-inflammatory markers were evaluated. In vitro immunomodulatory properties of different concentrations of S. obovata extract were determined in LPS-stimulated RAW 264.7 cells by nitrite quantification and in LPS-stimulated CMT-93 cells by cytokine production (IL-6 and TNF-alpha).

S. obovata showed intestinal anti-inflammatory effect, as evidenced by the reduction of macroscopic score. The extract also decreased colonic MPO activity and increased glutathione content. S. obovata extract reduced the colonic expression of the pro-inflammatory cytokines IL-1β, IL-6, IL-12a and IL-23 and the adhesion molecule ICAM-1, as well as of the chemokine MCP-1. S. obovata extract was also able to significantly up-regulate the expression of the markers of intestine epithelial integrity: villin and the mucin MUC-3. Moreover, it displayed immunomodulatory properties in vitro since they inhibited nitrite production induced by LPS in RAW cells and decreased IL-6 and TNF-alpha production.

The extract of Satureja obovata showed intestinal anti-inflammatory activity in the TNBS model of rat colitis and in vitro studies. This beneficial effect can be related with its antioxidant properties as well as with the downregulation of the immune response, which can result in the improvement in the intestine epithelial barrier.

Keywords: TNBS, colitis, *Satureja obovata*, immunomodulatory properties, cytokines

P164: NK2 receptor but not NK1 receptor antagonist (GR159897, RP67580) increases release of angiogenic chemokine from breast carcinoma

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Substance P (SP) has a widespread distribution in both central and peripheral nervous systems. SP and its receptors (neurokinin-1 receptor; NK1-R and neurokinin-2 receptor; NK2-R) play a significant role angiogenesis, and inflammation. MIP2 is the mouse homologue of CXCL2 which induces angiogenesis and recruits neutrophils. SP is known for its inflammatory effects especially under acute conditions, hence SP receptor antagonists are likely to inhibit inflammatory factors such as MIP-2. The goal of this study was to determine the effects of NK1-R and NK2-R antagonists on MIP-2 secretion from highly metastatic breast carcinoma cells.

We used heart (4THM) and brain metastatic (4TBM) cells of 4T1 breast carcinoma cells which were originally obtained from spontaneously formed breast cancer in a Balb-c mouse. Later 4THM and 4TBM cells were treated with different concentrations (10µM and 30µM) of NK1-R antagonist RP67580 and NK2-R antagonist GR159897. Changes in cell proliferation were determined by using WST-1. Changes in secretion of MIP-2 were determined using ELISA. The effects of antagonists on phosphorylation of p38 were also examined using western Blot.

RP67580 and GR159897 at 30µM concentration (relatively high dose) inhibited cell growth. Surprisingly GR159897 (30µM) increased secretion of MIP-2 and phosphorylation of p38 demonstrating that SP and related neurokinins may inhibit release of angiogenic/inflammatory chemokines from cancer cells.

Even though NK2-R antagonist inhibited cell proliferation, NK2R antagonist-induced increases in MIP-2 secretion from cancer cells, may increase local inflammation and angiogenesis counteracting its anti-tumoral effects.

This study is supported by TUBITAK, Grant no: 214S389.

Keywords: breast cancer, neurokinin receptor 1, neurokinin receptor 2, p38, MIP-2

P165: In-vivo effects of NK1 and NK2 receptor antagonists on metastatic breast carcinoma

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Neurokinin 1 and neurokinin 2 receptors are mammalian tachykinin receptors. Activation of these receptors play an important role in angiogenesis, inflammation and cancer, hence inhibitors may have anti-tumoral, anti-metastatic effects. The goal of this study was to determine the effects NK1-R and NK2-R antagonists (RP67580, GR159897 respectively) on in vivo growth and dissemination of breast carcinoma.

Liver metastatic (4TLM) and brain metastatic (4TBM) cells of 4T1 breast carcinoma (10000 cells/mouse) injected into the mammary fat pad of Balb-c mice and two days after inoculation of the tumor cells, treatments were started as 5 times a weeks (0,1-1 ve 4mg/kg). Changes in systemic metastasis (lung and liver) evaluated microscopically and macroscopically 26 days after injection of cells.

RP67580 and GR159897 at 0,1- 1- 4mg/kg concentration did not affect primer tumor growth in both 4TLM and 4TBM injected mouse. Surprisingly we observed tumor-type dependent changes in liver metastasis. Spesifically NK1R antagonist decreased liver metastasis in 4TLM-injected group but not in 4TBM injected mice. On contrary increased the effects of antagonist in liver metastasis were different in 4TLM injected mice, NK1-R antagonist NK1R antagonist at 4 mg/kg dose increased liver metastasis in 4TBM injected mice.

Because of differences we observed in liver metastasis of different subset of breast carcinoma cells, NK1R antagonist should be considered in treatment after through evaluation of possible factors involved in outcome of the treatment.

This study is supported by TUBITAK, Grant no: 214S389.

Keywords: breast cancer, NK1-R antagonist, NK2-R antagonist, metastasis

P166: Dietary galacto-oligosaccharides improve budesonide treatment efficacy in house dust mite-induced asthma in mice by enhancing suppression of pulmonary Th2 driving mediators

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The standard treatment for airway inflammation in allergic asthma makes use of glucocorticosteroids, such as budesonide. Previously, we showed dietary non-digestible galacto-oligosaccharides (GOS) to suppress symptoms in a murine model for HDM-induced asthma.

We aimed to study combined dietary GOS and budesonide treatment on allergic asthma in mice.

BALB/c mice were sensitized and challenged with HDM while being fed a diet containing 0, 1 or 2.5 w/w% GOS. Budesonide (500ug/kg) was either or not instilled oropharyngeally. On day 14, airway resistance was determined and leukocyte subtypes were analyzed in the broncho-alveolar lavage fluid (BALF). Mast cell activation was assessed and chemokines and cytokines in lung homogenates and T-helper cell subtypes were phenotyped in lung cell suspensions by means of flow cytometry.

HDM allergy increased airway responsiveness, budesonide treatment prevented this in mice fed the control or GOS diet. BALF leukocyte numbers were enhanced in HDM allergic mice. Budesonide treatment reduced the number of lymphocytes and eosinophils in HDM allergic mice, while the GOS diet reduced the number of eosinophils only. In mice fed GOS and treated with budesonide the lung inflammation, specifically eosinophilic infiltration, was largely abolished. Both GOS as well as budesonide reduced mast cell activation. The combination of GOS and budesonide, most effectively suppressed HDM induced increase in IL-33, CCL17, CCL22 and IL-13 in lung homogenates and the frequency of lung Th2 cells.

Dietary intervention using GOS may be a novel way to further improve the effectiveness of anti-inflammatory drug therapy in asthma.

Keywords: asthma, oligosaccharides, budesonide, house dust mite

P167: The role of urotensin-II and its receptors in sepsis induced lung injury under diabetic conditions

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This study aimed to investigate the potential role of Urotensin-II receptors in sepsis induced lung injury in diabetic mice via using Urotensin-II receptor agonists and antagonists.

A total of 110 male CD1 mice were used in this study. Diabetes was induced through intraperitoneal administration of Streptozotocin at a single dose of 200 mg/kg-body weights in the diabetes and the diabetes-sepsis groups. One month after diabetes induction, the cecal ligation and puncture (CLP) induced polymicrobial sepsis model was applied in diabetic and non-diabetic mice. Low and high doses of Urotensin-II agonist (HU-II) and antagonist (palosuran) were administered one hour after sepsis induction. HU-II administration repeated in two hours intervals. Biochemical, molecular, and histopathologic examinations were performed 6 hours and 12 hours after sepsis.

Regarding to the mRNA expression and immunohistochemistry results of TNF-alpha, IL-1β, IL-6 and NF-κB, it was observed that cytokine levels increased in both time points in the diabetes and the sepsis groups compared to the healthy group and this increase was higher in the diabetes-sepsis groups. Our biochemical (SOD, GSH, MDA) and histopathological findings also supported these results. All increased parameters were reduced dose-dependently by Palosuran, an urotensin receptor antagonist, administration. mRNA expression of Urotensin-II and its receptor were examined in the lung tissue. We have found that Urotensin-II and Urotensin receptor levels which increased in the damaged tissue were significantly reduced by palosuran administration.

This study has shown that Urotensin-II and Urotensin-II receptors contribute in the aggravation of sepsis-induced lung injury in diabetic mice and palosuran prevents this damage by antagonizing this receptor.

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Keywords: diabetes, sepsis, lung, urotensin-II, palosuran

Drugs for Infectious Diseases

P168: Evaluation of the antibacterial activity of the solvent fractions of the leaves of Rhamnus prinoides L'Herit (Rhamnaceae)

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The objective of study was to advance further the claimed antibacterial activity of Rhamnus prinoides, a medicinal plant used traditionally for treatment of infectious diseases.

The solvent fractions of the plant were obtained by successive soxhlet extraction with chloroform and methanol followed by maceration of the marc with water. The antibacterial activity of the solvent fractions was evaluated on seven bacterial species using agar well diffusion at different concentrations (780mg/ml, 390mg/ml and 195mg/ml) in the presence of positive control and negative control. The minimum inhibitory concentration of the solvent fractions was determined by resazurin based microtiter plate serial broth dilution method.

Methanol and chloroform fractions revealed antibacterial activities against the growth of test bacterial strains with varying antibacterial spectrum and the susceptible bacterial species were Staphylococcus aureus, streptococcus pyogen and streptococcus pneumoniae. The average minimum inhibitory concentration value of the methanol and chloroform fractions ranged from 8.13 mg/ml to 32.5 mg/ml and from 8.13 mg/ml to 16.25 mg/ml, respectively.

The methanol and chloroform fractions demonstrated significant antibacterial activities against the growth of pathogenic bacteria which indicates the potential for development of antibacterial compounds.

Keywords: antibacterial activity, minimum inhibitory Concentration, Rhamnus prinoides

P169: In vitro susceptibility patterns of clinically important Trichophyton, Microsporium and Epidermophyton species against four antifungal drugs

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In recent years, although the incidence of dermatophytosis has increased considerably, data regarding susceptibility profiles against antifungal agents is still insufficient. Dermatophytes have different susceptibility patterns, and relative or absolute resistance. This study was designed with the aim of detecting in vitro susceptibilities of the agents of dermatophytosis to myconazole, itraconazole, terbinafine and nystatin.

Skin, hair and nail scarping specimens of patients, suspected of having dermatophytosis were collected and identified according to the cultural and microscopical characteristis. A total of 100 strains including Trichophyton rubrum (n=66), Trichophyton mentagrophytes (n=15), Microsporium canis (n=8), Trichophyton tonsurans (n=4), Epidermophyton floccosum (n=3), Trichophyton verrucosum (n=3), Trichophyton terrestre (n=1) were tested by determining minimal inhibitory concentrations(MIC) with myconazole, itraconazole, terbinafine and nystatin, by following the CLSI standards (M38-A2). Antifungals were prepared with a final concentration 0,0313-16 µl/ml. Three reference strains, Trichophyton mentagrophytes var. erinacei NCPF 375, Microsporium gypseum NCPF 580 and Aspergillus fumigatus ATCC 204305 were included as quality controls.

According to antifungal susceptibility testing, T. rubrum, T. tonsurans and E. floccosum had the highest MIC values to the tested antifungals. MIC values of T. rubrum isolates ranged between 0.0313- >16 µg/ml. Lowest MIC values obtained with itraconazole ranged between 0.0313- 0.5 µg/ml, 0.125- 0.5 µg/ml, 0.0625-2 µg/ml and 0,0313 µg/ml against T. mentagrophytes, T. verrucosum, M. canis and T. terrestre respectively. MIC ranges of myconazole were, 0.0625- 2 µg/ml, 1- 2 µg/ml, 0.0313- 4 µg/ml and 4 µg/ml against M. canis, T. verrucosum,T. mentagrophytes, and T. terrestre, respectively. Nystatin showed lower activity agaist the isolates tested.

Our study results demonstrated that, antifungal activities of myconazole, terbinafine and itraconazole against dermatophytes showed variation and nystatin had the lowest activities in general, indicating the importance of identification of infectious agents and determination antifungal susceptibilities in routine practice to avoid the treatment failure.

Keywords: dermatophytes, antifungal drug, susceptibility

P170: An altenative regime for the treatment of neurobrucellosis: Tigecycline combination

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Brucellosis is an endemic disease and still maintain its importance. Neurobrucellosis, is a serious complication of systemic brucellosis, having different clinical presentations. The aim of this study was to present a serious neurobrucellosis case who was successfully treated with tigecycline combination.

A 31-year-old male patient (shepherd), primarily had fatigue, weight loss, headache, muscle pain, fever, sweating, severe pneumonia symptoms and on subsequent days confusion had arisen. While transportation with the emergency ambulance, death had occured and after urgent intervention, the patient was hospitalized in the Intensive Care Unit.

After physical examination, collected blood and CSF specimens were examined microscopically, cultured and serologic tests were performed. All diagnostic methods showed the presence of Brucella melitensis, which was then confirmed by molecular tests.

After the isolation and identification of Brucella melitensis, dexamethasone, ceftriaxone,rifampicin and tigecycline combination therapy was applied, as pneumonia, sepsis and menengitis had developed due to brucellosis. On the sixth day of hospitalization high fever,pneumonia symptoms, confusion had declined and spontaneous respiration occurred, respectively. Determining significant clinical improvement ceftriaxone and tigecycline combination had stopped and therapy was continued with oral rifampin and doxycycline. The patient was discharged and in weekly checks, as the patient was found to be fully recovered from difficulty in walking and the ptosis. The therapy was completed in a total of six months.

Neurobrucellosis is a rare but a serious complication. Activity of tigecycline against Brucella strains, was shown formerly by in-vitro studies. To our knowledge this case is the first in-vivo example supporting these results. Our case was a late case, presenting short-term death, but the patient came back to life as a result of tigecycline combination therapy without any sequelae. With this presentation we aimed to show an alternative treatment regime.

Keywords: neurobrucellosis, tigecycline, treatment

P171: Effects of pioglitazone, a agonist of ppar-gamma, on mesenteric blood flow and organ damage in an experimental septic shock

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PPAR-gamma, a member of the nuclear receptor superfamily, is expressed by endothelial cells, T cells, macrophages which are activated in case of inflammation. PPAR-gamma ligands (agonists) shows anti-inflammatory effects by preventing T cell proliferation and release of various cytokines and mediators such as TNF and IL-6 which play an important role in the development of septic shock. In this study, effects of pioglitazone (Pio) which is a ppar-gamma agonist, on mesenteric injury by measuring the mesenteric blood flow (MBF) and organ damage histopathologically in an experimental septic shock model, cecal ligation and puncture (CLP), were investigated.

Swiss albino mice were allocated into four groups; sham (SH), cecal ligation and puncture (septic group, CLP), sham+Pio, CLP+Pio. At 2 h after Sham or CLP procedures 26 mice in these groups were given Pio (10 mg/kg, i.p) or its solvent (DMSO; 1 ml/kg, i.p.). After 20 h, mesenteric arterial blood flow (MBF) was measured with a Doppler ultrasound flowmeter. Liver, spleen and kidney damages were examined histopathologically.

MBF decreased in CLP group from 178±28 to 95±11 ml/min/kg ($p<0.01$). Pioglitazone couldn't prevent the decreasing of mesenteric blood flow in septic shock (90±46 ml/min/kg). In addition pioglitazone couldn't ameliorate the organ damage histopathologically in septic shock.

According to these results pioglitazone has no protective effect in sepsis. These findings may be related with pioglitazone dosage, route of administration, experimental model. It needs new studies with higher dosage, longer administration or different route such as i.v.

Keywords: pioglitazone, sepsis, mesenteric blood flow

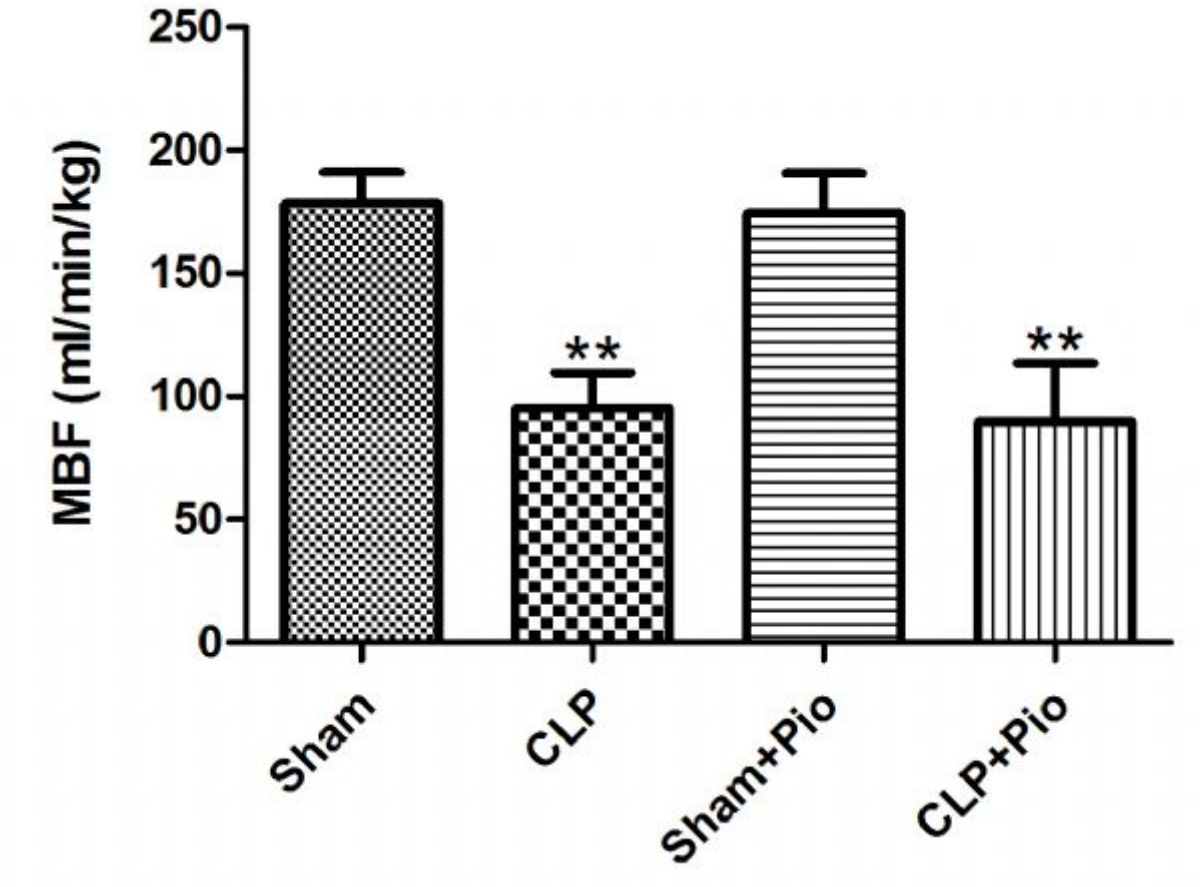

Figure 1. MBF. *Denotes significant difference between SH vs. other groups. (**, p<0.01). n=6-7 for the groups.

P172: Effects of PTDC, an inhibitor of NF- κB, on mesenteric blood flow and organ damage in an experimental septic shock

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NF-kB has regulatory role for releasing the NOS-2 mediated nitric oxide (NO) and inflammatory cytokines such as TNF, IL-1β, IL-6 which are responsible for formation of septic shock. So, inhibition of NF-kB may be protecitve in sepsis. In this study we investigated effects of pyrrolidine dithiocarbamate (PDTC) on mesenteric injury by measuring the mesenteric blood flow (MBF) and organ damage histopathologically in an experimental septic shock model that cecal ligation and puncture (CLP).

Swiss albino mice were allocated into four groups; sham (SH), cecal ligation and puncture (septic group, CLP), sham+PTDC, CLP+PTDC. At 2 h after Sham or CLP procedures 28 mice in these groups were given PTDC (150 mg/kg, i.p) or its solvent (DMSO; 1 ml/kg, i.p.). After 20 h, mesenteric arterial blood flow (MBF) was measured with a Doppler ultrasound flowmeter. Liver, spleen and kidney damages were examined histopathologically.

MBF decreased in CLP group from 170±35 to 92±16 ml/min/kg ($p<0.05$). PTDC couldn't prevent the decreasing of mesenteric blood flow in septic shock (98±22 ml/min/kg). In addition PTDC couldn't restore the organ damage histopathologically in septic shock.

According to these results PTDC has no protective effect in sepsis. These results may be related with PTDC administration route, dosage, experimental model. It needs new studies with higher dosage, longer administration or different route such as i.v.
Keywords: pyrrolidine dithiocarbamate, sepsis, mesenteric artery

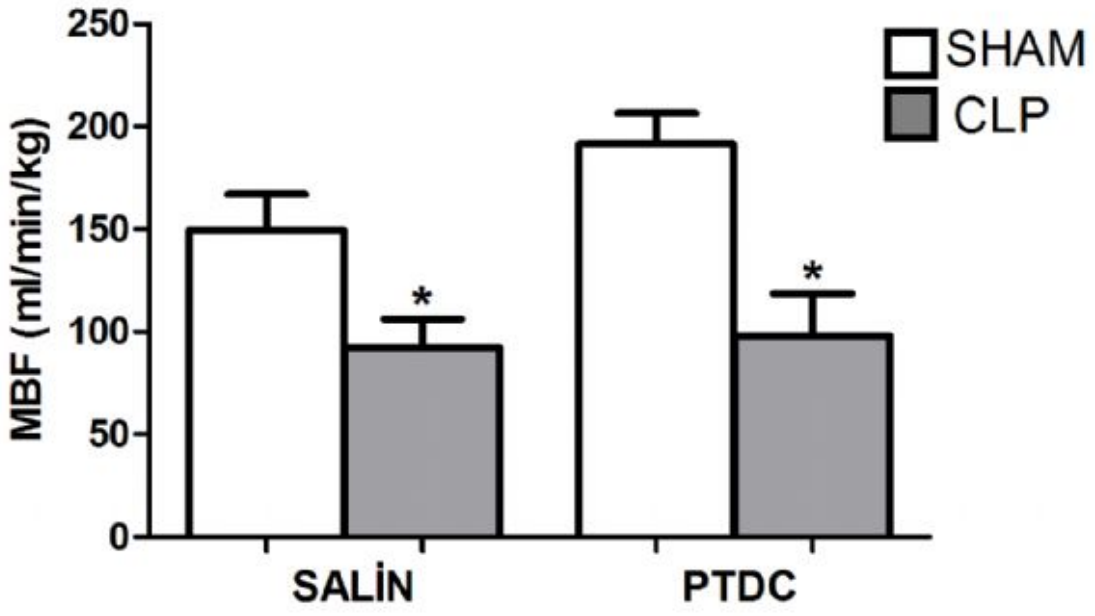

Figure 1. MBF. *Denotes significant difference between SH vs. other groups. (*, $p<0.05$). n=6-8 for the groups.

P173: Effects of Genistein, a tyrosine kinase inhibitor, on mesenteric blood flow and organ damage in an experimental septic shock

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Tyrosine kinase plays role in development and progression of septic shock via phosphorylation of proteins which are induce the release of inflammatory mediators such as TNF-alpha, IL-1β and IFN-γ. Phosphorylation of proteins by kinases also plays an important role in signal transduction pathway of these cytokines and mediators. Tyrosine kinase inhibitors prevent the release of proinflammatory cytokines via directly inhibition of the inflammatory cells. In this study we investigated effects of Genistein (Gen), a tyrosine kinase inhibitor, on mesenteric injury by measuring the mesenteric blood flow (MBF) and organ damage histopathologically in an experimental septic shock model that cecal ligation and puncture (CLP).

Swiss albino mice were allocated into four groups; sham (SH), cecal ligation and puncture (septic group, CLP), sham+Gen, CLP+Gen. At 2 h after Sham or CLP procedures 27 mice in these groups were given Gen (10 mg/kg, i.p) or its solvent (DMSO; 1 ml/kg, i.p.). After 20 h, mesenteric arterial blood flow (MBF) was measured with a Doppler ultrasound flowmeter. Liver, spleen and kidney damages were examined histopathologically.

MBF decreased in CLP group from 150±24 to 75±11 ml/min/kg ($p<0.05$). Genistein couldn't prevent the decreasing of mesenteric blood flow in septic shock (44±9 ml/min/kg). In addition Genistein couldn't restore the organ damage histopathologically in septic shock.

In accordance with these results Genistein has no protective effect in sepsis. These results may be related with Genistein administration route, dosage, experimental model.

Keywords: Genistein, sepsis, mesenteric blood flow

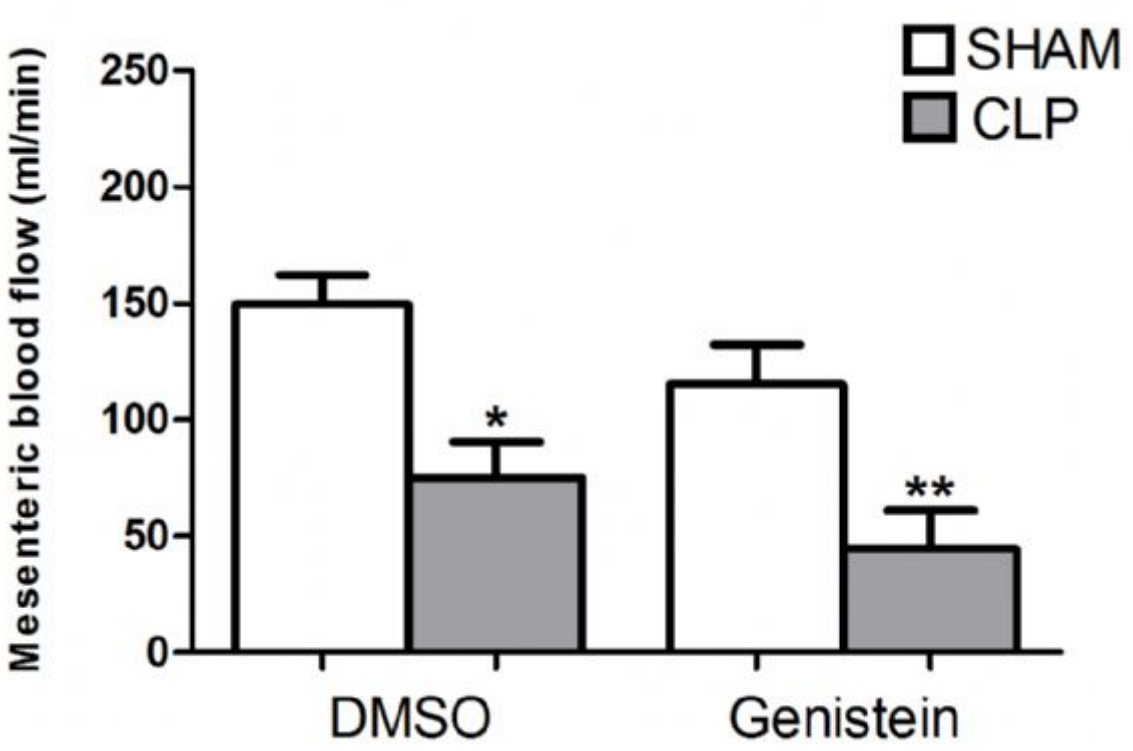

Figure 1. MBF. *Denotes significant difference between SH vs. other groups. (*, p<0.05). n=7-9 for the groups.

P174: Preventive effects of Thymoquinone on survival, vascular dysfunction and tissue injury in sepsis

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Thymoquinone (TQ) is a phytochemical compound found in the plant Nigella sativa and shows anti-inflammatory, antineoplastic, antioxidant effects. In a recent study it was showed that TQ improves survival and protects liver damage in endotoxemia which is an experimental septic shock model. We investigated effects of TQ on survival, vascular and tissue injury in a polymicrobial sepsis model induce by cecal ligation and puncture (CL) which is more appropriate clinical reality.

58 Wistar albino rats were divided into four groups; sham (SH), cecal ligation and puncture (septic group, CL), SH+TQ (SH TQ), CL+TQ (CL TQ). For 3 days rats were injected with TQ (1 mg/kg/day, i.p.) in SHTQ and CLTQ groups or its solvent (DMSO, 1 ml/kg/day, i.p.) in SH and CL groups. At 4th day animals were underwent CL and SH operations. After 20 hours the operations mesenteric arterial blood flow (MBF) and phenylephrine responses of isolated aortic rings were measured. Tissue damages were assessed biochemically.

TQ prevented the decrease MBF in septic rats. Phenylephrine responses of aortic rings decreased in septic rats that were partially prevented by TQ pretreatment. Survival rate decreased in CL groups ($p<0,001$) which was partially ameliorated by TQ. Serum biochemical parameters AST, ALT, LDH, BUN, Cr and TNF-alpha, IL-1β, IL-6 levels were increased in septic rats. The increase in biochemical parameters reversed by TQ. MDA levels in liver, lung, spleen and kidneys were increased and GSH levels decreased in septic rats. The increase in lung, spleen and kidney MDA levels and the decrease in liver, and kidney GSH levels were prevented by TQ. Liver MDA and spleen GSH levels partially ameliorated by TQ in septic rats.

TQ has preventive effects on sepsis mortality, vascular dysfunction and tissue injury possibly due to its antioxidant and anti-inflammatory properties.

Keywords: thymoquinone, sepsis, preventive effects

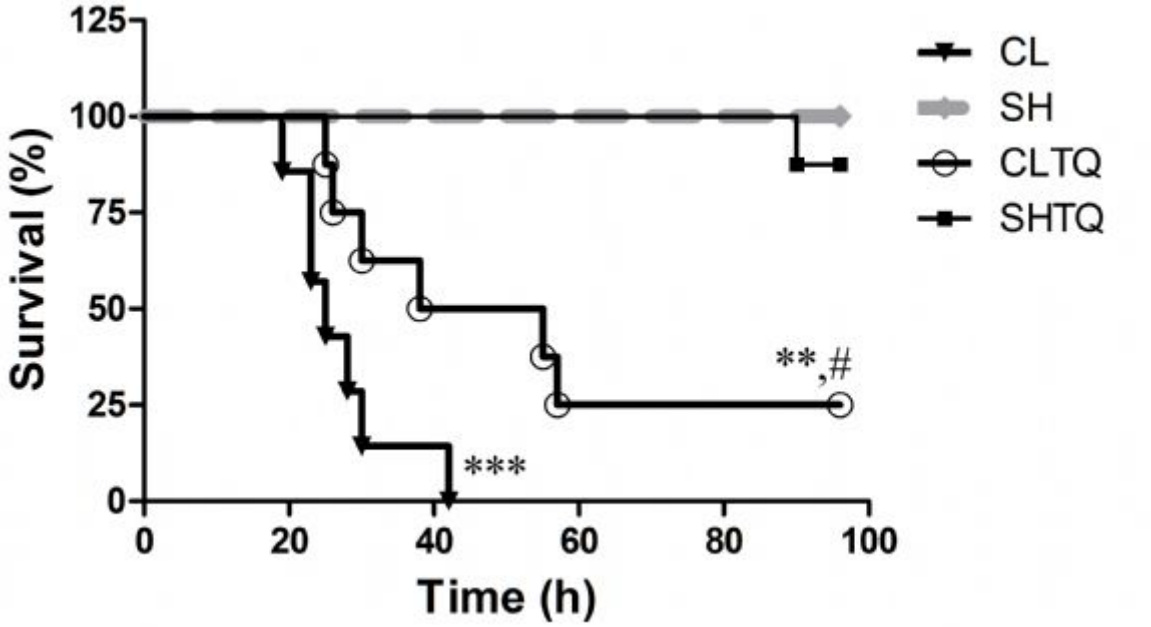

Figure 1. Survival rates. Survival rates obtained from rats challenged with caecal ligation and puncture (CL) and Sham (SH) procedures for 96 h. n=6-7 for the groups. Survival curves were compared by the Gehan Breslow Wilcoxon test. *Denotes significant difference between SH vs. other groups. #Denotes significant difference between CL vs. CLCCX groups. (#, $p<0.05$; **, $p<0.01$; ***, $p<0.001$).

Imaging Technics

P175: Evaluation of possible cytotoxic effects of Vanadium pentoxide on colon carcinoma cell line with a real time analyser

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It is obvious that tumor cells could be treated with various metal oxide nanoparticles such as V_2O_5. Vanadium exposure time is very important for its toxicity so our purpose is to investigate vanadium pentoxide on Colo-205 and also normal human fibroblast cells different doses depend on real time.

Colo-205 cells maintained in tissue culture flasks using Dulbecco's Modified Eagle's Medium supplemented with penicillin/streptomycin and 10% v/v fetal bovine serum.

Cytotoxic effect of V_2O_5 was monitored with Xcelligence Real-Time Cell Analyser (RTCA) as described by Uran et al (2010), with slight modifications. Optimal seeding concentration of Colo-205 cells were determined and cell proliferation, attachment and spreading were monitored every 15mins via the impedance of E-plate wells. 25.000 cells/well were seeded and approximately 24h post-seeding when the cells were in the log growth phase, we treated cells with V_2O_5 (200 µM, 150µM, 100 µM, 50 µM, 25 µM) replicated 4-times and the experiments were run for 95 hours.

The RTCA software performs cell index via the well impedance and calculates logarithmic half maximum effect of concentration

[log (IC_{50})] values at a given time point based on log concentration producing 50% reduction of cell index (CI) value relative to the control CI value (100%).

Statistical analysis were performed using GraphPad Prism software version 5.04 (GraphPad Software Inc., USA). One way ANOVA followed by Bonferrroni post-hoc test (95% confidence intervals).

After treatment of cells approximately 2-10 hours, V_2O_5 decreased the cell index comparing to control in a dose dependent manner on Colo-205 cells. V_2O_5 more decreases cell index on Colo-205 than normal human fibroblast cells.The cytotoxic effect of V_2O_5 started on Colo-205 cell line after 2 hours and lasted around 10 hours and dose range can be limited 50-100 µM in further studies.

Keywords: xCELLigence, Colo-205, vanadium pentoxide, colon cancer cells

P176: Real-time xCELLigence impedance analysis of the cytotoxicity of vanadium pentoxide on normal human fibroblast and MCF-7 cells

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Vanadium compounds are studied on cancer cells due to their low IC_{50}, anti-proliferative, pro-apoptotic properties and limit invasion and metastatic potential of neoplastic cells.

The aim of this study was to investigate cytotoxicity of vanadium pentoxide (V_2O_5) on human breast cancer cell line MCF-7 cells and normal human fibroblast cells at different doses in real time.

Cytotoxic effect of vanadium was monitored with Xcelligence Real-Time Cell Analyser (RTCA) as described by Uran et al (2010), with slight modifications. MCF-7 cells were seeded and cell proliferation, attachment and spreading were monitored every 15 minutes via the impedance of E-plate wells. 20.000 cells/well were seeded and approximately 24h post-seeding when the cells were in the log growth phase, we treated cells with V_2O_5 (200 µM,100 µM, 50 µM, 25 µM) and replicated 4-times and the experiments were run for 75 hours.

The xCELLigence technology uses electrical well impedance measurements from adherent cells and converts into CI. The RTCA software calculates logarithmic half maximum effect of concentration

[log (IC_{50})] values at a given time point based on log concentration producing 50% reduction of cell index value relative to the control CI value (100%).

Statistical analysis were performed using GraphPad Prism software version 5.04 (GraphPad Software Inc., USA). One way ANOVA followed by Bonferrroni post-hoc test (95 % confidence intervals).

The xCELLigence system is a reliable and efficient tool for real-time screening of the cytotoxic effect of compounds in cell-based in vitro assays. These results suggest that treatment of V_2O_5 on cells approximately 12 hours, decreased the cell index comparing to control with dose dependent manner on MCF-7 cells. V_2O_5 more decreases cell index (CI) on MCF-7 than normal human fibroblast cells for 48 hours treatment. It is the first profiling that using xCELLigence technology.

Keywords: xCELLigence, MCF-7, vanadium pentaoxide, cytotoxicity, fibroblast

P177: Cytotoxic effects of novel oxadiazole derivatives on MCF-7 breast cancer cell line

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Breast cancer is the leading female malignancy and second cause of cancer death in developed countries. Despite most patients present with early disease and are treated with surgery, often followed by adjuvant radiotherapy and chemotherapy. Nevertheless, 40–50 % of high-risk patients treated with adjuvant chemotherapy ultimately relapse due to drug resistance, including cross-resistance to structurally unrelated anti-cancer drugs. In this study, we aim to investigate the cytotoxic effects of novel oxadiazole derivatives on MCF-7 human breast cancer cell line.

Four different original oxadiazole derivatives were synthesized in Yeditepe University Department of Pharmaceutical Chemistry. To investigate the cytotoxic effects of these molecules MCF-7 was chosen and xcelligence system was used for determination. MCF-7 incubated for 24 hour in xcelligence system before addition molecules different concentrations (MB_1:100nM, 500nM, 1µM, 2.5µM, 5µM, 7.5µM and 10µM; $MB_{3,4,5}$:1µM, 5µM, 10µM, 25µM, 50µM, 75µM, 100µM). Cells viability was observed during 48 hours after molecules addition and IC50 values have been calculated via xcelligence software.

Cytotoxic effects of four novel oxadiazole derivatives (MB_1, MB_3, MB_4, MB_5) on MCF-7 determined with xcelligence system at 24 and 48 hours. According to preliminary results, IC_{50} values in 24thand 48th hours are respectively; $IC_{50}MB_1$:7,4 mM and 250 mM, $IC_{50}MB_3$:0,14 mM and 0,17 mM, $IC_{50}MB_4$:0,13 mM and 0,03mM, $IC_{50}MB_5$:0,53 mM and 1,4 M. According to these informations; Although MB_1 and MB_3 have no cytotoxic effect, MB_3 could be antiproliferative. MB_4 has the maximal cytotoxic effect among four molecules. MB_5 is cytotoxic also but not as strong as MB_4. The effects are seen earlier with high concentration. These novel molecules and datas provide new informations for anticancer studies. In further studies, we are planning to improve our research with these oxadiazole derivatives in other breast cancer cell lines.

Keywords: anticancer, human breast cancer, oxadiazole derivative, cytotoxicity, MCF-7

Pharmacology Education

P178: Kazakhstan National Medicines Formulary: building an evidence-based culture using expert resources and networks

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The role of a well-used and regularly updated National Medicines Formulary in encouraging rational use of medicines when used as a knowledge source for decisions is well documented. The aim of this project was to create a Kazakhstan National Medical Formulary (KNMF) in partnership with the Kazakhstan Ministry of Health and the World Bank. KNMF is a digital-first evidence-based resource that is easily accessed in all care settings. The EBN team built the first version of KNMF, and also created and trained an expert network of editors and advisors to provide continuous updating, development based on feedback, and strong governance links to stakeholders including education and professional bodies. KNMF was launched as an online free service in January 2016. This report offers early results on uptake, usage, impact and feedback, reinforcing the value of knowledge resources in creating an evidence-based culture in healthcare.

Keywords: formulary, rational use of medicines, evidence-based prescribing

P179: Objective structured clinical examinations (OSCE) improved communication skills of student of Pharmacology in Medicine and Podiatry degree

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Structured Clinical Examinations (OSCEs) are versatile multipurpose evaluative tools that can be utilized to assess health care professionals in a clinical setting including communication skills and ability to handle unpredictable patient behavior, which usually are not included in the traditional clinical exam. To designee and perform OSCEs by student is a novelty that really like to the students and may improve their arguing and planning capacities and their communication skills.

The study aimed to evaluate the impact of designing, developing and presenting. Structured Clinical Examinations (OSCE) by student in the communication skills development and in the learning of medicines in Medicine and Podiatry undergraduate students.

A one-year study in which students were invited to voluntarily form groups (4 students maximum). Each group has to design and perform an OSCE (10 min maximum) showing a clinical situation/problem in which medicines' use was needed. A clinical history, camera, a mobile-phone's video editor, photos, actors, dolls, simulators or whatever they may use was allowed. The job of each group was supervised and helped by a teacher. The students were invited to present their work to the rest of the class. After each OSCE performance the students were encouraged to ask questions if they wanted to do it. After all the OSCEs performances the students voluntarily answered a satisfaction survey.

Students of Pharmacology of Medicine degree and Podiatry degree, N=80, 53.75% female, 21±2.3 years old were enrolled. 26 OSCEs showing a clinical situation or clinical problem were made. The average time spent by students in making the OSCE was 21.5±9 h. The percentage of students which were satisfied with this way of presentation of the OSCE was 89.7%.

Objective Structured Clinical Examinations (OSCE) designed and performed by student of Pharmacology of the Medicine and Podiatry Degree improved their communication skills.

Keywords: Objective Structured Clinical Examinations (OSCE), communication skills, assessment

P180: Erythropoietin accelerates tumor growth through increase of erythropoietin receptor (EpoR) in vitro and vivo as well as vascular endothelial growth factor receptor (Flt-1) expression in DLD-1 and Ht-29 xenografts

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Anemia is a relatively common symptom coexisting with colorectal carcinoma. The role of Epo in colon cancer has not been clearly shown. The aim of this study was to assess the effects of Epo therapy on colorectal carcinoma cells both in in vitro and in animal models. Human colon adenocarcinoma cells DLD-1 and Ht-29 were cultured in medium with Epo beta (1, 10 and 100 IU/ml). Cell proliferation was measured with an automated cell counter. Expression of erythropoietin receptor (EpoR) mRNA, a serine/threonine-specific protein kinase (Akt) mRNA and their proteins were assessed by RT-PCR and confocal microscopy, respectively. Mice were inoculated with adenocarcinoma cells and treated with a therapeutic dose of Epo.

We identified that Epo/EpoR triggers downstream signaling via Akt, which promotes colon cancer cell growth and proliferation. Epo, and high levels of phosphorylated EpoR, directly accelerates tumor growth through its proliferative and proangiogenic effects. This study demonstrated that Epo had enhanced carcinogenesis through increase of EpoR, vascular endothelial growth factor receptor (Flt-1) expression and thereby contributed to tumor development. These results suggest that both EpoR-positive and EpoR-negative cancer cells could be regulated by exogenous Epo.

Keywords: erythropoietin, erythropoietin receptor, vascular endothelial growth factor receptor, colon cancer

P181: Evaluation of patients' attitudes regarding medicine use for dental health problems

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There are critical worries that nearly half of the medicines are used irrationally by the society. The rationality of medicine usage for dental problems is not investigated adequately from the patient's perspective. It was aimed to evaluate the patients' attitudes regarding their medicine utilization for dental health problems.

A face-to-face survey was conducted on 107 patients who applied to dental clinics (DCs) at a dentistry faculty or an oral and dental health center in Istanbul. Patients' demographic characteristics and their attitudes about medicine usage were questioned.

It was found that 26.9% of the respondents declared they used medicine prior to apply a DC. Patients stated that they had used analgesics (75%), antibiotics (10.7%) and other drugs before applied to DCs. When it was asked "whom did you consult before using dental medicines which were already kept at your home?", 53.5% of the patients stated that they did not consult anyone and 25.6% of them declared that they contacted with their dentist. The most of the participants (82%) pointed out that they kept dental medicines which were left from their previous treatments, for using in future and 49% of them also declared they threw it into the garbage.

The present study revealed information about the attitudes and requirements of patients from different DCs. Their attitudes about dental medicine usage seem far away from being rational. This could be associated with some findings such as high level tendency to make self-medication even it includes antibiotic, unnecessary medicine possession at home and throwing problems. Therefore, patients should be educated especially in general principles of rational use of medicine and they should be informed properly about medicine by their healthcare providers in dentistry practices.

Keywords: dentistry, irrational drug usage, pharmacoepidemiology

P182: An evaluation of knowledge and attitudes of second year students of Akdeniz University Medical Faculty about rational drug use

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Rational drug use (RDU) is defined by WHO as "where patients receive medications appropriate to their clinical needs, in doses that meet their own individual requirements for an adequate period of time, and at the lowest cost to them and their community. There are some roles for doctors such as diagnosis of patient's situation carefully, defining goals of medical treatment, making a decision for treatment, choosing appropriate drug and prescribing, giving information about drugs to the patients, watching, evaluation and consulting of medical treatment during RDU. In this study, we aimed to evaluate of knowledge and attitudes of second-year students of Akdeniz University Medical Faculty about RDU.

100 students were given a questionnaire to assess their knowledge about RDU. This questionnaire included following questions: What does RDU mean? Where did you learn this term? Did you get any education about RDU?, What did you do with drugs with a past expiry date? Do you want to take a lesson about RDU? The questionnaire was applied to second-year medical students who had not got previous lessons about RDU.

All students (55 female, 45 male) answered the questionnaire. It was determined that 85% did not get any previous education about RDU, 6% had no idea about RDU, 24% described RDU as using only appropriate doses of drugs while 70% gave correct answers about the meaning of RDU. 56% declared that they heard and learnt about RDU through social media. 90% stated that they throwed away drugs with a past expiry date in the garbage and only 4% brought them to the nearest pharmacy store. Only a quarter (25%) of students wanted to take lessons about RDU.

Based on these findings it was suggested that all health care suppliers, particularly the medical students should get RDU education both during and after their graduation.

Keywords: appropriate drug dose, education, questionnaire, rational drug use

P183: Investigation the use of over-the-counter and herbal medication in a Turkish university population

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Over-the-counter (OTC) and herbal medication may be associated with an elevated risk of adverse health outcomes resulting in hospitalizations and sometimes death. This study investigates the use of OTC and herbal medication in a Turkish University population.

A cross-sectional study was carried out with 846 representative individulas who are student or worker in Marmara University Göztepe Campus in Istanbul. The participants were selected randomly from 29575 students and workers. With a selffilled questionnaire, soscio-demografic features, prescribing and OTC medications used in the three weeks prior to the study, diseases, primary health care visits were asked. In addition 2-item brief depression inventory was applied.

58.6% of the participants were female and the mean age was 24±7.94. 80.6% of the participants was student. Among the 846 participants 13% of the participants were using at least one medication during the study. 31.3% of the participants had used at least one OTC drugs and 19% herbal drugs during the last 12 months. OTC drugs or herbal use was not associated with sex, working or income status. The most frequently used OTC drugs were analgesics/NSAIDs and symptomatic medications for common cold. 5% of the participants reported at least one advers drug reaction. Use of OTC and herbal medication were associated with the having depression symptoms ($p>0.001$, $p=0.001$).

According to our study use of OTC and herbal drugs may be related with depression symptoms. It should be emphasized the importance of the biopsychosocial approach in Clinical Pharmacology education.

Keywords: over the counter drugs, herbal, drug use, university campus, depression

Pharmacokinetics and Drug Metabolism

P184: Bioavailability and metabolism products of andrographolide in A23187 induced-rabbit

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Andrographolide is the main bioactive component of Andrographis paniculata (Burm. F.) Nees) which has been traditionally used as a pain reducer in Indonesia. Previous study showed that boiled water of Andrographis paniculata herbs, calculated as andrographolide, administered in healthy rabbits, was fastly absorbed from the stomach (t_{max} 1 hour), distributed in the circulation system (t_{max} 1.5 hours) and metabolized in the liver (t_{max} 2 hours), in subsequent process. In this work, we investigated the chromatogram profile of andrographolide in A23187 induced-New Zealand rabbits' urine and faeces. Prior to be treated, the animals were given orally 40 mg andrographolide. Urine and faeces were collected during 2 x 24 hours then were extracted using a mixture of ethyl acetate-water (1:1). The water extract was further analyzed using reversed-phase HPLC with methanol-water (55:45) as mobile phase, flow rate 1ml/min. Detection was set at λ = 227 nm. HPLC chromatogram showed that andrographolide was not detected in the urine while compounds with higher polarity were observed at 1.5 to 3 minutes. Andrographolide was still detected in faeces along with a more nonpolar compound. It could be concluded that andrographolide showed a good bioavailability in rabbit. This compound was metabolized and excreted in the form of more polar compounds in urine and a more nonpolar compound in faeces.

Keywords: antiinflammation, calcium ionophore, conjugation, drug metabolism, HPLC

P185: P-glycoprotein mediated pharmacokinetic interaction between talinolol and barnidipine

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Co-administration of P-glycoprotein (P-gp) substrates and inhibitors may cause drug interactions leading to side/toxic effects. Barnidipine is an antihypertensive drug and an inhibitor of P-gp in vitro and talinolol is a beta-blocker and a probe substrate of P-gp. The present in vivo study was designed to investigate the effects of single and repeated doses of barnidipine on the pharmacokinetics of talinolol in rats. In single-dose study, talinolol (20 mg/kg) alone and with barnidipine at low (1 mg/kg) and high dose (10 mg/kg) were administered to rats orally. In repeated-dose study, rats were treated with barnidipine (1 mg/kg/day) for four days orally then with talinolol (20 mg/kg p.o., on day 5). Rats in control group were treated with excipient for four days and then talinolol alone on day 5. Blood samples were collected at 0.5, 1, 2, 4, 6h after dosing and plasma talinolol levels were determined by HPLC. C_{max} values of talinolol elevated by 10% ($p>0.05$) and 110% ($p<0.05$), and plasma AUC_{0-6h} values increased by 33% ($p>0.05$) and 46% ($p<0.05$) following low and high single doses of barnidipine co-administration, respectively. In repeated-dose study, C_{max} and AUC_{0-6h} of talinolol increased by 131% ($p<0.05$) and 130% ($p<0.05$) respectively following low dose of barnidipine co-administration as compared to control. In addition to these results, double-peaks were observed after 0.5h and 4h with talinolol when co-administered with single or repeated low doses of barnidipine. There may be a coupling between the occurrence of double-peak phenomenon and inhibition of P-gp which shows regional differences in the expression/protein level in intestine. Increment of talinolol bioavailability upon both low and high doses of barnidipine co-administration may be due to P-gp inhibition. Higher increase of talinolol plasma AUC_{0-6h} values due to the repeated doses of barnidipine may be explained by downregulation of P-gp.

Keywords: talinolol, barnidipine, pharmacokinetics, P-glycoprotein, double-peak phenomenon

P186: Sex factors in the pharmacokinetics of itopride in healthy human volunteers

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Sex differences in pharmacokinetics have been described in many drugs (Soldin *et al.* Clin Pharmacokinet 2009; 48: 143-57; Schwartz Clin Pharmacokinet 2003; 42: 107-21). The aim of our study was to investigate the influence of sex on the pharmacokinetics of prokinetic drug itopride.

Itopride was administered to 18 healthy men (18-48 years, 62-85 kg) and 16 healthy women (20-53 years, 58-88 kg) in a single dose of 50 mg (one tablet) after 10-hour fasting. Blood samples (5 ml) were drawn 0-36 hours after the administration. Plasma itopride concentrations were determined by an HPLC-MS/MS method.

Calculated pharmacokinetic parameters (arithmetic mean±standard deviation, except for t_{max} data which are given as median and range) are presented in Table 1. Unpaired t-test was used for statistical evaluation of logarithmically transformed pharmacokinetic parameters (men *versus* women).

There were no statistically significant differences between men and women in any non-adjusted pharmacokinetic parameter. However, after adjustment of the parameters AUCs to the body weight significant differences were found.

The pharmacokinetics of itopride seems to be influenced by the sex in healthy human volunteers; the extent of bioavailability is about by 20% lower in women.

Keywords: itopride, pharmacokinetics, sex, gender

Table 1. Pharmacokinetic parameters.

Parameter	Unit	Men	Women	p
$AUC_{0\text{-}inf}$	[ng.h/ml]	799 ± 200	680 ± 336	0.0722
$AUC_{0\text{-}t}$	[ng.h/ml]	750 ± 206	630 ± 333	0.0700
C_{max}	[ng/ml]	254 ± 85	249 ± 110	0.6150
t_{max}	[h]	0.75 (0.33-1.25)	0.5 (0.33-1.25)	-
$t_{1/2}$	[h]	5.76 ± 1.20	5.94 ± 1.58	0.7042
$AUC_{0\text{-}inf,\ adj}$	[ng.h/ml]	836 ± 230	671 ± 324	0.0274
$AUC_{0\text{-}t,\ adj}$	[ng.h/ml]	785 ± 237	621 ± 323	0.0296
$C_{max,\ adj}$	[ng/ml]	267 ± 101	245 ± 106	0.4086

adj … body weight-adjusted parameter (calculated by normalizing for 70 kg of body weight)

P187: The frequency of monitoring phenytoin therapeutic concentration range and re-evaluation of the corrected phenytoin levels

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Phenytoin is an antiepileptic drug which is eliminated by CYP2C9, CYP2C19 (less important: CYP2C18, CYP3A4) enzyme. Therapeutic concentration range is very important due to the narrow therapeutic drug levels. Targeted therapeutic concentration range is 10–20 mg/L (40–80 lmol/L) in blood serum for adults and children older than three months. Phenytoin has highly variable pharmacokinetics due to its complex of binding to Albumin (%90).

A retrospective study was conducted in all inpatients (n=72) that including phenytoin treatment at the university hospital in 2015. 11 different departments were examined to monitor therapeutic concentration range during phenytoin treatment. Corrected phenytoin levels were calculated from total phenytoin concentration by winter-tozer equation according to albumin level for hypoalbuminemic patients.

Phenytoin level monitoring for a year in the hospital (n=315) have checked for hypoalbuminemia and 90 results are calculated again because of hypoalbuminemia. After reconsideration 25 of the 76 patients evaluated as therapeutic range, according to the calculation results, not in the target range actually in the toxic range. There have been found a significant difference ($p \leq 0.01$) between the laboratory result and the corrected calculation of the clinical pharmacist. Also there have been found a significant difference ($p \leq 0.01$) between the drug monitoring of phenytoin level of different inpatient service. The %83.3 of phenytoin-treated patients in the intensive care unit at 2015 is monitored for phenytoin level beside most of the services do not check for phenytoin level for phenytoin-treated patients even once.

In conclusion, phenytoin-treated patients should monitored for phenytoin level for monitoring of the treatment and for avoiding the subtherapeutic or toxic levels of phenytoin. The results have to recalculate if necessary by considering the albumin level and renal failure of the patients.

Keywords: phenytoin, therapeutic concentration range, corrected phenytoin levels

P188: Pharmacokinetics of opioids in chronic low back pain (CLBP) treatment: preliminary data on cytochrome p450 2D6 (CYP2D6)

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Chronic Low Back Pain (CLBP) is a significant problem worldwide and inadequately treated remains a major cause of suffering and dissatisfaction in pain therapy. Although opioids are recognized as an essential tool in CLBP, it is still unclear how to identify opioid responders and onset of side effects that frequently occur, together with risk of drug addiction, affecting patients' adherence. The CYP2D6 gene is highly polymorphic across the human population. Understanding opioid metabolism and disposition is essential for assessing risk of toxicity, providing additional information on therapeutic failure.

A cohort of 90 patients with CLBP (enrolled in our FP7 Project) is tested correlating oxycodone and codeine side effects to SNPs involved in pharmacokinetics of CYP2D6 in order to identify the patients with low compliance (for side effects and no efficacy). We detects, using xTAG technology on the Luminex® 200™ xMAP® platform, a panel of nucleotide variants, including gene rearrangements associated with the deletion (*5) and duplication genotypes. The test determines the diplotype of each sample and detects the complexity of CYP2D6 nucleotide variants across different populations.

Either poor or extensive/ultra-rapid CYP2D6 metabolisers are exposed to side effects of opioid analgesics but in whom 2:1 has shown that variants on *1/*4 and *1/*41 alleles have significantly product a benefit in respect to variations to *2/*41 alleles in the group chronically treated with opioids. In particular SNPs 1661G>C and 4180G>C, detected in the allele *41, can predict a reduced enzyme's activity to determining side effect events in painful patients.

Predicting individual opioids' effectiveness and safety through CYP profile variants to perform genotype/phenotype correlations useful to stratify interindividual variability of opioid response, could be a new biomarker to identify the personalized treatment.

Keywords: CYP2D6, chronic pain, opioids response

P189: Clopidogrel markedly increases the plasma concentrations of pioglitazone: Evidence for strong inhibition of CYP2C8 by clopidogrel

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Pioglitazone, a peroxisome proliferator-activated receptor γ (PPAR- γ) agonist, undergoes hepatic cytochrome P450 (CYP) 2C8-mediated biotransformation to its main metabolites, hydroxypioglitazone (M-IV), and its secondary metabolite, ketopioglitazone (M-III). The adenosine diphosphate (ADP) receptor inhibitor clopidogrel is metabolized to clopidogrel acyl-β-D-glucuronide, which acts as a strong time-dependent inhibitor of CYP2C8 in humans. Therefore, we found it important to investigate the effect of clopidogrel on the pharmacokinetics of pioglitazone.

In a randomized crossover study, ten healthy volunteers ingested either 300 mg of clopidogrel on day 1, and 75 mg on days 2 and 3, or placebo. Pioglitazone 15 mg was administered 1 h after placebo and clopidogrel on day 1. Plasma concentrations of pioglitazone, clopidogrel, and their main metabolites were measured up to 72 h.

When compared with placebo, clopidogrel increased the area under the plasma concentration-time curve ($AUC_{0-\infty}$) of pioglitazone 2.1-fold (p<0.001, 90% CI 1.8-2.6) and prolonged its half-life from 6.7 to 11 h (p=0.002). The peak concentration of pioglitazone was unchanged, but the concentration at 24 h postdose, representing the trough concentration in once daily dosing, was increased 4.5-fold (range 1.6- to 9.8-fold;p<0.001, 90% CI 3.2-6.5) by clopidogrel. The metabolite M-IV-to-pioglitazone $AUC_{0-\infty}$ ratio was 49% (p<0.001, 90% CI 0.40-0.59) of that during the control phase, indicating that clopidogrel inhibited the CYP2C8-mediated metabolism of pioglitazone.

Clopidogrel markedly raises the plasma concentrations of pioglitazone and impairs its biotransformation, consistent with strong inhibition of CYP2C8 by clopidogrel. In consequence, concomitant use of clopidogrel may increase the risk of fluid retention and other concentration-related adverse effects of pioglitazone.

Keywords: clopidogrel, pioglitazone, CYP2C8, pharmacokinetics, drug interaction

P190: Prediction of CYP2D6 phenotype from the risperidone and its active metabolite 9-OH-risperidone concentration ratio in children with conduct disorders

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Risperidone, an antipsychotic drug, that is effective in conduct disorders (e.g. disruptive disorders, oppositional defiant disorder) in children. Risperidone is catalyzed to its active metabolite 9-OH-risperidone (9-OHR) by CYP2D6. In this retrospective study, we investigated whether the relationship of CYP2D6 activity and the R/9-OHR ratio plasma concentration in conduct disorder children treated with risperidone.

We have included in 26 children, aged 4-15 years, with conduct disorders treated with risperidone at the doses of 0,5 – 6 mg/d. Patients concomitantly used substrates or potent inhibitors of CYP2D6 were not included to this study. The phenotype of CYP2D6 was determined by assaying the metabolic ratio of dextromethorphan (DEM) and its metabolite dextrorphan (DOR) in the urine collected for 8 h after a single 30 mg oral dose of DEM. According to this test, patients were classified into four phenotypic groups for CYP2D6: ultrarapid (UM), extensive (EM); intermediate (IM), and poor (PM) metabolizers. Plasma concentrations of R and 9-OHR and DEM were assayed by using LC/MS/MS.

The R/9-OHR ratio in PM patients was significantly higher (3,55 ± 0,06;n=6) than those of IM, EM and UM patients (P<0, 001). CYP2D6 IM patients have significantly less R/9-OH ratio compared with UM patients; 0,09 ± 0,007, n=6 and 0,22 ± 0,08, n= 8, respectively (P<0.05). However, no difference was found between EM (0,13 ± 0.015, n=6) and IM or UM patients. The plasma concentration/dose ratio of 9-OHR in IM and PM patients was about half (4,96 ± 0,4 and 5,34 ± 0,06 nmol L mg, respectively) compared with UM and EM patients (8,79 ± 1,6 and 8,72 ± 1,2 nmol L mg, respectively) (P< 0.01).

These findings suggested that the R/9-OHR ratio may help for individualization of dose and concomitant use with other CYP2D6 substrates of risperidone in clinical practice.

Keywords: risperidone, CYP2D6, phenotype, metabolism

P191: β-adrenoceptor mediated antiproliferative effect in MB 231 breast cancer cells does not depend on the inhibition of extracellular regulated kinase 1/2 activity

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Triple negative breast cancer cell lines do not express estrogen, progesterone and epidermal growth factor receptor2. These cell lines express high level of β-adrenoceptor (AR). β-AR stimulation induces de-phosphorylation of extracellular regulated kinase 1/2 (ERK1/2) and antiproliferative effect in MB231 breast cancer cells. In this study, we examined the involvement of ERK1/2 inhibition in β-AR mediated antiproliferative effect in MB231 cells.

Protein levels were measured by Western-blot analysis. Proliferation and viability assays for MDA-MB231 cells were performed by ICELLigenceTM system and WST 1 assays.

Incubation with β2-AR agonists (Terbutalin, Clenbuterol, Formeterol) inhibited cell proliferation. The antiproliferative effect was observable at 72 and 96h incubation of MB231 cells with β2-AR agonists. Stimulation of MB231 cells with terbutalin resulted in an inhibition of ERK1/2 phosphorylation. This response disappeared after 24h stimulation with terbutalin. There was no inhibitory effect of β2-AR agonists on ERK1/2 phosphorylation at 48, 72 and 96 h incubation with the agonists. We incubated cells for 24h with terbutalin and washed the cells with medium to remove terbutalin and measured cell proliferation at 48, 72 and 96 h. In these conditions, antiproliferative effect of terbutaline was not obtained at 72 and 96h.

Our study showed that stimulation of β-adrenergic receptors resulted in a decline in pERK1/2 level and cell proliferation. Terbutalin mediated de-phosphorylation of pERK1/2 was not observable after 24h stimulation. Long term stimulation (72 -96 hours) by terbutalin resulted in a decrease of cell proliferation and 24 h stimulation with terbutaline was not enough to produce antiproliferative effect in MB231 cells.

We reached the conclusion that the inhibition of MDA-MB231 cell proliferation does not depend on pERK1/2 inhibition and that some other mechanisms are involved in β-adrenergic receptor mediated inhibition of the proliferation.

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Keywords: beta adrenoceptor, ERK1/2, MB231, breast cancer, proliferation

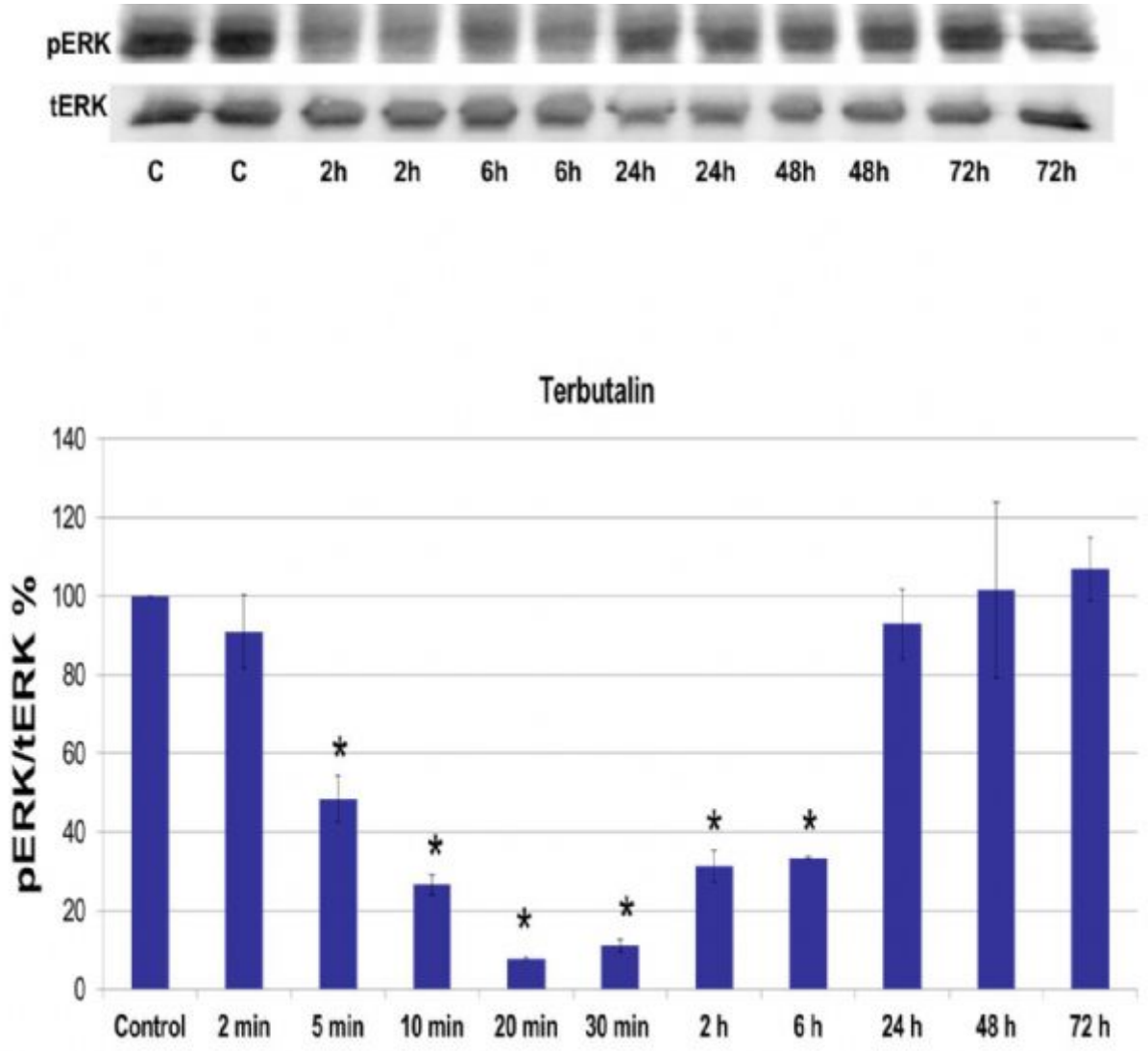

Figure 1. Terbutalin stimulation and ERK1/2 de-phosphorilation pattern in MB231 cells.

Clinical Pharmacology

P192: The effects of the antiepileptic drugs on the levels of thyroid hormones, Vitamin B12 and folate

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The effects of the antiepileptic drugs on hormones, vitamin B_{12} and folate have been well verified. But these studies were about old antiepileptics which are enzyme inhibitors and inductors. There are very limited numbers of studies with the “new” antiepileptics. In this study, antiepileptics like valproate and carbamazepine were compared with new antiepileptics such as oxcarbazepine, levetiracetam and topiramate about the effects on thyroide hormones, folate and vitamin B12.

Patients with partial epilepsy were recuited for the study. They were epileptic patients for at least one year in antiepileptic drugs. Total number of 63 patients; 15 for carbamazepine, 15 for valproate, 15 for oxcarbazepine, 9 for levetiracetam, 9 for topiramate were arranged in different drug groups. Two different control groups; first as healthy (n=15) and second as epileptic but no use of drugs (n=15) were gathered.

The levels of folate were low (P=0,001) in the epileptic controls than the healty controls. Carbamazepin group has lower folate levels (P<0.05) than the healty controls. When patients on valproate compared with the healthy controls; folate was low(P<0,001) in the valproate group. In comparison with the epileptic group, there were high levels of vitamin B12 (P<0.05) on valproate group. In comparison of oxcarbazepine group with the healthy controls; FT4 (P<0.05) and folate (P=0,001) were low in the oxcarbamazepine group. In levetiracetam group lower vitamin B12 and folate levels (P<0.05) than healthy controls were detected. In comparison with the epileptic controls, there were no difference in statistical significance in the parameters. The topiramate group had lower levels of folate (P<0.05) in comparison with the healthy controls.

Result of our study suggested that the new antiepileptics have no negative effects on the levels of thyroid hormones, vitamin B_{12} and folate. Some observed pathological effects were found as statistically insignificant in comparison with epileptic control patients.

Keywords: antiepileptic, thyroid, folate, vitamin-B_{12}

P193: Utilization of generics in antipsychotic prescribing for outpatients of AHEPA Hospital in Greece during the years of financial crisis

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Under the current financial crisis in Greece, an effort has been made to increase generic prescribing, in order to lower medicinal cost[1,2,3]. The purpose of this work was to study trends in utilization of generics in antipsychotic prescribing for outpatients of a tertiary Hospital of Thessaloniki, during the years of the financial crisis.

Two samples of antipsychotics prescriptions corresponding to the first four months of the years 2009 and 2015 were collected from the archives of the Psychiatry Outpatient Department of the AHEPA Hospital in Thessaloniki, Greece. All proprietary names of antipsychotics and their relative ratios in the prescriptions were estimated, and the percentage of generics in prescriptions was calculated. The amount of prescribed medicines was estimated in Defined Daily Doses (DDDs) of the reference drug and its generics.

The total number of prescriptions increased from 21,879 DDDs in 2009 to 47,373 DDDs in 2015. Generic prescribing increased dramatically from 2009 to 2015, corresponding to 24% of total antipsychotics prescriptions in 2015 and 4% of total antipsychotic prescriptions in 2009. The percentage of generics was high for olanzapine (61%), risperidone (60%) and amisulpride (60%) in 2015, while in 2009 the percentage of generics was high only for risperidone (19%).

The total number of prescriptions and the percentage of generics in antipsychotic prescribing were much higher in 2015 than in 2009. These results reflect an increase in the number of people seeking medical advice in the National Health System, and an increase in the efforts of Greek physicians to lower medicinal cost.

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Keywords: antipsychotics, generics

P194: Trends in generic use of antidepressants during the financial crisis in Greece

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Under the current financial crisis in Greece, an effort has been made to increase generic prescribing, in order to lower medicinal cost[1,2,3]. The purpose of this work was to study trends in utilization of generics in antidepressants sales in the market of Thessaloniki, the second largest city in Greece, during the years of the crisis.

Two samples of antidepressants registered sales corresponding to the years 2012-2013 and 2014-2015 were collected for the study. The samples corresponded only to a small amount of sales from the market of Thessaloniki. All classes of antidepressants and their relative ratios in the sales were estimated, and the percentage of generics in the sales of each medicine was calculated out of a variety of brand names in each class of antidepressants. The amount of medicines was estimated in Defined Daily Doses (DDDs) of the reference drug and its generics. The comparison of the two samples was made by using the statistical package SPSS.

Generic use corresponded to 26% of total sales of antidepressants in the sample of the years 2012-2013 (43,523 DDDs) and 32% of total sales in the sample of the years 2014-2015 (43,620 DDDs). During the last two years the percentage of generics increased from 36% to 52% in sertraline sales, from 44% to 56% in venlafaxine sales and from 53% to 65% in citalopram sales.

Under the financial crisis in Greece, an increase in generic use was observed in antidepressant sales in the market of Thessaloniki.

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Keywords: generics, antidepressants

P195: Nurses' knowledge, opinions and attitudes toward clinical research – a cross sectional survey at Dokuz Eylul University Hospital

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As a part of multidisciplinary team in clinical researches, nurses have a significant role at each level of clinical research from planning and conducting to finalising and reporting the research. In spite of their critical roles in clinical research, however, only little is known about their knowledge, opinions and attitudes toward clinical research. We aimed to investigate nurses' knowledge, opinions and attitudes toward clinical research.

The data was obtained from a cross sectional survey conducted among 291 nurses at Dokuz Eylul University Hospital, Turkey. Knowledge, opinions and attitudes of the respondents and pairwise comparisons among each factor have been analysed by employing descriptive statistics, independent t-test, ANOVA and correlation analysis.

The results indicated that 8.6% of the respondents had experience in clinical research and only 3.8% of the respondents had undertaken relevant training in clinical research. The mean of knowledge level of the respondents regarding clinical research was 10.1±4.1 (max. 19). The respondents, who have relevant clinical research training, experience and also patients consulting about clinical research, had significantly higher knowledge score than the rest of the respondents ($p<0.05$). The average positive attitude score of the respondents was 5.8 ±1.7 (max.8). There was a significantly positive correlation between the knowledge level and the attitude scores toward clinical research ($r =0.35$, $p<0.01$). Both knowledge and attitude scores of the respondents had a significant impact on nurse's opinions corresponding to specialisation, education and participation in clinical research ($p<0.05$).

These results state that the nurses have insufficient knowledge about clinical research. The positive correlation between knowledge and both attitudes and opinions of the nurses support the reasoning which increasing in the clinical research training for the nurses might result in more positive attitudes and opinions as well as participations about clinical research.

Keywords: nurses, clinical research, knowledge, attitude, opinion

P196: Pharmacovigilance perception and adverse drug reaction reporting of physicians in an university hospital

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Spontaneous Adverse Drug Reaction (ADR) reporting is an important tool in national Pharmacovigilance (PV) system. Under-reporting of ADRs by physicians is a common problem. Therefore, we analysed the knowledge and perception about PV and ADR reporting attitudes of physicians in Dokuz Eylul University Hospital (DEUH) in Izmir.

A cross-sectional, observational, questionnaire-based study was conducted. Physicians, who has been working in DEUH as clinicians, completed a structured questionnaire. Correct answers were graded as one point and false answers were graded as zero point. If all questions were answered correctly, the total score was 27.

Total number of the physicians completed the questionnaire was 203. Most of the physicians were residents (%55.2, n=112) followed by Professors (33.0%, n=67). More than half of the physicians (52.2%, n=106) had not a knowledge about the existence of national regulations about ADR reporting. Previously trained physicians were significantly aware of the presence of a PV contact point in DEUH (χ^2= 20.497, p<0.0001) and the national PV center (χ^2=28.328, p<0.0001). Only 15.8% of the physicians had reported an ADR previously. The average knowledge score of the physicians about PV was 16.0±0.3. There were no difference in PV knowledge among physicians according to academic degrees.

Knowledge about PV and spontaneous ADR reporting rate by physicians who have been working in DEUH were found to be quite low. Main reasons of the low ADR reporting rate may be the lack of knowledge about the importance of PV and and how to report an ADR. In order to improve the participation of physicians in spontaneous ADR reporting, it might be necessary to improve their knowledge about PV by training.

Keywords: pharmacovigilance (PV), adverse drug reaction (ADR), physician, training

P197: Use of hormonal contraceptives in Thessaloniki, Greece

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The purpose of this work was to study sales of hormonal contraceptives in a sample from the medicines market of Thessaloniki, the second largest city in Greece.

A sample of hormonal contraceptives registered sales corresponding to the years 2014-2015 was collected for the study. The sample corresponded only to a small amount of sales from the market of Central and Eastern Thessaloniki. All kinds of systematic and regular hormonal contraceptives in the sales were estimated, and the consumption of contraceptives was expressed in months of contraception. Emergency contraceptives were not included in the study. The statistical package SPSS was used for statistical analysis.

In the study sample, the sales of systematic oral contraceptives corresponded to 3,825 months of contraception out of a total of 4,725 months of contraception (81%). The most common type of oral contraceptives was the combination of ethinylestradiol and drospirerone. The sales of intrauterine systems of hormonal contraception were very limited (less than 3% of sold boxes) but corresponded to 900 months of contraception (19%), thanks to the extended release of levonorgestrel and the long duration of contraception of these systems (5 years).

The sales of hormonal contraceptives in the market of Thessaloniki were low. The percentage of intrauterine hormonal contraception was very limited.

Keywords: hormonal contraceptives, oral contraceptives, intrauterine contraceptives

P198: Hypoglycemic properties of polyphenol concentrate from Cabernet Sauvignon collection of grapes of Kazakhstan region on the model of alloxan induced diabetes in rats

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Investigate hypoglycemic properties of polyphenol concentrate from Kazakhstan selection of Cabernet Sauvignon collectiongrapes on the model of acute alloxan diabetes in rats.

Study of hypoglycemic activity of polyphenol concentrate was conducted on 12 male rats weighing 160 ± 20 g. Insulin-dependent diabetes was induced by intraperitoneal administration of alloxan (Sigma, USA) a single dose of 160 mg / kg of animal body weight after rats pre-trial detention in conditions of hunger during the day (while maintaining access to water). The occurrence of diabetes was monitored by the dynamics of blood glucose levels, taken from the tail vein of rats. Formation of diabetes occurred within 4 days, after which the animals were divided into two treatment groups: control and experimental. The rats in the experimental group were administered polyphenol concentrate at a dose 0.5 ml for 5 days. Rats in the control group received placebo (drinking water) in equivolume amounts. After 5 days of administration of polyphenol concentrate blood glucose level was determined on the device for measuring blood glucose «Gamma Mini» (Gamma, UK). Statistical analysis was performed using «Statistica 6.0» program.

Average initial glucose reading before the experiment was 6.0±0.4 mMol/l, which is within the limits of physiological norm for this type of laboratory animals. After 4 days alloxan injection the blood glucose level in rats was significantly increased and reached 29,2±2,7mMol/l in the control group and 28.4±3.4 in the test group, indicating the development of hyperglycemia. After 5 days of administration of the polyphenol concentrate the blood glucose level was 13,0±2,9mMol/l, whereas in the control group, glucose level was 23,7±4,1mMol/l.

These results shows that concentrate of polyphenols derived from Cabernet Sauvignon collection of grape, at course action in the conditions of the model showed pronounced hypoglycemic properties.

Keywords: polyphenols, diebetes

P199: Clinical trials in Turkey registered at clinicaltrials.gov

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Clinical trials are studies performed to investigate the safety or efficacy of medicine. For human medicines, these studies are carried out in human volunteers.

We want to give information about ClinicalTrials.gov website and how can we use this and also characteristics of clinical studies in Turkey.

ClinicalTrials.gov is a Web-based resource that provides patients, their family members, health care professionals, researchers, and the public with easy access to information on publicly and privately supported clinical studies on a wide-range of diseases and conditions. The Website is maintained by the National Library of Medicine(NLM) at the National Institutes of Health(NIH). Information on ClinicalTrials.gov is provided and updated by the sponsor or principal investigator of the clinical study. Studies are generally submitted to the Website (that is, registered) when they begin, and the information on the site is updated throughout the study There are 210,411 clinical trials in the World. 8645 of these clinical trials are localized in Middle East region included Turkey. There are 2091 clinical trials in Turkey (last reviewed in October, 2015). We categorized these studies according to study types, funder types, phases and intervention types. 1672 studies are interventional, on the other hand 409 studies are observational. Most of these studies are funded by industries. 31, 222, 787 and 387 studies are phase I, II, III and IV respectively.

Clinical trials are laid out to let us what works (and what doesn't) in medicine and health care. They are the most important route to learn what serves best in treating diseases like cancer. ClinicalTrials.gov is a free online database to help the public and researchers learn more about clinical trials, today contains identifications, locations, types and other important informations about more than 210,411 studies. Therefore, it is important to know how we can use this website.

Keywords: ClinicalTrials.gov, clinical trials, medicine, study phases

P200: What is the additional monitoring drugs?

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Additional monitoring is a new method for marked medicines which are needed to follow up very closely. According to this method, some medicines are described as “additional monitoring drugs”. There are inverted black triangle symbol and description “ this medicine is subject to additional monitoring” in the Summary of Pruduct Characteristics. It is used in european union countries since 2013. All medicines are carefully monitored after they are placed on the market. If a medicine carried the Inverted Black Triangle symbol, this means that it is subject to intensive monitoring. This is because we have relatively limited information about their safety from clinical trials as these trials generally involve only small numbers of eligible patients who take the medicine for a relatively short period of time. In Turkey, Legislation of Drug Safety is published on 15th April, 2014, including additional drug monitoring.

Turkish Medicines and Medical Devices Agency make list of additional monitoring drugs and publish on their website. There are 119 medicines under additional monitoring.

Inverted Black Triangle symbol does not mean that medicine is unsafe. Black Triangle (additional monitoring status) is always assigned to medicine if: ıt contains a new active substance; new medicines or vaccines authorised on or after January 2011 are assigned Black Triangle. It is biological medicine, such as vaccine or medicine derived from plasma, it has been given conditional approval or approved under exceptional circumstances. The company that markets the medicine is required to carry out additional studies: for instance, to provide more data on long-term use of the medicine, or on a rare side effect seen during clinical trials.

Therefore, it is important to continue to monitor closely the use of these drugs in clinical practice to improve the knowledge on their long-term safety and their place in treatment of disease.

Keywords: additional monitoring drug, inverted black triangle symbol, long-term safety

P201: Rational use of methylphenidate in ADHD in Umraniye, Istanbul

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Attention Deficit Hyperactivity Disorder (ADHD) is vastly overdiagnosed and red prescription (RP) obliged for the drug methylphenidate. In this study, the prescriptions of controlled drugs (CD) in Umraniye, Istanbul were evaluated. The aim of this retrospective study was to investigate if there is any irrational use of methylphenidate.

Prescriptions of CD written in last three months of 2014 were investigated in the archives of Local Directorate of Health. 416 (25%) of 1685 (100%) RP were retrospectively evaluated and 60 patients' drug history investigated from September 2014 to December 2015 from the aspect of drug-drug interaction, overdose, psychiatry consultation frequency, month of birth and demographic data by analysing the patients electronic system datas.

In 357 (86%) of 416 met were prescribed. In randomly chosen sample 48 (80%) patients were prescribed methylphenidate. 304 (55,2%) visits was to psychiatry department between January 2014 and December 2015 for 48 patients. According to the drug histories of sample: age mean(M):11,7 standart deviation(SD): 3,03; first admistration age minimum(min) was 5 years maximum(max) was 17 years (M:9,1; SD:3,01); how many years used results was min 2 years, max 5 years (M:2,58; SD:1,05); overdosed(60mg) was 45,2% drug-drug interaction was 35,4%, overdosed and drug-drug interaction have not determined were 17%, psychiatry consultation frequency throught two years min was 1, max was 24 (M:7,33; SD:5,02); total visits throught two years min was 1, max was 43 (M:12,5; SD: 9,38). Daily dose min was 10 mg, max 108 mg. Most of the prescriptions were written for men(71%). 44,12 per cent of men's month of birth was June-July-August. There was pozitif correlation(spearman correlation coefficient rho:0,302; p:0,042); between men's psychiatry visits frequency and month of birth (June-July-August).

The results confirm the accurance of an irrational use of methylphenidatet and series of inadequate practices related to ADHD medication.

Keywords: rational use of drug, methylphenidate, ADHD, red prescription, pharmacoepidemiyology

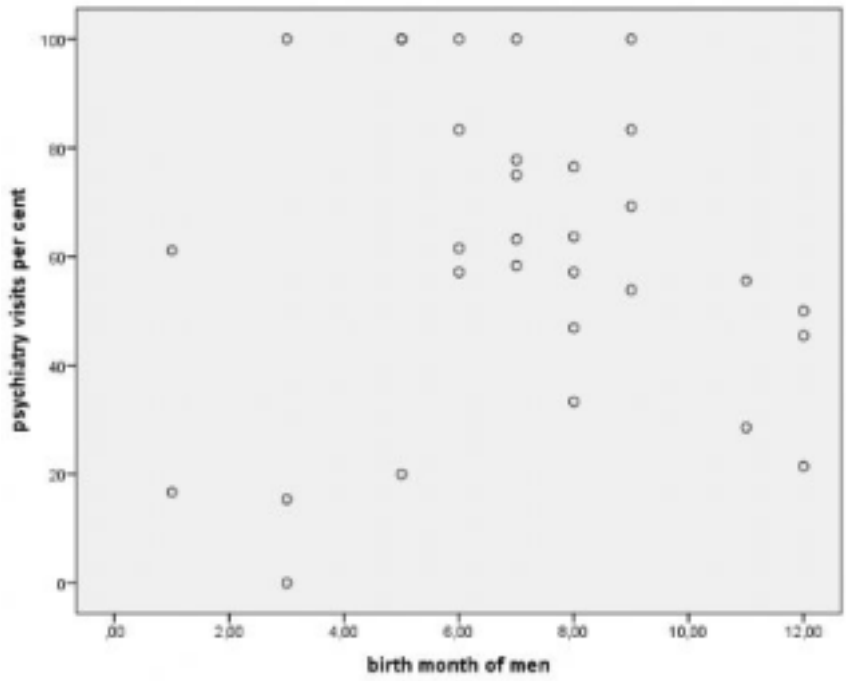

Figure 1. Correlation between psychiatry visits per cent and birth month of men. There was pozitif correlation between men's psychiatry visits per cent and month of birth.

Table 1. Correlation statistics of interaction and overdose. CC, Correlation Coefficient Spearman rho. *There was positive correlation between interaction and psychiatry visits and all visits frequency. There was positive correlation between overdose and duration of drug use.*

		gender	age	duration of drug use	psychiatry visits	psychiatry visits per cent
interaction	CC	-0,1	0,141	0,192	,440**	-0.014
	p	0,5	0,34	0,19	0,002	0,926
	N	48	48	48	46	45
overdose 60mg	CC	0,009	-0,346	-,412*	-0,333	0.054
	p	0,961	0.057	0,021	0,072	0,776
	N	31	31	31	30	30
		all visits	interaction	overdose 60mg	interaction and overdose	first admistration age
interaction	CC	,472**	1	-0,029	0,699**	0,049
	p	0,001		0,876	0	0,74
	N	48	48	31	31	48
overdose 60mg	CC	-0,331	-0,029	1	,694**	-0,224
	p	0,069	0,876		0	0,227
	N	31	31	31	31	31

P202: Self-medication practices with antibiotics among Greek medical students

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Self-medication with antibiotics is a major reason for the spread of antibiotics resistance. The aim of the present study was to assess practice and perception of self medication among medical students. A questionnaire-based, cross-sectional survey, concerning economic conditions and health practices of antibiotic self administration, for the previous 6-months time period, was conducted in Faculty of Medicine, School of Health Sciences, Aristotle University of Thessaloniki, from January to April 2015. A total of 700 copies of questionnaires were distributed. A total of 531 forms were completed and returned (response rate 75.8%). According to the results, 414 (77.9%) students had a monthly income of less than 300 euro, while 97 (18.3%) students did not have any health insurance. Out of 531 students that responded, 81 (15,9%) were self-medicated with antibiotics the last 6 months. The principal symptoms for seeking self medication with antibiotics were pain, cough and fever as reported by 48 (59%) students. Out of 81 self medicated students only 41 (50.6%) were completed the full course of antibiotic regimen while 37 of them (45.7%) were taking wrong dosages during the treatment period and 24 (29%) reported adverse events related to antibiotics. The main reason reported for not visiting a medical doctor, 67 students (82.7%), was the prior use of the same antibiotic in the past. Male gender and older age were identified as independent risk factors of self medication with antibiotics. Self administration with antibiotics is common between medical students in Greece and can often lead to wrong dosages or adverse events. Strict regulations on antibiotic sales and education reinforced by further health care reform are recommended.

Keywords: self medication, antibiotics, medical students, adverse events

P203: Evaluation of potential drug–drug interactions in cardiovascular surgery intensive care unit patients

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Patients in the intensive care unit (ICU) are prescribed large number of medications and predisposed to development of drug–drug interactions (DDIs) that may complicate disease severity. The software used within hospital network identifies DDIs with any severity rating and warnings may be disregarded by the physicians.

We aimed to identify the prevalance of potential DDIs and categorize main drug classes involved in severe DDIs and to highlight clinically significant DDIs in ICU patients.

This cross-sectional study included 168 patients who has been hospitalized in cardiothoracic surgery ICU on December 2015. The drugs administered during the first 24 hours of ICU hospitalization are analysed retrospectively. The presence of potential DDIs was identified by using Lexi-Interact database and categorized according to severity: A (unknown), B (minor), C (moderate), D (major), and X (contraindicated). Interactions with a severity rating of D and/or X were considered clinically important DDIs for further analysis.

The mean age was 60.3 years with male predominance (70.3%). From 1588 drugs, 85 pharmacologically active substances were prescribed and 39 lead to major (D) and contraindicated (X) interactions. D and X interactions are detected in 55 patients (32.7%). Drug classes responsible from these interactions are cardiovascular drugs (39.5%), central nervous system drugs (21%), blood formation, coagulation and thrombosis drugs (10.5%) and respiratory tract agents (7.9%). Pharmacodynamic interactions showed predominance in relation to pharmacokinetic interactions. The combinations of those agents that should be avoided are due to possible QTc-prolonging effect, additive effects and possible increased risk for gastrointestinal ulceration and bleeding.

This study showed the clinical significance of DDIs in ICU patients. Drug classes responsible for interactions in cardiovascular surgery ICU are frequently prescribed cardiovascular drugs and awareness of physicians about patient safety should be increased and attention should be given to the warnings supplied by software.

Keywords: drug-drug interactions, intensive care unit

Table 1. Drug classes, agents associated with D and X interactions, and the frequency of interactions

Drug classes	Specific medications (frequency of interactions)
Cardiovascular drugs	Metoprolol (26), Amiodarone (25), Diltiazem (20), Dopamin (9), Valsartan + Hidroklorotiyazid (8), Captopril (5), Furosemide (3), Ramipril (3), Digoksin (2), Propranolol (3) Spironolakton + Hidroklorotiazid (2), Calvedilol (1), Adrenalin (5), Noradrenalin (3)
Central nervous system agents	Fentanyl (23), Tramadol (12), Midazolam (7), Rocuronyum (2), Dexmedetomidine (1), Phenytoin (1), Quetiapine (3), Chlorpromazine (1)
Blood formation, coagulation, and thrombosis	Warfarin (11), Clopidogrel (1), Cilostazol (2), Acetylsalicylic acid (8)
Respiratory tract agents	Ipratropium (5), Salbutamol (3), Theophylline (5)
Anti-infective agents	Cefazolin (2), Ciprofloxacine (1)
Gastrointestinal drugs	Pantoprazole (1), Metoclopramide (1)
Miscellaneous	Methylprednisolone (8), Diphenhydramine (2), Colchicine (1), Dexketoprofen (3), Diclofenac (1), Atropin (1)

P204: Assessment of drugs that may prolong QT and/or cause *torsades de pointes* in the intensive care unit patients of a university hospital

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QT-interval prolongation is associated with an increased risk of a characteristic life-threatening cardiac arrhythmia, known as ''*torsedes de pointes* (TdP)''. Medications which can lead to QT-interval prolongation play important role in critically ill patients with potential risk factors for improvement of TdP. This study aimed to evaluate the use of medications which have potential for QT-interval prolongation in surgical intensive care unit (ICU) patients of a university hospital.

Organized consultation reports for ICU patients by medical pharmacology department in Marmara University Hospital for 12 months (from January 1st 2015 to December 31st 2015) were retrospectively analyzed. Available TdP risk categories of medications were identified according ''*CredibleMeds*''[(1)], with updated lists of specific medications that prolong the QT interval which the agents are classified with known, possible and conditional TdP risks (Table 1).

Of 305 ICU patients, 228 were treated with at least one (n=111) or more than one drug (n=117) which may have a risk of TdP. The most common medications with known risk of TdP were domperidone (13%; n=30), amiodarone (8%, n=18), and propofol (7%, n=16). The most common medications with possible risk of TdP were olanzapine (2%; n=4), granisetron (1%, n=2), aripiprazole (1%, n=2). The most common medications with conditional risk of TdP were pantoprazole (47%, n=108), metoclopramide (33%; n=75), and amantadine (13%; n=29).

The medications should be carefully evaluated before ordered because they may interact and cause QT prolongation and/or *torsades de pointes* especially the ones that may not be essential for the treatment of critically ill patients such as domperidone, metoclopramide and pantoprazole.

(1) https://crediblemeds.org/ (last access: March 15 2016).

Keywords: drug interaction, ICU patients, QT prolongation, *torsades de pointes*

Table 1. *The list for the drugs that prolong QT and/ or cause torsades de pointes used in the ICU unit of Marmara University Hospital (the number of the patients are given in the parenthesis).*

Known TdP Risk	Possible TdP Risk	Conditional TdP Risk
Domperidone (30) Amiodarone (18) Propofol (16) Escitalopram (15) Ciprofloxacin (12) Fluconazole (11) Moxifloxacin (9) Ondansetron (9) Donepezil (2) Levofloxacin (1) Sotalol (1)	Olanzapin (4) Granisetron (2) Aripiprazole (2) Dexmedetomidine (1)	Pantoprazole (108) Metoclopramide (75) Amantadine (29) Furosemide (27) Quetiapine (23) Hydrochlorothiazide (8) Sertraline (4) Fluoxetine (2) Trazodon (1) Hydroxyzine (1) Ivabradine (1) Itraconazole (1)

P205: Venlafaxine side effects on urogenital system caused early diagnosis of prostate cancer: A case report

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Venlafaxine is a seratonine-norepinephrine reuptake inhibitor (SNRI) antidepressant used for the treatment of major depression and anxiety disorders. It's effect on noradrenerjic system causes side effects including difficulty starting and maintaining a steady stream of urine, dysuria and painful ejeculation.

Prostate cancer is the development of cancer in the prostate. Early prostate cancer usually has no clear symptoms. Sometimes cause symptoms including difficulty starting and maintaining urination, hematuria, and dysuria.

Side effects of venlafaxine on urogenital sytem are similar to symptoms of bening prostatic hyperplasia(BPH) and prostat cancer. No matter if these symptoms are evident or not before starting treatment with venlafaxine, urination problems, dysuria and painful ejeculation occurs, measurement of PSA is important for early diagnosis of prostate cancer.

A 45 years old male was referred to our clinic for treatment of social anxiety disorder and accompanying irritabl bowel syndrome. Venlafaxine was prescribed starting 35mg/kg. After 15 days, the dose changed to 150 mg/kg. 2 weeks after difficulty in starting urination, dysuria and painful ejeculation occured. These symptoms are thought as velafaxine side effects. To consider every possibility urological consultation performed. Prostate volume, PSA level and free/total PSA ratio was found 6ml, 4.1, 0.20 respectively. After ciprofloxacine treatment serum PSA level re-analyzed and found 4.3. Prostate biopsy was performed, result was “prostatic intraepithelial neoplasia”. Because of early diagnosis nevre-sparing prostate surgery planned for our patient.

Venlafaxine has side effects including painful ejeculation, difficulty starting and maintaining a steady stream of urine and dysuria. These symptoms are also accompany BPH and prostate ca. This case showed us no matter if the patient have urogenital symptoms before venlafaxine treatment or not, PSA test must be performed after 40 to exclude or to diagnose prostate cancer, or bening prostate hyperpasia if symptoms including painful ejecaulation, dysuria and diffuculty during urination occur.

Keywords: venlafaxine, benign prostate hyperplasia, prostate cancer

P206: Antipsychotic prescribing for inpatients of AHEPA hospital in Greece

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Under the current financial crisis in Greece, an effort has been made to lower medicinal cost[1,2,3]. The purpose of this work was to study antipsychotic prescribing and utilization of generics in patients of a tertiary Hospital of Thessaloniki.

A sample of antipsychotics prescriptions corresponding to the first six months of the year 2015 was collected from the archives of the Psychiatry Department of the AHEPA Hospital in Thessaloniki, Greece. All proprietary names of antipsychotics and their relative ratios in the prescriptions were estimated, and the percentage of generics in prescriptions was calculated. The amount of prescribed medicines was estimated in Defined Daily Doses (DDDs) of the reference drug and its generics. The comparison of the two samples was made by using the statistical package SPSS.

Prescriptions of haloperidol corresponded to more than half of total prescriptions (53%). Risperidone, olanzapine and quetiapine were the other three most prescribed antipsychotics, corresponding to 33% of total prescriptions. Generic prescribing corresponded to 15% of total antipsychotic prescribing (1,188 DDDs out of 7,724 DDDs). The percentage of generics was around 50% for risperidone and olanzapine.

In the study sample, older and cheaper antipsychotics or generics were prescribed in most cases. These results reflect the efforts of Greek physicians to lower medicinal cost under the current financial crisis in Greece.

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Keywords: antipsychotics, generics

P207: Use of antihypertensives in Thessaloniki, Greece

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The purpose of this work was to study utilization of antihypertensives in the market of Thessaloniki, the second largest city in Greece.

A sample of antihypertensives registered sales corresponding to the year 2015 was collected for the study. The sample corresponded only to a small amount of sales from the market of Thessaloniki. All classes of antihypertensives and their relative ratios in the sales were estimated, and the percentage of generics in the sales of each medicine was calculated out of a variety of brand names in each class of antihypertensives. The amount of medicines was estimated in Defined Daily Doses (DDDs) of the reference drug and its generics. The comparison of the two samples was made by using the statistical package SPSS.

Out of a total number of 121,020 DDDs of registered sales, angiotensin II receptor antagonists corresponded to 32%, calcium channel blockers corresponded to 24%, beta blockers corresponded to 16%, ACE inhibitors corresponded to 15% and diuretics corresponded to 12% of antihypertensives. Generic use corresponded to 15% of total sales of antihypertensives.

Angiotensin II receptor antagonists, calcium channel blockers, beta blockers, ACE inhibitors and diuretics were the most prescribed antihypertensives in the study sample of registered sales in the market of Thessaloniki.

Keywords: antihypertensives, generics, DDDs

P208: Evaluation of the effect of lemon juice on blood pressure in hypertensive patients

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Complementary and alternative medicine is very common among hypertensive patients. The present study aimed to evaluate the effectiveness of lemon juice to lower blood pressure.

Twenty nine patients who admitted to a research outpatient clinic with at least 6 months of hypertension history and treated with at least one antihypertensive medication were included. Patients were randomly allocated to lemon juice or water groups. Lemon juice (25%) is prepared just before drinking. Blood pressure measurements were done before (basal) and 5^{th}, 15^{th} and at 30^{th} minute after lemon juice or equal volume of water intake.

When compared with the basal values, patients who were given lemon juice had lower systolic blood pressure at 5^{th},15^{th} and 30^{th} min. Control patients who were given water had lower systolic blood pressure at 5^{th} and 15^{th}, and diastolic blood pressure at 15^{th} min. When compared with the basal values, patients who were given lemon juice had lower heart rate at 15^{th} and 30^{th} and control patients who were given water had decreased heart rate at 5^{th}, 15^{th} and 30^{th} min.

Both lemon juice and water decreased systolic blood pressure within first 15^{th} minutes possibly due to doctor and environmental silent effect. The decrease in heart rate in both groups supports this suggestion. Decrease in systolic blood pressure is sustained at 30^{th} minute in lemon juice given patients, but not in control patients. Although lemon juice is frequently used among Turkish hypertensive population our study results is not supporting its use.

Keywords: hypertension, complementary and alternative medicine, clinical pharmacology

P209: Drug exposure before and/or during pregnancy: Take-home messages from small data

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Pregnant women may need pharmacotherapy both to cure various diseases and to overcome some pregnancy-induced symptoms. Present study was designed to investigate the properties of drug exposure before and/or during pregnancy regarding to drug spectrum (ATC/DDD, 2016), pregnancy risk categories of drugs (FDA), number of drugs, drug doses, duration of drug use and the pregnancy week during exposure. For this purpose, our consultation reports within ten years period (2004-2014) were evaluated retrospectively. Seventy-three patients were applied for consultation, one of them was for the drug use before pregnancy, 51 of them (69%) was for drug use during pregnancy, others were before and during pregnancy. According to our data, paracetamol, pseudoephedrine, ciprofloxacin, hydroxyzine were the most frequent drugs used during pregnancy. Nervous system drugs, C (53%) and B (31%) pregnancy risk groups of drugs accounted for a major part of the data. The disposition of other risk groups were A (3%), D (4%) and X (7%). Median number of drugs per person was 2 (1-12) at the time of application. With the exception of one patient, all patients were exposed to drugs at therapeutic or subtherapeutic doses and with usual duration of therapy or shorter than this. Drug exposure was in the early period of pregnancy, median pregnancy week was 7(0-20). The results suggest that the frequency of drug exposure during accidental pregnancy is high in our patient population resulting in drug exposure during first trimester of pregnancy. Besides, polypharmacy seems not rare among our patients, as well. In conclusion, we can say that prescribing drugs for women in reproductive age needs to avoid unnecessary use of drugs and it is important to choose drug treatments for them judiciously considering accidental pregnancy possibility.

Keywords: drug exposure, drug use, pregnancy

P210: Evaluation of potential drug-drug interaction among the end stage renal failure patient of Cerrahpasa Medical Faculty Nephrology Transplant Unit

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Polypharmacy is commonly associated with chronic kidney disease (CKD) patients and this population is always at risk of complex medication regimens. The present study was design to estimate the drug-drug interactions (DDIs) through prescription patterns of drugs given to the patients of Nephrology Transplant Unit of Cerrahpasa Medical Faculty.

A total of 96 patients were included in the study. DDIs among the every combination of prescribed drug were analysed using the Thomson Reuters Micromedex System.

The following study reported 149 interactions making 2.16 interactions per prescription with incidence rates of 69.7%. Approximately 4.1% of interactions were of major severity, 75.1% were of moderate severity and 20.8% of interactions were classified as minor. Most frequent interactions were found between iron and aluminum, calcium or magnesium containing products (21.37%), calcium channel blockers and beta blockers (8.96%), aspirin and aluminum, calcium or magnesium containing products (7.58%). Most commonly reported clinical outcomes of the drug interactions were the hypo or hypertension (39.24%), decreased drug efficacy (24.05%) and arrhythmia (9.49%). Aluminum, calcium or magnesium containing products drugs (33.10%) constitute the major class of drugs involved in interactions. The data showed 49% pharmacodynamic and 42.94% pharmacokinetic interactions.

We found that drug interactions mainly affect the drug plasma concentration and secondly lead to hypertensive- hypotensive effects. Close monitoring of CKD patients is required to decrease morbidity and clinical drug interactions.

Keywords: chronic kidney disease, drug interactions, polypharmacy

P211: A comparative study on efficacy of polymyxin B, neomycin and polymyxin B, neomycin, hydrocortisone in the treatment of otitis externa

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Acute otitis externa is a common condition involving inflammation of the ear canal. The acute form is caused primarily by bacterial infection, with Pseudomonas aeruginosa and Staphylococcus aureus the most common pathogens. Acute otitis externa presents with the rapid onset of ear canal inflammation, resulting in otalgia, itching, canal edema, canal erythema, and otorrhea, and often occurs following swimming or minor trauma from inappropriate cleaning. Tenderness with movement of the tragus or pinna is a classic finding. Neomycin/polymyxin B/hydrocortisone preparations are a reasonable first-line therapy when the tympanic membrane is intact. Oral antibiotics are reserved for cases in which the infection has spread beyond the ear canal or in patients at risk of a rapidly progressing infection.

The aim of the study is to compare the efficacy of polymyxin B, Neomycin and Polymyxin B, Neomycin, Hydrocortisone in the treatment of Otitis externa.

This study was carried out in the Department of ENT, Nepal Medical College Teaching Hospital (NMCTH), Attarkhel, Kathmandu, Nepal from August 2012 to May 2014. This, prospective randomized study included patients with otitis externa. Patients of all age groups and both gender were included in this study. Patients were randomized into two groups; Group A patients, who received Polymyxin B, Neomycin and Group Bpatients who received Polymyxin B, Neomycin, Hydrocortisone. Pack soaked either with polymyxin B neomycin/ hydrocortisone and applied for 48 hours. If not recover again applied for next 48 hours. The study will be conducted on the patient diagnosis, clinical features- signs:1. Tragaltenderness. 2. Circumduction tenderness

Thus, the efficacy was compared between two drug groups.

In comparison to polymyxin B + neomycin group, hydrocortisone group exhibited statistically significant effectiveness at 48 hours of treatment ($p<0.05$), but in cure rates at 96 hours, no statistical significant difference was observed between two groups ($p>0.05$).

Polymyxin B + neomycin + hydrocortisone group showed higher and faster cure rates than polymyxin B + neomycin group in the treatment of OE at 48 hours follow up.

Keywords: otitis externa, tragal tenderness, circumduction tenderness

Pharmacology in Special Populations

P212: The risk of drug interaction and polypharmacy in geriatric patients

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As a result of extending life expectancy, number of elderly people is rising in the world. The incidences of geriatric illnesses are increasing by extended life expectancy. When looked through the developed countries, the rate of polypharmacy seen is notified as; Iceland 41%, USA 43,4%, Avustralia 35,8%, Italy 46,8%. A study of 2500 people participated from USA, showed that the use of multiple medications, is mostly seen at women aged 65 years or over, 23% of them using minimum 5 drugs a day, 12% of them minimum 10 drugs a day.

In the retrospective study, 100 in-patients of university hospital, aged over 65, participated. Patients are chosen randomized and their medical records are taken from the hospital medication record system. The amount of patients' concurrently taken drugs are examined in terms of polypharmacy and their interactions are checked. Patient's use of medication are examined according to The Beers, START and STOPP criteria.

It was determined a significant correlation between service where patients receive treatment and the incidence of polypharmacy and hyperpolypharmacy (p=0,004). Polypharmacy and hyperpolypharmacy the most frequent in service respectively intensive care unit, internal medicine and cardiology services have been found. The study found that 81 % of them using minimum 5 drugs a day, 27 % of them using minimum 10 drugs a day. Also there are another significant correlation ($p \leq 0.01$) between the number of drugs used by the patients and risk of drug interactions.

The number of medications used by the patient and service where patients receive treatment is important to polypharmacy. Polypharmacy is inevitable in some services such as intensive care unit because of patient situation. Therefore, drug interactions and The Beers, START and STOPP criteria must be checked to protect from the negative consequences of polypharmacy.

Keywords: polypharmacy, geriatric patients, drug interaction

P213: Exposure to SSRI and SNRI antidepressants during pregnancy: Risk of fetus or newborn

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Maternal depression is associated with adverse pregnancy outcomes. Treating depression with agents like selective serotonin or noradrenalin reuptake inhibitors (SSRIs and SNRIs) has been associated with spontaneous abortion, preterm birth, low birth weight and congenital anomalies of the newborn. The aim of this study is to evaluate the exposure and consequences of SSRIs and/or SNRIs in women during pregnancy who were consulted to Medical Pharmacology Teratology Information Service at Marmara University between January 2014 and January 2016.

Consulted patients with depression who were using SSRIs and/or SNRIs (n=39) during or prior to pregnancy were included. Drug information, gestational history and demographic characteristics of the patients were recorded on the consultation day. The patients received feed-backs by several phone calls according to their expected delivery dates. Changes in the therapy, delivery outcomes of the pregnancies and neonatal health information were questioned.

The most common used antidepressants by the included patients were sertralin (28 %), escitalopram (25 %) and fluoxetine (7 %). Ten % of the patients were consulted for pregnancy planning and 90 % of them were already pregnant. All of the pregnant women discontinued their treatments when they learned that they are pregnant. Among the pregnant patients, 63 % reached term, 4 % had medical termination, 4% ended as stillbirth and spontaneous abortion occurred in 8 % of the group. One of newborns who exposed to escitalopram had tachycardia and the two who were exposed to bupropion had respiratory distress and stayed in the intensive care unit for 5 and 15 days. Twenty % of the patients could not be evaluated since their pregnancies were going on. In conclusion, this study may provide safety data for antidepressant use in pregnancy implying their use may affect neonatal health.

Keywords: pregnancy, teratogenicity, maternal depression

P214: Immunosupressive drug counseling for pregnancy in patients with solid organ transplant and fetal outcome

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Transplant patients have an increased risk of premature birth, having babies with low birth weight, cesarean birth or hypertensive disorders in pregnancy. The immunosuppressant therapy should be planned in transplant pregnant patients for the safety of pregnancy and considering the health of the mother, the fetus and the transplanted organ. We aimed to evaluate transplant patients consulted to our clinic for pregnancy planning and immunosuppressive drug exposure during pregnancy.

Database of Marmara University Medical Pharmacology Teratology Information Service between January 2013-February 2016 was retrospectively analyzed for transplant patients consulted for pregnancy planning or use of immunosuppressive drugs after organ transplantation during pregnancy. Two follow-up phone calls were arranged for each pregnancy outcome; at two weeks and one month after the expected delivery date. Changes in the therapy, delivery outcomes of the pregnancies and neonatal health information were questioned and documented.

Out of 587 pregnant women consulted to our clinic 6 (1.02 %) has been receiving immunosuppressive treatment due to organ transplantation. Of these 6 patients, 5 were pregnant and 1 of them was planning pregnancy. Five patients had renal transplant, 1 had liver transplant. Two out of 5 pregnancies resulted with healthy newborns delivered at full-term. Third patient with exposure to tacrolimus, azathioprine, prednisone and pantoprazole after kidney transplantation had spontaneous abortion at 3rd month of pregnancy. Fourth patient was on 16th week of her pregnancy period without any health problem of mother and fetus. The other pregnant women on tacrolimus, azathioprine, prednisone and everolimus therapy after kidney transplantation, had a medical termination at 2nd month of the pregnancy. She had urinary tract infection and vaginal bleeding.

To have safe and successful pregnancy period with lower rates of teratogenic risk for patients with solid organ transplantation is possible but it needs close monitoring and correct guidance for their immunosuppressive treatment.

Keywords: pregnancy, teratogenicity, organ transplantation

Pharmacogenetics / Pharmacogenomics

P215: Pharmacokinetic and pharmacodynamic analysis of glimepiride with CYP2C9 genetic polymorphism in healthy Korean Subjects

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Glimepiride is known to be metabolized by CYP2C9 and the effect of the single CYP2C9*3 variant could be important for drug therapy with glimepiride. The aim of this study was to investigate the effect of the single CYP2C9*3 variant on pharmacokinetics and pharmacodynamics of glimepiride in healthy Korean population. 428 Korean subjects were genotyped for CYP2C9 by polymerase chain reaction-restriction fragment length polymorphism (PCR-PFLP). Serum concentrations of glimepiride, hydroxyglimepiride (M1) and carboxyglimepiride (M2), after single oral dose of 2 mg administered to 31 individuals with CYP2C9*1/*1 (n=16) and CYP2C9*1/*3 (n=15) genotypes, were quantified by HPLC. Blood glucose was measured up to 4 hr after the drug administration. As shown in Table 1, the pharmacokinetic parameters of glimepiride such as area under the serum concentration-time curve from zero to infinity ($AUC_{0-\infty}$) and maximum concentration (C_{max}) were significantly different between CYP2C9*1/*1 and CYP2C9*1/*3 groups ($P<0.05$). The half-life ($T_{1/2}$) of hydroxyglimepiride (M1) was significantly longer in the CYP2C9*1/*3 group than in the CYP2C9*1/*1 group ($P<0.05$). The metabolic ratios (MR) of glimepiride concentration to hydroxyglimepiride (M1) and carboxyglimepiride (M2) concentration in serum were significantly lower in the CYP2C9*1/*1 group than in the CYP2C9*1/*3 group. The mean area under the effective blood glucose level-time curve from zero to 4 hr ($AUEC_{0-4h}$) of glimepiride in CYP2C9*1/*1 group was significantly lower than that in CYP2C9*1/*3 ($P<0.05$). The single CYP2C9*3 variant affects the disposition and hypoglycemic effect of glimepiride. The impact of the CYP2C9 genotype on glimepiride disposition and effect is likely to be clinically significant.

Keywords: pharmacokinetics, pharmacodynamics, glimepiride, glucose, CYP2C9*3

Table 1. Pharmacokinetic and pharmacodynamic parameters of glimepiride, hydroxyglimepiride (M1) and carboxyglimepiride (M2) in the different CYP2C9 genotype groups.

	Parameters	*1/*1 (n=16)	*1/*3 (n=15)	P
Glimepiride	AUC0-∞ (ng/mL·hr)	716.28±338.58	1311.86±447.10	0.001*
	C_{max} (ng/mL)	163.88±91.7	287.49±125.97	0.004*
	$T_{1/2}$ (hr)	2.17±0.46	2.49±0.42	0.082
M1	$AUC_{0-\infty}$ (ng/mL·hr)	275.62±94.36	294.21±66.53	0.429
	C_{max} (ng/mL)	47.74±16.11	43.96±12.95	0.813
	$T_{1/2}$ (hr)	2.51±0.63	3.05±0.65	0.006*
M2	$AUC_{0-\infty}$ (ng/mL·hr)	107.26±45.81	110.95±72.70	0.722
	C_{max} (ng/mL)	14.14±6.27	12.51±5.32	0.453
	$T_{1/2}$ (hr)	3.81±1.45	4.70±2.26	0.206
Glucose	$AUEC_{0-4h}$ (mg・hr/dL)	-29.07±16.09	-40.28±12.98	0.048*

P216: Frequencies of CYP2E1 and ALDH2 alleles and genotypes in Turkish head and neck cancer population

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Alcohol consumption and smoking habits are the risk enhancer factors for the squamous cell head and neck cancer. In our study we investigated the roles in genes polymorphisms at CYP2E1 and ALDH2 which are the responsible enzymes at metabolic pathways of these carcinogens and examined the susceptibility in individuals for squamous cell head and neck cancer. Comperable and matched with each other in point of epidemiological properties in cases (n=79) and healty controls (n=98), were genotyped by using multiplex PCR method for ALDH2 (rs671) and PCR-RFLP method for determining DraI (rs6413432), PstI (rs3813867), and RsaI (rs2031920) polymorphisms of CYP2E1 genes in people who are living in Denizli and its region. We only determined a statistically significant association for CYP2E1 RsaI polymorphisms on heterozygous (c1/c2) genotypes (OR= 7,79; %95 Cl, 0,92-179,64; p=0,04). The association of other polymorphisms under study was not observed. For ALDH2 and CYP2E1 genes polymorphisms our results are consistent with the studies which are conducted in Caucasian races. However, there are conflicting results in Caucasian races for RsaI/PstI polymorphisms. Consequently, c1/c2 genotypes for RsaI polymorphisms may be a risk factor in squamous cell head and neck cancer But, there are needed larger case groups to demonstrate the susceptibility more precisely.

Keywords: ALDH2, CYP2E1, squamous cell head and neck cancer, gene polymorphism

P217: Frequencies of P2RX7 alleles and genotypes in a Turkish Alzheimer's disease population

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P2RX7 which is a purinergic receptor and non-selective cation channel is widespread in the brain microglia. P2RX7 receptor is up-regulated in postmortem Alzheimer's Disease (AD) patients. This data support that P2RX7 pathway involved in the neurodegeneration. In our study, we investigated P2RX7 receptor gene -1513 A / C polymorphism association with apoptotic processes mediated by inflammation, pain and AD (a neuroinflammation process). Patients with similar epidemiology (n = 100) and control (n = 100) groups were parcipitated in this study.Genomic DNA extracted from peripheral blood samples of cases and controls was followed by restriction fragment length polymorphism. AD genotype frequencies for group AA and AC 69%,(CI) = 62-75; 31% (CI = 24-37) respectively. A allele frequency was 84.5% for the (CI = 79-89), and C allele was % 15.5 (CI = 10-20). In the control group, genotype frequencies were 36% (CI = 29-42) for AA and 64% (CI = 57-70) for AC. A allele frequency was 68% (CI = 61-74), C allele frequency was 32% (CI = 25-38). -1513 C allele has been associated with AD sensitivity (P = 0.0001, odds ratio (OR) for the wild-type allele = 2,565, 95% CI = 1.539 -4291). protective effect for AD was determined for 1513C allele (P = 0.0001, OR = 0.390 for the mutant C allele, 95% CI 0233-0650). P2X7 gene 1513A/C polymorphism is associated with AD.

Keywords: Alzheimer's disease, P2RX7, genetic polymorphism

P218: Investigation of the association between TRPM7 gene polymorphism and preterm birth

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Preterm or premature birth is defined as delivery of an infant before 37 completed weeks of gestation, and is the leading cause of neonatal death and infant mortality. Transient receptor potential melastatin 7 (TRPM7) is a Ca2+ and Mg2+-permeant channel protein. TRPM7 plays an important role in a large number of physiological and pathophysiological processes including cell growth/proliferation, adhesion and migration, motility and movements, oxidative stresss, and anoxic neuronal death. The aim of this study was to investigate a possible association between *TRPM7* gene polymorphisms and preterm birth in a Turkish population.

A total of 91 women in preterm labor and 96 women in term labor with similar age and sex were enrolled to this study. Genomic DNA from the participants was analyzed by a BioMark 96.96 dynamic array system (Fluidigm, South San Francisco, CA, USA). For calculation of the significance of differences in genotype and allele frequencies, the chi-square test or Fisher's exact test was used.

There were significant changes in the genotype (CC, 97.8%; CG, 2.2%; GG, 0%) and allele (C, 98.9%; G, 1.1%) frequencies for the *TRPM7* rs77165588 polymorphism in preterm birth when compared to the controls (CC, 88.5%; CG, 0%; GG, 11.5%, $p=0.0017$; C, 62.2%; G, 37.8%, $p<0.0001$). However, no associations were found with the *TRPM7* rs55924090 (Ile459Thr), rs34181677 (Thr201Ser), rs8042919 (Thr1482Ile), and rs62021060 polymorphisms.

Our results are the first to demonstrate that *TRPM7* gene rs77165588 polymorphism may modify individual susceptibility to preterm birth.

Keywords: polymorphism, preterm birth, TRPM7

P219: Lack of association between insulin-like growth factor-1 receptor polymorphism and Alzheimer's disease

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It is interesting that how oxidative stresss can cause cells to go into apoptosis in both normal ageing and in neurodegenerative disorders. Previous research has investigated insulin-like growth factor-1 (IGF-1) as being involved in the pathogenesis in Alzheimer's disease (AD) by protecting the neurons through reducing neuronal susceptibility to oxidative stress. IGF-1 receptor (IGF-1R) polymorphisms which alter cerebral and systemic levels of IGF-1, may alter the function of the receptor. We genotyped the IGF-1R gene by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) to assess whether this gene polymorphism can be linked to AD. We used leukocyte DNA from 100 patients with AD and a control group consisting of 100 individuals without a history of progressive neurological disorders. Analysis of gene frequencies (dbSNP;rs2229765; for A and G alleles; for AA, AG, GG genotypes) revealed no significant difference between AD patients and controls ($P>0.05$). Our results suggest that the alleles of IGF-1R may no be involved in the pathogenesis of AD.

Keywords: Alzheimer's disease, IGF-1 receptor, genetic poymorphism

P220: Effects of a CYP3A4 inhibitor on the pharmacokinetics of tramadol in relation to *CYP2D6* genotype

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Tramadol is a centrally acting analgesic indicated for the management of moderate to moderately severe pain. It is metabolized by CYP2D6 to its pharmacologically active metabolite O-desmethyltramadol (ODT), but the involvement of CYP3A4 in the biotransformation was also shown. Thus the coadministration of CYP2D6 or CYP3A4 inhibitor may reduce the clearance of tramadol increasing the risk of adverse drug effects such as serotonin syndrome. The aim of this study was to investigate the effects of a CYP3A4 inhibitor on the pharmacokinetics of tramadol in different *CYP2D6* genotypes.

Forty-four healthy volunteers were selected and classified according to *CYP2D6* into three groups (*CYP2D6*wt/*wt* (**wt =*1* or **2*), *CYP2D6*wt/*10* and *CYP2D6*10/*10*). Each subject received a single oral dose of 100 mg tramadol in control phase. In the study phase, clarithromycin 500 mg was administered once daily for five days and clarithromycin 500 mg and tramadol 100 mg on day 6. Blood samples were collected up to 36 hours after tramadol intake and its plasma concentrations were determined by LC-MS/MS.

In control phase, oral clearance (CL/F) and AUC_{inf} of *CYP26D*wt/*wt* and *CYP2D6*wt/*10* were 48.1 ± 11.3 L/hr, 2161.1 ± 389.1 ng·hr/mL and 38.9 ± 11.7 L/hr, 2728.4 ± 593.5 ng·hr/mL, respectively. In study phase, the pharmacokinetic parameters significantly increased or decreased to 43.3 ± 10.7 L/hr, 2453.7 ± 700.5 ng·hr/mL and 34.9 ± 11.8 L/hr, 3115.6 ± 857.5 ng·hr/mL, respectively. The *CYP2D6*10/*10* group showed only a significant increase of AUC_{inf} ($P<0.05$) between the two phases. Those pharmacokinetic parameters were also significantly different between the three genotype groups, in the control phase as well as in the study phase.

In conclusion, *CYP2D6* genotype has significant effects on the pharmacokinetics of tramadol and ODT, and the CYP3A4 inhibitor also showed significant effects on the clearance and plasma exposure of tramadol.

Keywords: tramadol, pharmacogenetics, CYP2D6

P221: Coadministration of a CYP3A4 inhibitor and risperidone in relation to *CYP2D6* genotype

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Risperidone is a widely used atypical antipsychotic indicated for the treatment of schizophrenia and for the treatment of moderate to severe manic episodes associated with bipolar disorders. Risperidone is extensively metabolized in the liver by CYP2D6, like several antipsychotics, to its pharmacologically active metabolite 9-hydroxyrisperidone, and to a lesser extent by CYP3A4. Substances strongly inhibiting CYP3A4 may influence the pharmacokinetics of the risperidone active antipsychotic fraction. The aim of this study was to investigate the effects of a CYP3A4 inhibitor on the pharmacokinetics of risperidone in different *CYP2D6* genotypes.

Thirty-seven healthy volunteers were selected and classified according to *CYP2D6* into three groups (*CYP2D6*wt/*wt* (**wt = *1* or **2*), *CYP2D6*wt/*10* and *CYP2D6*10/*10*) for the control group and thirty-four for the study group. The subjects of the control group received a single oral dose of 2 mg risperidone. The subjects of the study group received clarithromycin 500 mg twice a day for five days and clarithromycin 500 mg and risperidone 2 mg on day 6. Blood samples were collected up to 48 hours after tramadol intake and its plasma concentrations were determined by LC-MS/MS.

In control phase, C_{max} and AUC_{inf} of *CYP26D*wt/*wt*, *CYP2D6*wt/*10* and *CYP2D6*10/*10* were 11.3 ± 7.3 ng/mL, 33.4 ± 28.8 ng·hr/mL and 17.7 ± 7.0 ng/mL, 74.2 ± 56.6 ng·hr/mL and 21.6 ± 7.0 ng/mL, 121.0 ± 52.0 ng·hr/mL, respectively, and they were significantly different between the three genotype groups. Also half-life in *CYP2D6*10/*10* were significantly higher and oral clearance significantly lower than that in*CYP2D6*wt/*wt* or *CYP2D6*wt/*10*. But there were no significant differences between the control and study phase in any of the three genotype groups.

In conclusion, *CYP2D6* genotype has significant effects on the pharmacokinetics of risperidone and to a lesser extent to 9-hydroxyrisperidone, but the CYP3A4 inhibitor has not.

Keywords: risperidone, pharmacogenetics, CYP2D6

P222: Effect of *CYP2D6*10* allele on the pharmacokinetic parameters of tolterodine

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Tolterodine is a competitive muscarinic receptor antagonist indicated for the symptomatic treatment of urge incontinence and/or increased urinary frequency and urgency as may occur in patients with overactive bladder syndrome. Tolterodine is extensively metabolized in the liver. After oral administration tolterodine is subject to CYP2D6 catalyzed first-pass metabolism in the liver, resulting in the formation of the 5-hydroxymethyl (5-HMT) derivative, a major pharmacologically equipotent metabolite. As *CYP2D6*10* allele is the most common allele in the Asian population, we investigated the effect of *CYP2D6*10* allele on the pharmacokinetics of tramadol and its active metabolite.

Forty-two healthy volunteers were selected and classified according to CYP2D6 into three groups (*CYP2D6*wt/*wt* (**wt* = **1* or **2*, n=14), *CYP2D6*wt/*10* (n=14) and *CYP2D6*10/*10* (n=14)). Each subject received a single oral dose of 2 mg tolterodine after overnight fasting. Blood samples were collected up to 24 hours after tolterodine intake and its plasma concentrations were determined by LC-MS/MS.

C_{max} and AUC_{inf} of *CYP2D6*wt/*wt*, *CYP2D6*wt/*10* and *CYP2D6*10/*10* were 0.3 ± 0.2 ng/mL and 4.9 ± 4.3 ng•hr/mL, 0.6 ± 0.5 ng/mL and 7.6 ± 6.0 ng•hr/mL, 1.1 ± 0.7 ng/mL and 13.4 ± 8.1 ng•hr/mL respectively. While the two pharmacokinetic parameters above and also the oral clearance were significantly different between the three groups, half-life was not. The pharmacokinetic parameters of the metabolite 5-hydroxymethyl tolterodine were not significantly different between the three genotype groups.

In conclusion, *CYP2D6* polymorphism showed significant effect on the pharmacokinetics of single-dose tolterodine and the *CYP2D6*10* allele showed a significantly decreased enzyme activity but significant differences of 5-HMT between the genotype groups could not be determined.

Keywords: tolterodine, pharmacogenetics, CYP2D6

P223: The Frequency Distributions of *CYP3A4*22* in Turkish Population

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Optimal pharmacotherapy is much far away from sufficiency, although, important development is obtained in most of the fields of clinical pharmacology. Interindividual variability of drug responses may cause insufficient drug therapy or undesirable drug effects. Today,the main factors responsible for the variability of interindividual drug response are genetic factors.Interindividual differences observed in the efficacy and toxicity of many drugs are associated with genetic polymorphisms or mutations of drug metabolizing enzymes, especially phase I cytochrome P450 (CYP) enzymes.Due to these polymorphisms interindividual and interethnic differences of enzyme activities may lead to ineffective drug therapy or drug toxicity. *CYP3A4*22* was recently discovered through its association with low hepatic CYP3A4 expression and CYP3A4 activity,and showing effects on statin,tacrolimus and cyclosporine metabolism.

We collected blood samples,with the attendance of 8 centers from different places of our country,from 158 healty people for preliminary evaluation.Our goal is studying total of 800 blood samples which including 100 for each center.DNA was extracted from whole blood by a modified the method of salt precipitation.Single-nucleotide polymorphisms (SNPs) were analysed using an automated TaqMan Real-Time PCR system.

Of the cohort of 158, 140 (87,2 %) recipients were *CYP3A4 *1/*1* (wild-type),while 18 (12,8 %) were **1/*22* heterozygote. *CYP3A4*22* allele frequency was 6,04%.Allele frequencies are similar with recent studies in Caucasian population (5-7 %).Any statistically significant differences as for age, sex, and other laboratory factors were not detected in healthy individuals ($p>0.05$).

In conclusion, identification of genetic polymorphisims of drug metabolizing enzymes will provide us important information of the success of pharmacotherapy and predict the ineffectiveness of drug therapy and/or side effects of many drugs. In the light of expected data,it is predicted that patients will benefit from a more effective drug treatment.

This study is supported by Gaziantep University Scientific Research Projects Governing Unit, Project No: TF.13.35.

Keywords: pharmacogenetics, pharmacogenomics, pharmacotherapy, pharmacoeconomy, phase 1 CYP450 enzymes

P224: Association with CYP2C9 polymorphism in head and neck cancer

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The aim of this study is to investigate the association of polymorphism in cytochrome P450 2C9 (CYP2C9) with head and neck squamous cell carcinoma (HNSCC). Seventy eight patients suffering from head and neck squamous cell carcinoma and 100 healthy control subjects were genotyped for CYP2C9*2 and CYP2C9*3, leading to poor metabolizers (PMs) by PCR-based RFLP. The CYP2C9*1 allele frequencies were 0.67 in controls and 0.58 in cases (P=0.04). The frequencies of the CYP2C9*2 allele were 0.18 and 0.08 among controls and cases (P=0.01). The frequencies of the CYP2C9*3 allele were 0.15 and 0.34 among controls and cases (P=0.0001). The CYP2C9*1/*1 genotype frequency was not significantly higher in the control group (39%) compared to that of the cases (28%) (P = 0.15). The CYP2C9*1/*3 genotype frequency was significantly higher in the cases (54%) compared to that of the control group (24%) (P = 0.0001). The CYP2C9*1/*2 genotype frequency was significantly higher in the control group (32%) compared to that of the cases (3%) (P = 0.0001). The CYP2C9*2/*3 genotype frequency was significantly higher in the cases (15%) compared to that of the control group (5%) (P = 0.02).The data suggests a significant association of the CYP2C9 polymorphism with HNSCC.

Keywords: CYP2C9 genetic polymorphism, squamous cell carcinoma of the head and neck

P225: The role of ROCK1 and ROCK2 gene polymorphisms in preeclampsia

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Preeclampsia (PE) is one of the key complications of pregnancy, identified by hypertension and proteinuria or end organ dysfunction after 20th week of gestation. PE complicates 2-8% pregnancies worldwide, with significant maternal and fetal morbidity and mortality. The exact cause of preeclampsia is currently unknown but genetic factors can play a role in the pathogenesis. The aim of this study was to investigate the possible role of Rho-kinase (*ROCK1* and *ROCK2*) gene polymorphisms in patients with preeclampsia.

A total of 96 pregnant women with PE and 96 women with normal pregnancy, admitted to the Department of Gynecology and Obstetrics at University Hospital, were included to this study. All participants have undergone routine control and genotyping tests. Data on socio-demographic characteristics in addition to the maternal and neonatal outcomes of pregnancy were collected. DNA was extracted from whole blood and polymorphisms were analyzed by using BioMark HD dynamic array system. Chi-square test was used for statistical analysis.

We found that the allele and genotype frequencies for *ROCK1* gene rs2271255, and *ROCK2* gene rs6755196, rs1515219, and rs965665 polymorphisms were significantly different in patients when compared to the control group. T allele of rs2271255 (56.0% vs. 7.2%, $p<0.0001$), A allele of rs6755196 (21.5% vs. 12.2%, $p=0.0288$), T allele of rs1515219 (43.1% vs. 27.6%, $p=0.0023$), and G allele of rs965665 (87.1% vs. 70.3%, $p=0.0001$) were observed at high frequencies in patients.

To the best of our knowledge, these results are the first to demonstrate that *ROCK1* and *ROCK2* gene polymorphisms may modify individual susceptibility to PE. Identification of *ROCK* gene polymorphisms in pregnant women may contribute to the understanding of genetic risk for PE development.

This study is supported by Gaziantep University Scientific Research Projects Governing Unit (TF.11.17).

Keywords: genetic polymorphism, preeclampsia, rho-kinase, ROCK

P226: Investigation of receptor of advanced glycation end product gene polymorphisms in cardiac syndrome X patients

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Cardiac Syndrome X (CSX) is defined as an angina-like pain despite positive treadmill exercise tests and normal coronary arteriograms. Although the pathogenesis of Cardiac Syndrome X is not entirely highlighted, coronary microvascular dysfunction is suggested to be the main reason for the visible symptoms in many patients having angina attack without a significant cardiac disease. In addition, inflammation is known to be involved in endothelial and vascular dysfunction. Thus, recent researches have shown that inflammation plays a significant role in the pathogenesis of Cardiac Syndrome X.

The receptor of Advanced Glycation End Product (RAGE) takes mainly part in the response to acute and chronic stress. Upregulation of RAGE in diabetes, vascular, inflammatory and neurodegenerative diseases is observed and it is thought to play role in pathogenesis and complications of these diseases.

On this context, our study compares CSX patients and control subjects from Turkish population in the terms of 429T/C, -374T/A and G82S single nucleotide polymorphisms, which have been previously evaluated in many researches due to their relationship with many cardiovascular diseases.

As a result of our study, no significant difference was observed when CSX patients and control group were compared on the basis of genotypic distributions of those three RAGE genes ($p>0.05$).

Polymorphisms of -429T/C, 374T/A and G82S that are found in the RAGE gene are considered to have effects on RAGE mRNA and protein expression. Those polymorphisms may be related to increase of oxidative stresss and inflammation through RAGE activation. However, a meta-analysis, consisting of 17 studies with 4343 patients, 5402 controls, and examining -429T/C, -374 T/A and G82S SNPs in RAGE gene is consistent with our results with no statistically significant relationship between polymorphisms and cardiovascular diseases.

Keywords: cardiac syndrome X, inflammation, RAGE

P227: Association of eNOS, iNOS genetic polymorphisms with drug induced gingival hyperplasia

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Immunosuppressants, antiepileptics and calcium channel blockers may affect periodontal tissue and cause gingival hyperplasia through inflammatory processes. NO (nitric oxide) is an important mediator in inflammatory processes. eNOS (endothelial nitric oxide synthase) and iNOS (inducible nitric oxide synthase) are responsible for NO synthesis. Genetic polymorphisms of eNOS and iNOS may alter NO action in tissues.

In this study, we aimed to investigate association of eNOS (G894T, T-786C, VNTR), iNOS (Ser608Leu, C11743T) genetic polymorphisms with gingival hyperplasia in patients using cyclosporine A, phenytoin, nifedipine, and diltiazem.

A total of 106 patients including cyclosporine A (n=36), phenytoin (n=25), nifedipine (n=25), diltiazem (n=20) users were included in the study. Among these 106 patients; 39 (37%) patients had gingival hyperplasia in various degrees compared to 67 (63%) patients who did not have this adverse effect. Patients were genotyped by using PCR and RFLP. Statistical analysis for comparison of frequencies among groups was performed by using chi-square test.

Carriers of the wild type (GG) genotype for eNOS G894T genetic polymorphism was associated with a lower rate of gingival hyperplasia. GG genotype frequencies were 41% in patients with gingival hyperplasia compared to 61% in patients without gingival hyperplasia (p=0.045). Genotype frequencies for eNOS T-786C and VNTR had no significant difference among the groups.

There was no significant association among iNOS Ser608Leu and C-1173T genotypes and occurrence of gingival hyperplasia.

GG genotype in eNOS G894T genetic polymorphism might have a protective role against drug induced gingival hyperplasia.

This study is supported by Hacettepe University Research Grant (No: 004 D03 201 002).

Keywords: eNOS, iNOS, gingival hyperplasia, pharmacogenetics

P228: Association of multidrug resistance protein 1 (MDR1) with drug induced gingival hyperplasia

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Multidrug resistance protein 1 (MDR1) substrates such as cyclosporine A, phenytoin, nifedipine and diltiazem affect periodontal tissue and cause gingival hyperplasia. MDR1 genetic polymorphisms may change transporter activity and has been associated with efficacy or adverse effects of drugs.

In this study, we investigated association of *MDR1* genetic polymorphisms *C3435T*, *G2677T/A* and *C1236T* with gingival hyperplasia in patients using cyclosporine A, phenytoin, nifedipine and diltiazem.

A total of 106 patients were included in the study. The patients have been receiving one of cyclosporine A (n=36), phenytoin (n=25), nifedipine (n=20) and diltiazem (n=20). In various degrees, 39 (%37) patients had gingival hyperplasia and 67 (%63) patients did not have this adverse effect after at least for 6 months of drug use. Patients were genotyped by using PCR-RFLP. Statistical analysis for comparison of frequencies among the groups was performed by using chi-square test.

In the patients using phenytoin (n=25), carriers of T allele for *MDR1 C1236T* genetic polymorphism (n=20) was associated with a lower rate of gingival hyperplasia as compared to the patients with CC genotype (n=5). T allele frequencies were 55% in the patients with gingival hyperplasia and 100% in the patients without gingival hyperplasia (p= 0.009). There were no significant differences among the genotype groups for *C3435T*or *G2677T/A* polymorphisms in the patients with and without gingival hyperplasia.

T allele for *MDR1 C1236T* genetic polymorphism might have a protective role against drug induced gingival hyperplasia in the patients using cyclosporine A, phenytoin, nifedipine and diltiazem.

This study is supported by Hacettepe University Research Grant (No: 004 D03 201 002).

Keywords: genetic polymorphism, MDR1, p-glycoprotein, gingival hyperplasia

P229: Evaluation of CYP2C19 activity at colchicine treated patients

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Colchicine has an anti-inflammatory effect by inhibiting of chemotaxis and phagocytic activity of neutrophils. Therefore, it is used in plenty of autoimmune diseases such as Familial Mediterranean Fewer, Systemic lupus erythematosus and Behçet's disease. Cytochrome P450 enzyme system is responsible for metabolism of many drugs. Among them, CYP2C19 enzyme has a crucial role in the metabolism of some drugs such as Proton pump inhibitors and various antiepileptic. In humans CYP2C19 enzyme activity can be measured by using lansoprazole as a probe drug.

The aim of this study is to evaluate colchicine's effect on CYP2C19 activity in humans, in order to do this, we will compare 2C19 activity before and after the colchicine treatment in two-week period

Six patients (4 Behçet's Disease, 2 osteoarthritis) who have been prescribed colchicine for the first time included in the study. A single dose of 30 mg lansoprazole is given to patients in order to evaluate CYP2C19 activity before and after 2nd week of treatment. Blood samples collected at 3rd hour after administration. Lansoprazole and OH-Lansoprazole(metabolite) levels measured by HPLC in prepared plasma samples.

[Lansoprazole]/[OH-Lansoprazole] metabolic ratio is used to evaluate CYP2C19 activity. Metabolic ratios calculated before and after colchicine treatment compared within patients by using Wilcoxon test.

Lansoprazole / OH-Lansoprazole metabolic ratios (Mean±SD(range)) were calculated as 20.0±11.7 (0.8-36.1) and 17.2±12.6 (1.5-37.6) in pre and after the colchicine treatment, respectively (p=0.43).

Metabolic ratio of Lansoprazole/OH-Lansoprazole were similar in pre and after colchicine treatment. These results show that colchicine doesn't have a significant effect on CYP2C19 activity in humans. These results were similar to our previous study about colchicine's effect on CYP2C9 activity in humans.

Keywords: colchicine, CYP2C19 activity, lansoprazole, OH-lansoprazole, HPLC

Drug Safety and Toxicology

P230: Antiproliferative effect of doxorubicin in fetal glomerular mesangial cells

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Drugs including antibiotics, anti-inflammatory, antiepileptic, and anticancer agents that are administered to pregnant women and preterm newborns have been shown to derange kidney development and induce kidney injury. The mesangium, an important component of the renal glomerulus is formed by the glomerular mesangial cells (GMCs) and their surrounding matrix. GMC hyperproliferation and death are hallmarks of several kidney dysfunctions, including diabetes, lupus nephritis, Berger's disease, and membranoproliferative glomerulonephritis. However, the role of GMC dysregulation in the mechanisms that underlie the pathological actions of nephrotoxic drugs in newborns are unresolved. In the present study we examine the effect of anticancer agent doxorubicin on fetal GMC survival in vitro.

GMCs were derived from preterm newborn pig kidneys that were harvested at gestational day 105 (91% of term). Renal glomeruli, isolated by serial sieving of cortical homogenates were decapsulated and cultured in DMEM containing mesangial cell growth supplement, 2% FBS, and 1% penicillin/streptomycin, generating a pure population of GMCs within 4 weeks. Growth kinetics of the cells were studied in real time using the IncuCyte ZOOM live content microscopy system.

Doxorubicin (0.001 - 10 µM) caused concentration- and time-dependent cell death and inhibition of proliferation over 72 h. Attenuation of cell growth was significant above 0.1 µM by 16h ($p<0.05$). At 0.001 - 0.003 µM, doxorubicin attenuated growth steadily but insignificantly for up to 72 hr, whereas, proliferation was essentially absent in cells treated with 0.3 - 10 µM doxorubicin within 52 h. Confocal microscopy indicated that doxorubicin stimulates reactive oxygen species (ROS) generation in GMCs. Doxorubicin-induced ROS generation was reduced by ROS inhibitor N-acetylcycsteine.

These data suggest that doxorubicin reduces fetal GMC proliferation and causes GMC death, possibly by inducing oxidative stresss. Doxorubicin-induced fetal GMC death may contribute to its fetal nephrotoxic activity when administered prenatally.

Keywords: doxorubicin, fetal renal toxicity, mesangial cells, oxidative stresss

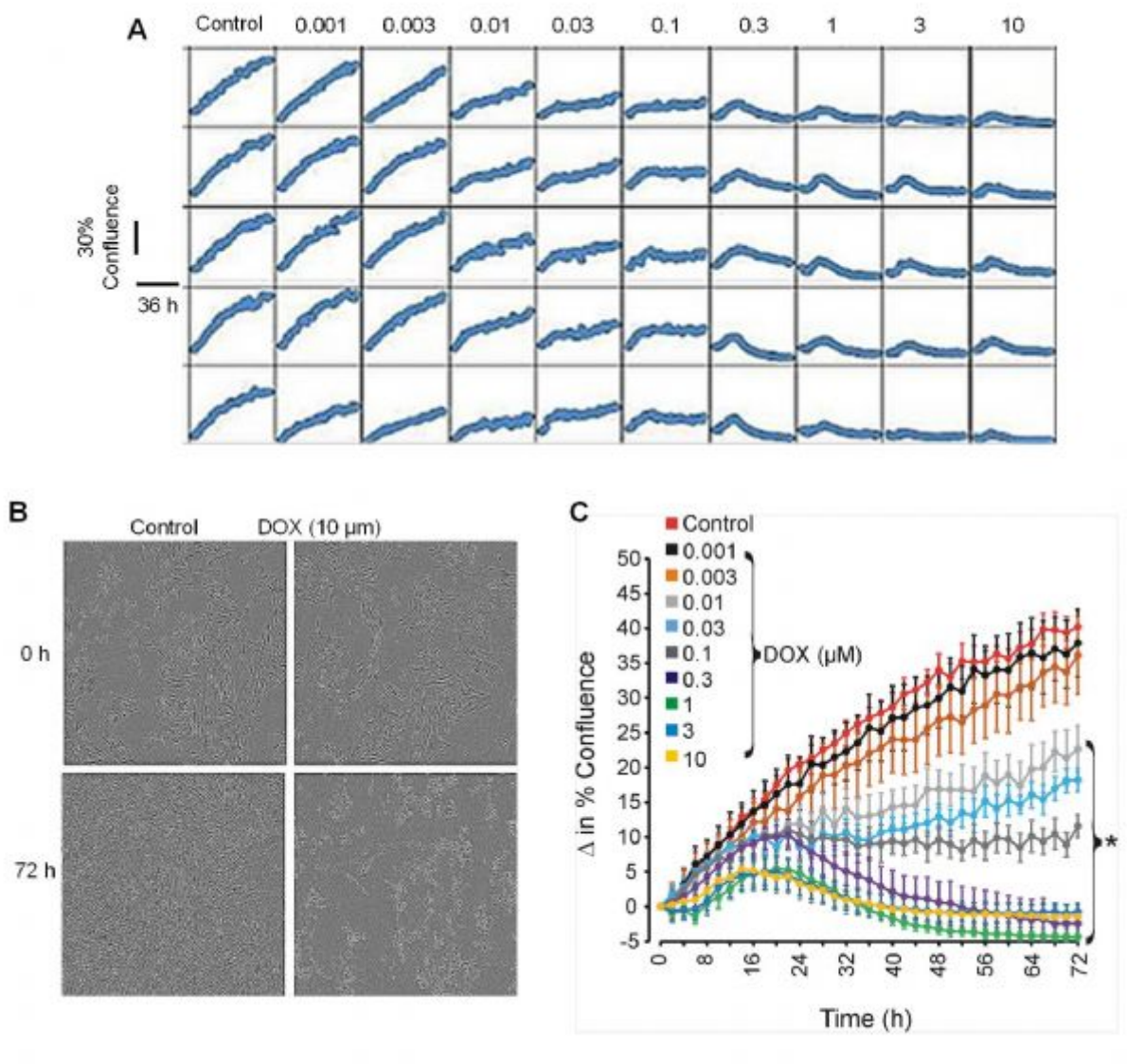

Figure 1. Doxorubicin attenuates growth of fetal glomerular mesangial cells

P231: Allergic response assessment in immunized mice with effective adjuvanted toxoid

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Allergic response and adverse events after vaccine administration are commonly reported and constitute a common problem in clinical practice. The most frequent reactions after immunization are cutaneous and systemic reactions particularly after injection of vaccines toxoids.The aim of this study, was to characterize the adverse events and allergic response induced by detoxified antigen associated to alum adjuvant used as vaccine preparation against scorpion envenomation. Animals were immunized subcutaneously with three doses of antigen preparation at 2 weeks intervals. For assessment of potential adverse events, the weight, fever, local cutaneous reaction and cardio-respiratory signs of each mouse was monitored weekly during the immunization schedule. Eosinophil cell count, serum eosinophil peroxidase activity and histological study of mast cell degranulation at the subcutaneous injection site were also assessed. On the other hand, a specific immunoglobulin IgE, IgG, IgG1, IgG2 titer and the protective effect against scorpion venom-toxicity were evaluated after immunization.

During the immunization schedule, antigen preparation was well tolerated and no serious adverse events were observed. However, granulama formation was monitored after immunization indicating an inflammatory cells infiltration at the injection site. Furthermore, immunized mice showed low levels of specific IgE against antigen preparation compared to the controls. Similarly, the degree of mast cell degranulation and eosinophilic infiltration were lower in immunized mice when compared to the non-immunized mice or envenomed mice. All these results indicate that antigen administration does not induce a severe allergic reaction on mice. On the other hand, vaccine preparation induced a positive IgG_1/IgG_2 ratio that increased after each injection that showed a general shift toward a Th2 response. Interestingly, immunization with adjuvanted detoxifed antigen protects mice against envenomation. The protective immunity is associated with circulating antibodies against antigen preparation.

The vaccine preparation induces a potent specific immune response and protection without serious allergic response and adverse events.

Keywords: detoxified antigen, alum adjuvant, adverse events, allergic response, protection

P232: The investigation of possible protective effect of melatonin on oxidant injury in streptozotocin induced diabetic rats intestine

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Oxidative stress, along with inflammation is one of the major causative factors leading to diabetic complications. The purpose of this study is to investigate the possible protective effects of melatonin treatment against oxidative injury in intestinal tissues of STZ-induced diabetic rats.

Sprague-Dawley rats weighing 250-300g were rendered diabetic by injecting streptozotocin

[60 mg/kg, intraperitoneally (i.p.)]. Rats with a plasma glucose level between 150 and 350 mg/100 ml within 48 hours after the STZ injection were considered diabetic. Control group was given SF only. Melatonin (10 mg/kg, i.p.) and/or insulin (6 U/kg, subcutaneously (s.c.)) were administered for 8 weeks. At the end of the study, plasma glucose levels of all rats were measured and rats were decapitated. Intestinal tissue samples were taken for the determination of malodialdehyde (MDA) and gluthatione (GSH) levels and superoxide dismutase (SOD), myeloperoxidase (MPO) and caspase-3 activities.

In diabetic rats, increased plasma glucose levels were significantly decreased with insulin, and the insulin + melatonin combination treatment. Diabetes mellitus caused significant decreases in GSH levels and SOD activities, and increases in MDA levels, and MPO and caspase-3 activities. On the other hand melatonin and insulin+melatonin treatment significantly reversed all these biochemical parameters.

The results demonstrate that melatonin reduces tissue injury caused by diabetes due to its antioxidant properties. On the other hand, melatonin is unable to regulate blood glucose levels on its own. Besides, insulin treatment is incapable of ameliorating the oxidative damage. The present data demonstrate that melatonin, through its free radical scavenging and antioxidant properties, attenuates diabetes-induced oxidative organ injury, suggesting that melatonin when combined with insulin may have a potential benefit in diabetes mellitus treatment to minimize the concomitant oxidative injury.

Keywords: diabetes, intestinal epithelium, melatonin, antioxidant effect

P233: Protective effect of hypericum perforatum extract on gentamicin induced neprotoxicity in mice

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Hypericum perforatum, is a plant which blooms between july and september at farms, borders of roads and woods, top of hills and grasslands, whose anti-inflammatory effects are shown in various studies. Due to this reason, we planned a study to examine the protective effects of Hypericum perforatum on nephrotoxicity caused by gentamicin. For this purpose, 100 mg/kg gentamicin and 70 mg/kg Hypericum perforatum extract are administered to mice for 9 days and cyclooxygenase-2, phospholipase A_2 and inducible nitric oxide synthase enzymes in their kidneys are analyzed by using ELISA method. Gentamicin administration increased the expression of cyclooxygenase-2, phospholipase A_2 and inducible nitric oxide synthase enzymes. Hypericum perforatum administration to mice that are administered gentamicin previously decreased the rate of increase of the cyclooxygenase-2 and iNOS caused by genetamicin while it didn't have any effect on phospholipase A_2 increase. In conclusion, our study shows that the gentamicin administration causes nephrotoxicity and the use of Hypericum perforatum extract can be helpful against this toxic effect

Keywords: nephrotoxicity, Hypericum perforatum, cyclooxygenase-2, phospholipase A_2, inducible nitric oxide synthase

P234: Protective effect of alpha-linolenic acid on gentamicin induced neprotoxicity in mice

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Alpha-linolenic acid is a carboxylic acid, whose anti-inflammatory and anti-oxidant effects are shown in various studies. Due to this reason, we planned a study to examine the protective effects of alpha-linolenic acid on nephrotoxicity caused by gentamicin. 100 mg/kg gentamicin and 70 mg/kg alpha-linolenic acid are administered to mice for 9 days and cyclooxygenase-2, phospholipase A_2 and inducible nitric oxide synthase enzymes in their kidneys are analysed by using ELISA method. Gentamicin administration increased the expression of cyclooxygenase-2, phospholipase A2 and inducible nitric oxide synthase enzymes. Alpha-linolenic acid administration to mice that are administered gentamicin previously decreased the rate of increase of the cyclooxygenase-2 and iNOS caused by gentamicin while it didn't have any effect on phospholipase A_2 increase. Our study shows that the gentamicin administration causes nephrotoxicity and the use of Alpha-linolenic acid can be helpful against this toxic effect.

Keywords: nephrotoxicity, alpha-linolenic acid, cyclooxygenase-2, phospholipase A_2, inducible nitric oxide synthase

P235: Antipsychotic exposure in an emergency service

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To analyse the antipsychotic medication exposures' distribution and to evaluate severity of the clinical findings in typical and atypical antipsychotics exposures on the admisssion to Department of Emergency Medicine in Dokuz Eylul University Hospital (EMDEU) between 1993 and 2012.

Data related to the demographics, type of exposure, distribution according to the reason, amount of the exposed antipsychotics, clinical findings, length of hospital stay and outcome of the patients exposed to typical and atypical antipsychotics were analysed retrospectively from the patient charts. Chi-square and Fisher's exact tests were used to compare the groups.

The rate of antipsychotic medication exposure was 2.4% in adult poisonings. Mean age of the patients was 31.8±1.8 with female predominancy. Most of the antipsychotic exposures were intentional (93.9%). Atypical antipsychotics (77.6%) were the most exposed antipsychotics. Frequently exposed atypical and typical antipsychotics were quetiapine (50.0%) and chlorpromazine (36.4%), respectively. Toxic amount of antipsychotic ingestions were common both in typical (54.5%) and atypical (57.9%) antipsychotic exposures. Tachycardia (51.0%, n=25) was the most common symptom in typical (54.5%) and atypical (50.0%) antipsychotic exposures followed by unconsciousness (36.7%, n=18), nausea (26.5%, n=13), and hypotension (16.3, n=8). Gastric decontamination was applied to the 57.1% of the patients. There was a significant correlation between elapsed time after the application to EMDEU and duration of hospital stay (r = 0.373, p= 0.008). All of the patients exposed to antipsychotics were recovered.

Overdoses with atypical antipsychotics are more frequent than typical antipsychotics. Atypical antipsychotic exposure did not cause fewer clinical findings compared to typical antipsychotics. Therefore patients ingested toxic doses of all antipsychotics should be monitored closely.

Keywords: antipsychotic, typic, atypic, poisoning, clinical findings

P236: Cardiac safety of long acting muscarinic receptor antagonists in the treatment of chronic obstructive pulmonary disease

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Chronic obstructive pulmonary disease (COPD) is the third leading cause of death worldwide; it is a condition characterised by progressive inflammation of the airways due to emphysema and bronchitis. Ipratropium bromide, a short acting muscarinic receptor antagonist is widely prescribed for COPD, as well as long acting Aclidinium, Tiotropium and Umeclidinium (LAMAs). These drugs inhibit the action of muscarinic receptors involved in airway bronchoconstriction, resulting in airway dilation. Recently, clinical studies assessing the use of anti-muscarinics, have suggested an increased risk of stroke and myocardial infarction, thus highlighting cardiovascular risk.

The current study aimed to assess the cardiac safety profiles of selected COPD drugs (LAMAs), using in vitro models of myocardial ischaemia-reperfusion (I/R) injury. Following 20 minutes of stabilisation, Langendorff hearts, from male Sprague Dawley rats, were subjected to regional ischaemia (35 minutes) and subsequent reperfusion (120 minutes) in the presence of Aclidinium, Tiotropium or Umeclidinium bromide (10 µM-0.1 nM). Following reperfusion, hearts underwent triphenyltetrazolium chloride (TTC) staining to assess infarct/risk ratio (IR%).

The administration of Aclidinium (10 µM, 10 nM, 1 nM), Tiotropium (10 µM-0.1 nM) or Umeclidinium bromide (100 nM - 1 nM) during reperfusion, significantly increased infarct/risk ratio (%) compared with I/R controls (67.1 ± 1.8%, 67.4 ± 3% and 69.56 ±1.02% vs. 50.8 ± 3.9%, $p<0.001$, $p<0.0001$ and $p<0.0001$ respectively, at 10 nM). This study is the first to show that the administration of LAMAs in a pre-clinical whole heart model may exacerbate myocardial injury during I/R. The cellular mechanisms responsible for mediating the observed LAMA induced injury are yet to be elucidated and require further study.

Keywords: long acting muscarinic receptor antagonists, ischaemia-reperfusion, cardiac safety

P237: Effects of prenatal citalopram exposure on motor function and coordination of rat offspring

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Pregnancy is a period that is characterized with complex hormonal and psychosocial lifestyle changes. Therefore depression, anxiety disorders, obsessive compulsive disorders can develop and due to restriction of use of psychotropic drugs, existing serious psychiatric disorders are frequently intensified during the pregnancy. Selective serotonin reuptake inhibitors (SSRIs) are the most commonly used medications in the treatment of depression, panic and phobic disorders as well as obsessive compulsive disorders in pregnancy. However, there is insufficient data on the impact of developmental characteristics and motor functions of infants which were prenatally exposed to these drugs. The aim of this study is to investigate the effect of prenatal exposure to citalopram on reflex and motor functions of rat pups.

Pregnant Sprague-Dawley rats were used in the experiments. Rats were divided into 3 groups and when treatment groups received 5 and 20 mg/kg/d citalopram by orogastric gavage from gestational days 1 to 18, control group received same volume of saline (2 ml/kg/d). Developmental parameters were monitored as pinna detachment, incisor eruption, fur development and eye opening. Righting reflex (postnatal day-PND 2-6), negative geotaxis (PND 3,5,7,9) and grip response (PND 3-7) were evaluated as measures of the development of reflexes.

The gestation duration of rats and the number of pups born alive was not statistically different among any groups. When rats compared by physical landmark developments such as weight gain, pinna detachment, incisor eruption, fur development and eye opening there was no significant difference between treatment and control groups. Righting reflex, negative geotaxis and grip response assessments of pups was not significantly different from control group at any day.

The results of study have been thought that intrauterine exposure to citalopram has no effect on coordination and motor development of rat pups.

This study is supported by the Ondokuz Mayis University Research Fund, PYO.TIP. 1904.15.024.

Keywords: citalopram, teratogenicity, rat

P238: The comparison of resveratrol and silibinin in preventing alpha amanitin-induced liver toxicity: Preliminary findings

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Alpha amanitin (alpha-AMA) containing mushroom poisonings are resulted in severe liver toxicity. Therefore, we aimed to investigate the efficacy of resveratrol (R), a known antioxidant and a hepatoprotective agent, on the alpha-AMA- induced hepatotoxicity and to compare R with silibinin (Sil), a well-known antidote for the alpha-AMA- induced hepatotoxicity.

Acute hepatotoxic dose of alpha-AMA that increased liver function tests (AST and ALT) and developed liver damage histopathologically was determined as 1.4 mg/kg, intraperitoneally in Balb/c mices. There were six experimental groups: 1.Control: alpha-AMA (n=6), 2.alpha-AMA+DMSO (solvent of R and Sil, n=4), 3.alpha-AMA+R (SAR, simultaneously, n=8), 4. alpha-AMA+R (12AR, 12 hours after alpha-AMA administration, n=7), 5.alpha-AMA+R (24AR, 24 hours after alpha-AMA administration, n=6), 6.alpha-AMA+Sil (SAS, simultaneously, n=3). While R administration was repeated with 12 hour intervals, Sil administration was repeated with 6 hour intervals. Mice were sacrificed under ether anesthesia after a 48 hour observation period. Liver function tests and liver damage scores were evaluated.

AST levels were significantly decreased in SAR (2191 ±281.2 U/L) or 12AR (2266 ±823.4 U/L) groups compared the control group (7577 ±1713 U/L $p<0.01$, $p<0.01$). Histopatological liver damage score was significantly decreased in SAR (1.3 ±0.2), 12AR (1.2 ±0.1) and 24AR (1.2 ±0.3) groups compared to control group (2.6 ±0.2, $p<0.001$).

Late onset of the signs and symptoms of the poisonings after ingestion of the alpha-AMA containing mushrooms causes a delay in starting treatment and decreases the success of the treatment. According to the preliminary findings of our experiments, resveratrol seems to be effective as silibinin for reversing hepatotoxicity when used simultaneously or early after the alpha-AMA exposure.

This study is supported by the Scientific and Technological Research Council of Turkey (TUBITAK, No: 114S927).

Keywords: alpha-AMA, resveratrol, hepatotoxicity, silibinin

P239: α1- and β-adrenoceptor mediated transactivation of epithelial growth factor receptor in DU145 prostate cancer cell lines

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In our previous studies, we showed that α1(α1a,b,d-AR) adrenergic receptor stimulation cause transactivation of epidermal growth factor receptor (EGFR) in CHO cells. (JPET,347.47-56,2013). In this study, we investigated the EGFR transactivation with the stimulation of α1- and β-AR especially in DU145 cancer cell lines, which express both of α1- and β-AR.

Protein levels (EGFR, pEGFR, ERK1/2, pERK1/2) were measured by Western-blot analysis. Dynamic assessment of proliferation and viability assays for DU145 cells were performed by ICELLigence TM system and WST 1 assays.

Stimulation of DU145 prostate cancer cells with phenylephrine (10μM, 2, 5, 10, 30min) or terbutaline (10μM, 5, 10,min) caused phosphorylation of EGFR and ERK1/2. Phenylephrine stimulated EGFR phosphorylation was antagonized by SRC inhibitor PP2 (10μM, 30min), calmodulin inhibitor W7 (10μM, 30min) and EGFR kinase inhibitor AG1478 (10μM, 30dk). Terbutaline stimulated EGFR phosphorylation was antagonized by SRC inhibitor PP2 (10μM, 30min), PI3K inhibitor LY294002 (10μM, 30min), EGFR kinase inhibitor AG1478 (10μM, 30dk), but not by PKA inhibitor H89 (10-5M, 30 min). Phenylephrine increased DU145 cell proliferation very slightly (% 5-10), but terbutalin did not.

In DU145 prostate cancer cell line, α1 or β2-AR stimulation increases ERK1/2 activity and transactivates EGFR. Results have shown that, SRC/PI3K pathway plays regulatory role on the β2 -AR mediated transactivation but the same cannot be said for PKA pathway. Also we reached the conclusion that, SRC and calmodulin have an important role on α1-AR mediated EGFR transactivation. Our studies showed that α1 and β2-adrenergic receptor stimulation do not have a very significant effect on cellular proliferation in DU145 prostate cell lines.

This study is supported by TUBİTAK project No: 113S396.

Keywords: DU145 prostate cell lines, EGFR, pERK, adrenoceptors

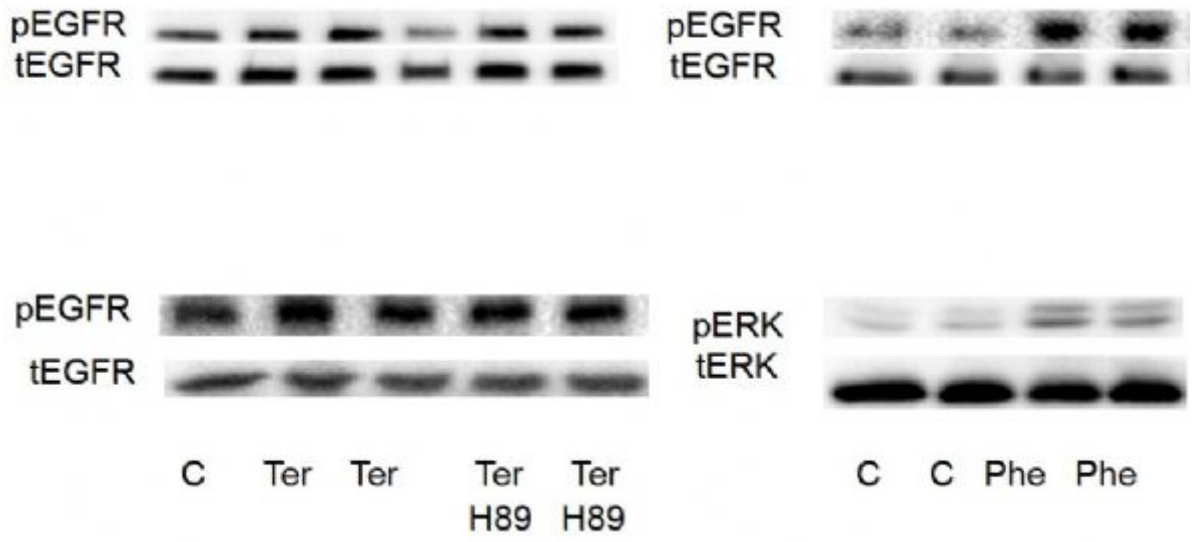
pEGFR
tEGFR
pEGFR
tEGFR
pEGFR
tEGFR
pERK
tERK
C
Ter
Ter
Ter H89
Ter H89
C
C
Phe
Phe

Figure 1.

P240: Contribution of rho/rho-kinase signalisation pathway to gentamicin induced nephrotoxicity

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One of the main causes of acute renal failure is aminoglycoside nephrotoxicity. nephrotoxicty defined as renal failure. Nephrotoxicity is evident charecteristic of tubular necrosis. The studies showed that inhibition of Rho-kinase signaling pathway in diabetes and kidney damage caused by renal ischemia is useful. Similar pathological conditions, such as diabetes and renal ischemia in the nephrotoxic aminoglycosides are observed. Therefore, we planned to examine the effect of gentamicin which is an aminogylicozid induced nephrotoxicity on rho/rho kinase signalisation pathway. For this purpose 100 mg/kg gentamicin are administered to mice for 9 days and rhoA and rho-kinase enzyme and activity in their kidneys are analyzed by using ELISA method. Gentamicin administration increased the expression rhoA and rho-kinase enzyme and its activity.

Keywords: mice, gentamicin, nephrotoxicity, rhoA and rho-kinase

P241: Antioxidant provocation of aspirin and vitamin C against consumption of corn syrup on rat liver

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Corn syrup (CS) is made by an industrial enzymatic isomerization of glucose to fructose and studies have shown that chronic consumption of fructose over daily requirement causes specially non-alcoholic fatty liver disease (NAFLD), hyperlipidemia, obesity, resistance to insulin, metabolic syndrome, type 2 diabetes and goute. In this study, we aimed to research the protective effect of Aspirin (ASA) and vitamin C (Vit C) against to liver damages which are caused by consuming corn syrup.

In our study, Sprague Dawley rats (250-300g/male) are divided into 5 groups each contain 8 rat. CS form were supplied with drinking water (30% of F30). Groups were; Control, CS, CS+ASA (10 mg/kg/d), CS+Vit C (200 mg/kg/d) and CS+ASA+Vit C (10 mg/kg/d ASA+ 200 mg/kg/d Vit C). Rats in all groups were sacrificed at the end of 6 weeks following the last application after 24 hours. Histological analysis was revealed liver tissue changes. Blood samples were examined biochemical parameters.

Serum cholesterol and AST levels found significantly ($p<0.05$) decreased in groups CS+Vit C and CS+ASA+Vit C according to group CS; ALT and triglyceride levels significantly ($p<0.05$) decreased in group CS+ASA+Vit C according to group CS. TNF-alpha which responsible for inflammatory cytokines significantly ($p<0.05$) decreased by ASA and Vit C than group CS. OSI (Oxidative stress Index) in hepatic tissue significantly ($p<0.05$) reduced by ASA and Vit C than group CS. We determined the hepatic injury and lipoidosis at the end of histopathological examinations.

We observed a remarkable improvement in the liver function tests, lipid profile and serum levels of the proinflammatory cytokine (TNF-alfa) with the implementation of aspirin and vitamin C. Histopatologicals results were compatible with biochemical parameters. As a conclusion, use of these antioxidants may an important promising option for preventing (NAFLD) and related diseases due to consumption of CS.

Keywords: vitamin C, aspirin, corn syrup, liver damage, NAFLD

P242: May vitamin C and aspirin be protective against pancreatic damage caused by consumption of corn syrup?

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Fructose (F) which is an essential ingredient of processed ready-made foods, is a chemical sugar achieved from corn starch and more preferred than glucose for creating a feeling of safety. Chronic consumption of high-fructose corn syrup (HFCS) causes several problems such as insulin resistance, type 2 diabetes, metabolic syndrome and Nonalcoholic fatty pancreas disease (NAFPD). The goal of the study was to investigate pancreatic damage induced by chronic HFCS consumption and the protective effects of Aspirin (ASA) and VitaminC (VitC) on pancreatic cells.

In our study, Sprague Dawley rats (250-300g/male) are divided into 5 groups each contain 8 rat. F form were supplied with drinking water (30% of F30). Groups were; Control, F, F+ASA (10 mg/kg/d), F+VitC (200 mg/kg/d) and F+ASA+VitC (10 mg/kg/d ASA+ 200 mg/kg/d VitC). Rats in all groups were sacrificed at the end of 6 weeks following the last application after 24 hours. Histological and biochemical analysis was revealed pancreatic tissue changes and blood samples.

Fasting blood sugar and insulin levels found significantly ($p<0.05$) decreased in group F according to control group. Aspirin and VitaminC were not sufficient affect to the change in HOMA-IR scores which is an indicator of insulin resistance. Amylase which is an indicative of damage to pancreas was significantly ($p<0.05$) decreased by treatment of ASA and VitC than group F. Significant decrease of lipase levels was not determined ($p>0.05$). OSI was not significantly ($p>0.05$) reduced by treatment of ASA and VitC than group F. The histological changes were observed to be benefical with use of ASA and VitC.

We obtained the results which support that development of nonalcoholic pancreatic steatosis depends on corn syrup consumption. As a result, this study indicated that ASA and VitC applications for treatment of HFCS induced pancreatic lesions has not adequate.

Keywords: vitamin C, aspirin, fructose, pacreatic damage, NAFPD

P243: The risk of drug interaction in patients taking antihypertensive medications

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Spending on treatment of hypertension is a large part of health care costs of countries. More than 15 prescriptions per year are written for each patient and also patients receive 4-5 different drugs avaregly. One of cases that make difficult to treatment is drug interaction and patient compliance because of polypharmacy. Investigation of patient's drugs treatments in terms of drug interactions is very important to anticipate the positive or negative consequences of drug interactions. The aim of the study is to evaluate the drug interactions in patients taking antihypertensive medications.

A retrospective study was conducted in 100 adult patients taking antihypertensive medication who are hospitalized in the cardiology department. Patients are selected randomly and patients' informations were collected from the hospital medical record system. All the patients checked for drug interactions during treatment and the data recorded as ¨contraindicate¨ "serious-use alternative" "significant-monitor closely" "moderate" or "minor".

The average of the medication used by patients was found 9.6 per day. There is no risk of drug interaction in 8% of patients. 1164 possible interactions were identified in the study. These interactions respectively 1 contraindicate, 81 serious-use alternative, 835 significant-monitor closely, 0 moderate, 248 minor out of 1164 possible interactions. It was determined a significant correlation between the number of total drugs using and the number of drug interactions ($p \leq 0.01$). In the current study, findings showed that, 13% of patients using less than five drugs a day, 42% of patients using 5-9 drugs a day, and 45% of patients using more than 10 drugs a day.

Drug interactions may negatively affect the treatment, leading to adverse drug reactions. Consequently, the control of drug interactions in patients with hypertension is important to avoid potential medication errors.

Keywords: drug interactions, polypharmacy, medication errors

P244: The risk of drug interactionin hospitalized patients using clarithromycin

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Clarithromycin is a macrolide antibiotic which is often used in the antimicrobial therapy. Also it is very important for drug interaction because it is a potent inhibitor of CYP3A4. The pharmacokinetic changes in concomitant medication use was observed in the studies may lead to cause an increase in the concentration of the other drug3. Especially when used in combination with drugs that are substrates of CYP3A4 such as statin by causing an increase of statin plasma concentrations as rhabdomyolysis has led to more frequent occurrence of side effects.

100 adult and pediatric inpatients in the university hospital that taking clarithromycin treatment, evaluated for the retrospective study. Patients selected randomly by using the hospital's medication record system. All the patients checked for drug interactions during treatment.

It was determined average 3,65 drug interactions for a patient, 1,53 of them is serious. It was observed a significant difference between different inpatient services. Cardiology clinic is at the top with average 5.36 interactions. It also has to be considered that average age of the inpatients is higher at this clinic. Geriatric population has greater risk of the polypharmacy. It was determined a significant correlation between the number of total drugs using and the number of drug interactions ($p \leq 0.01$). Also there are another significant correlation ($p \leq 0.01$) between the number of total drugs and the importance of interactions for each classification such as serious-use alternative, significant-monitor closely, moderate and minor.

Monitoring for drug interaction to review the drug choice according to the interactions and pharmacist consultation for drug interaction for the physicians may reduce the number of medical events caused by medicines. Therefore clinical pharmacists are vital for improving inpatient treatment if they should check for drug interactions.

Keywords: drug interaction, clarithromycin, polypharmacy

P245: Follow-up results of maternal and paternal exposure to isotretionin

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Isotretinoin, that is a derivative of vitamine A, is a well known therapeutic agent in the treatment of acne. However, isotretinoin is contraindicated during pregnancy due to the risk of teratogenicity. The FDA pregnancy category of isotretinoin is X. The aim of the present study was to evaluate the outcome of the risk of teratogenicity in female patients who was exposed to the isotretinoin and the risk of recurrent abortus in a female in whom her husband was on isotretinoin therapy.

The outcomes of maternal and paternal exposure to isotretinoin therapy were retrospectively evaluated in patients admitted to the Medical Pharmacology Teratology Information Unit of the Marmara University Hospital between October, 2013 and February, 2016. Of 587 patients in this period, 5 female and 1 male patients were on isotretinoin therapy. Due to the maternal and paternal exposure, they were included in our analysis and evaluated by a follow-up call for the outcome of the pregnancies and neonatal health information.

The ages of the female patients receiving isotretinoin treatment ranged between 26 and 35 years old, with a mean of 29.6 years of age. Three of these 5 pregnancies ended with medical termination and the other two with Caesarean birth. One female patient in whom her husband exposed to isotretinoin is now at 7-month pregnancy. Before starting the isoretinoin therapy, its potential teratogenic effect should be explained to female or male patients in childbearing age. Thus, the isoretinoin therapy should be combined with an effective contraception in both female and male patients.

Keywords: pregnancy, isotretionin, teratogenicity

P246: Evaluation of enoxaparin-acetylsalicylic acid interactions used in patients in intensive care units

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The use of anticoagulants and antithrombotic drugs are important in the treatment of patients in intensive care units. The most frequently used drugs are acetylsalicylic acid, a nonsteroidal antiinflammatory drug for antithrombotic therapy and enoxaparin, a low molecular weight heparin for anticoagulant therapy. However, combined use of these drugs is considered as 'category C' drug interaction which is defined as 'Clinically, which can cause significant interactions and it is necessary to be careful during treatment'since they may show additive effect and increase the risk of bleeding. The aim of this study is to evaluate enoxaparin and aspirin usage in the surgical intensive care unit of Marmara University Education and Research Hospital.

Database of Medical Pharmacology Department is retrospectively analyzed for consultations requested by surgical intensive care unit in between 07.01.2015-10.02.2016. Patient records with anticoagulant and/or antithrombotic drugs are evaluated for drug-drug interaction.

There were 345 consultation reports for surgical intensive care unit. Out of 345 reports 220 (63.8 %) had enoxaparin, 36 (10.4 %) had acetylsalicylic acid and 30 (8.7 %) had enoxaparin-acetylsalicylic acid combination. Given some patients required more than one consultation, the number of patients treated with enoxaparin was 139, with acetylsalicylic acid was 20 and with enoxaparin-acetylsalicylic acid was 16. There were 7 patients who were treated with 3 drug combination including enoxaparin- acetylsalicylic acid –clopidogrel. All the patients were closely monitored for coagulation and bleeding parameters such as INR, PT and PTT. There were not any complications due to bleeding that could result from drug interaction.

Combination of enoxaparin and acetylsalicylic acid in surgical intensive care unit can be successfully used without any complication under close monitoring of the patient.

Keywords: polypharmacy, drug interaction, drug safety

P247: The levels of immunosuppressive drugs, tacrolimus and cyclosporine-a measured in therapeutic drug monitoring laboratory

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Therapeutic drug monitoring (TDM) of immunosuppressive drugs in transplantation patients is essential to prevent rejection of organs due to inadequate therapy or drug toxicity caused by high drug levels. The aim of this study is to evaluate the results of concentration measurements of most frequently used immunosuppressive drugs - Tacrolimus and Cyclosporine-A, in our TDM laboratory.

In this study the results of 1297 blood samples which were processed in our laboratory in year 2015 (between 01 January and 31 December 2015) were evaluated. Drug levels were analyzed using CEDIA (Cloned Enzyme Donor Immuno Assay- Thermo Scientific™) method (MGC 240 Benchtop Analyzer) and drug levels were classified according to therapeutic window for Tacrolimus (subtherapeutic, therapeutic and toxic) and aimed therapeutic ranges for Cyclosporine-A, because this drug can be used in various of autoimmune disease as well. The therapeutic window for Tacrolimus is considered as 5-20 ng/ml and ranges for Cyclosporine-A are 0-50 ng/ml, 50-100 ng/ml, 100-300 ng/ml and over 300 ng/ml.

The ratio of therapeutic levels were higher than subtherapeutic and toxic ratios; the subtherapeutic, therapeutic and toxic level ratios for Tacrolimus(n= 685) were found as 35,62%, 60,15% and 4,23%, respectively. By the way the ratio of therapeutic ranges for cyclosporine-a (n=612) was 19,60% for 0-50 ng/ml; 29,90% for 50-100 ng/ml, 39,55% for 50-100 ng/ml and 10,95 % for over 300 ng/ml.

Therapeutic drug monitoring is important for efficacy of drug with safety usage and minimum toxicity. The high ratios for subtherapeutic drug levels indicate that it is important to follow these patients up closely especially for dose optimisation and prevent inadequate therapy. Hence there must be a good coordination between the clinic and the laboratory.

Keywords: therapeutic drug monitoring, immunosuppressive drugs, tacrolimus, cyclosporine-a

P248: The protective role of resveratrol on serum total sialic acid and lipid-boundsialic acid in female rats with chronic fluorosis

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In the present study, the effect of resveratrol on serum total sialic acid (TSA) and lipid bound sialic acid was investigated in the rats exposed to chronic fluoride. The study was administered using 32 female Sprague Dawley rats weighing 200-250 g. Rats were divided into four groups (n=8/group). Group I comprised the control group, group II was treated with NaF (10 mg/lt/day), group III was treated resveratrol (50 mg/lt/day) and group IV was treated NaF+resveratrol for 90 days period. Total sialic acid (TSA) and lipid-bound sialic acid (LSA) were determined in serum samples. Statistical analysis showed that the NAF group was significantly higher than the control group with regards to LSA and TSA levels ($p<0.01$, and $p<0.05$ respectively). Whereas, the Resveratrol group was also significantly lower than the NaF group regarding LSA and TSA levels ($p<0.05$ and $p<0.05$ respectively), Moreover, no significant differences in LSA and TSA levels were observed in the Resveratrol + NAF groups, as compared to the control group ($p>0.05$). The present study demonstrated significantly positive and beneficial effect of resveratrol on the concentration levels of LSA and TSA in serum.

Keywords: fluorosis, resveratrol, TSA, LSA, female rat

P249: Determination of Microcystin Toxin in *Chroococcus minutus*

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In this study an investigation of microcystin toxin production by *Chroococcocus minutus* (Kützing) Nageli isolated from an freshwater reservoir.

Water samples were collected in Tokat Yeşilırmak river. One milliliter of water sample was inoculated in a petri dish of F2 medium. After visible cyanobacterial growth on medium,100 mL were spread onto solid BG-11 medium. Repeated streaking onto fresh solid medium and microscopic observations were applied until a monospecific culture was established and identified. This isolate was named. Cells were grown under a 12:12h light:dark cycle with white fluorescent illumination at 25 ℃. The culture was centrifuged at 12,000 g for 5 min and the cell pellet was lyophilized for HPLC (High Performance Liquid Chromatography). Chromatographic separation of microcystin forms (LR,RR,YR) were performed in the C18 reverse phase under 40 ^{0}C temperature and flow rate of 1 ml/min. The wavelength was set at 240 nm.

According to HPLC results, no microcystins detected in *Chroococcus minutus*

This result indicated that *Chroococus minutus* cultivated in BG 11were not produced microcystin toxin.

Keywords: *Chroococcus minutus*, microcystin, toxin, HPLC

P250: Effects of prenatal sertraline exposure on motor function and coordination of rat offspring

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Selective serotonin reuptake inhibitors (SSRIs) are the most commonly used medications in the treatment of depression in pregnancy. However, there are few data on the teratogenic effects of SSRIs. Sertraline is one of the most prescribed SSRIs all over the world. The aim of this study is to investigate the effects of prenatal exposure to sertraline on developmental characteristics, reflex and motor functions of rat pups.

12-14 weeks pregnant Sprague-Dawley rats were used in the experiments. Rats were divided into 3 groups and each rat was housed separately. When treatment groups were received 5 and 20 mg/kg/d citalopram by orogastric gavage from gestational days 1 to 18, control group was received same volume of saline (2 ml/kg/d). After birth duration of gestation, number of live and dead pups and gross malformation are evaluated. Developmental parameters were monitored as eye opening, pinna detachment, incisor eruption and fur development. Righting reflex (postnatal day-PND 2-6), negative geotaxis (PND 3,5,7,9) and grip response (PND 3-7) were evaluated as measures of the development of reflexes.

The gestation duration of rats and the number of pups born alive was not statistically different among any groups. When rats compared by physical landmark developments such as eye opening, pinna detachment, incisor eruption, fur development and weight gain, there was no significant difference between treatment and control groups. Righting reflex, negative geotaxis and grip response assessments of pups was not significantly different from control group at any day.

The results of study shows that prenatal exposure to sertraline has no effect on coordination and motor development of rat pups.

This study is supported by the Ondokuz Mayıs University Research Fund, PYO.TIP. 1904.15.023.

Keywords: sertraline, rat, teratogenicity

P251: Do we know enough about antibiotics?

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Being a specific group of drugs, antibiotics have been observed in their representation in self-treatment. The aim of our work is to examine and show the representation of antibiotic use, the way of application and the level of awareness.

The research was conducted by the method of interviewing students of Medical School in Podgorica. The data gathered by questionnaries were analyzed thoroughly, and according to the results of the research, antibiotics are second on the list in self-treatment, right behind analgesics. 51 examinee had received a recommendation from a doctor, while 52 of them used antibiotics without consultation. 44 heard of the phenomen of the resistance. More than half of examinees stop using antibiotics the first time they start feeling better. More than half of examinees are not familiar with the possibility of more precise diagnostic when using antibiotics. The largest number of examinees expects to get more information about antibiotics in the pharmacy.

The analysis has shown that self-treatment is present in high percentage, whereby the antibiotics are highly present. It is especially worrying that examinees stop using their medications after first improvements, while more than half of them are not familiar with the possibility of more precise determination of antibiotic therapy. Substantial percent of examinees thinks that there is too little information about antibiotics available, which means that one of our most important tasks is working on further education of population about the use of the antibiotics.

Research about the use of medications are extremely important, because they can point on the necessity of rationalizating the therapy, as well as the direction in which we should conduct the appropriate educational and regulatory measures, with the aim of the optimization of pharmacotherapy.

Keywords: antibiotics, therapy, drug

P252: On the toxicology of drug delivery systems: Polyamidoamine dendrimers inhibit angiotensin II-mediated EGFR and ErbB2 transactivation

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Drug delivery nanosystems are traditionally considered biologically “inert” but recent evidence suggests that beyond improving drug delivery, they can exert biological actions of their own. However, little is known of the effects of drug delivery polymer nanostructures on important signal transduction pathways. The effects of naked polyamidoamine (PAMAM) dendrimer delivery systems on Renin-Angiotensin-System (RAS) signaling via Angiotensin (Ang) II-mediated transactivation of the epidermal growth factor receptor (EGFR) and the closely related family member, ErbB2 (HER2) were investigated. In primary aortic vascular smooth muscle cells, a cationic generation (G) 5 PAMAM dendrimer dose- and time-dependently inhibited Ang II-mediated transactivation of EGFR and ErbB2 as well as their downstream signaling via extracellular-regulated kinase 1/2 (ERK1/2). Inhibition even occurred at non-cytotoxic concentrations, at short (1h) exposure times and was dependent on dendrimer generation (G7>G6>G5>G4) and surface group chemistry (amino- > carboxyl- > hydroxyl-). Mechanistically, cationic G5 PAMAM dendrimer inhibited Ang II-mediated transactivation of EGFR and ErbB2 via inhibition of the non-receptor tyrosine kinase, Src. This novel, early-onset, intrinsic biological action of PAMAM dendrimers as inhibitors of Ang II//Src/EGFR-ErbB2/ERK1/2 signaling pathway could have important toxicological and pharmacological implications in their clinical applications in nanomedicine.

Keywords: PAMAM, biological activity, EGFR, toxicity, angiotensin II

P253: Effect of hypericum perforatum extract on gentamicin induced apoptosis in kidney

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Hypericum perforatum, which have various names locally such as "sari kantaron, kanotu, kilicotu, mayasilotu, yaraotu", is a plant which blooms between july and september at farms, borders of roads and woods, top of hills and grasslands, and is a regulator of apoptotic mediators as shown in various studies. Also, it is known that gentamicin activates apoptotic mediators and causes necrosis in the kidney. Due to this reason, we planned a study to examine the protective effects of Hypericum perforatum on nephrotoxicity caused by gentamicin. For this purpose, 100 mg/kg gentamicin and 70 mg/kg Hypericum perforatum extract were administered to mice for 9 days and kaspase-3, bax ve bcl-2 protein in their kidneys were analyzed by using ELISA method. While, gentamicin administration increased the expression of kaspase-3, bax, it decreased the expression of bcl-2. Hypericum perforatum administration to mice, that were administered gentamicin previously, decreased the rate of kaspase-3, bax caused by gentamicin while it increased bcl-2. In conclusion, our study shows that gentamicin administration causes an increase of the proapoptotic mediators and a decrease of the antiapoptotic mediators while gentamicin plus hypericum perforatum extract treatment reversed the increase of proapoptotic mediators and the decrease of antiapoptotic proteins.

Keywords: gentamicin, nephrotoxicity, Hypericum perforatum, apoptosis

P254: Effect of alpha-linolenic acid on gentamicin induced apoptosis in kidney

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Flaxseed oil contains high amount of alpha-linolenic acid which is one of the fatty acids known as omega 3. Previous studies have shown the anti-oxidant and anti-inflammatory effects of this fatty acid and protected from cell damage by inhibiting apoptotic pathway. Also it is known that gentamicin activates apoptotic mediators and causes necrosis in the kidney. Due to this reason, we planned a study to examine the protective effects of alpha-linolenic acid on nephrotoxicity caused by gentamicin. For this purpose, 100 mg/kg gentamicin and 200 mg/kg alpha-linolenic acid were administered to mice for 9 days and kaspase-3, bax ve bcl-2 proteins in their kidneys were analyzed by using ELISA method. While, gentamicin administration increased the expression of kaspase-3, bax it decreased expression of bcl-2. Alpha-linolenic acid administration to mice that were administered gentamicin previously decreased the rate of kaspase-3, bax caused by gentamicin while, it increased bcl-2. In conclusion, our study shows that gentamicin administration causes an increase of proapoptotic mediators and a decrease of antiapoptotic mediators while gentamicin plus alpha-linolenic acid extract treatment reversed the increase of proapoptotic mediators and the decrease of antiapoptotic proteins.

Keywords: gentamicin, nephrotoxicity, alpha-linolenic acid, apoptosis

P255: Protective effect of hypericum perforatum extract on gentamicin induced ototoxicity

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Hypericum perforatum, which have various names locally such as St. John's worth, is a plant that blooms between july and september at farms, borders of roads and woods, top of hills and grasslands, and whose anti-inflammatory and antioxidant effects are shown in various studies. Due to this reason, we planned a study to examine the protective effects of Hypericum perforatum on ototoxicity caused by gentamicin. For this purpose, 100 mg/kg gentamicin and 70 mg/kg Hypericum perforatum extract were administered to mice for 9 days. On 9. and 10. days rotarod performance was assessed to evaluate the effect of gentamicin and Hypericum Perforatum extract on motor coordination of mice. Gentamicin treatment decreased the fall latency of mice while gentamicin together Hypericum Perforatum extract treatment increased the fall latency of mice. In conclusion, our study showed that Hypericum Perforatum extract can be useful to prevent gentamicin induced ototoxicity.

Keywords: gentamicin, ototoxicity, Hypericum perforatum, rotarod and motor coordination

P256: Uroprotective efficacy of oleuropein combined mesna in cyclophosphamide induced hemorrhagic cystitis in rats

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Cyclophosphamide (CP) is a drug used in the therapy of various types of malignancy and inflammatory diseases. Hemorrhagic cystitis is one of the serious side effects seen after treatment with CP. Oleuropein, has pharmacological effects including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, anti-atherogenic, anti-cancer activities. We investigated the possible enhanced protective effect of oleuropein combined mesna in CP induced hemorrhagic cystitis model in rats.

30 male Sprague Dawley rats were divided into of four groups. Group 1(Control) received saline single dose 0.5 ml (n=6). Group 2 (CP) received only cyclophosphamide single dose 100 mg/kg (n=8). Group 3 (CP+Mesna) received 100mg/kg CP and mesna in the dosage of 40 mg/kg was given 20 minutes before, 4 and 8 hours after administration of CP(n=8). Group 4 (CP+Mesna+Ole) received mesna in the dosage of 40 mg/kg was given 20 minutes before, 4 and 8 hours after administration of 100mg/kg CP and oleuropein (100 mg/kg/day) were administered for 2 days followed by CP (n=8). All rats were sacrificed 72 hours after the first application. Histological analysis was performed on the bladder.

CP+Mesna+Ole and CP+Mesna groups showed lower histological injury compared to CP group. In CP group, however, bladder stromal tissues showed neutrophil infiltration, hemorrhage and edema. There was no significant difference in histological appearances between the CP+Mesna+Ole group and CP+Mesna group.

This present experimental study was planned for the exhibition of the efficacy of oleuropein combined mesna in the prevention of cyclophosphamide-induced hemorrhagic cystitis. The outcomes of the study display that there was no noteworthy difference between CP+Mesna+Ole group and CP+Mesna group. The effectiveness of unaccompanied oleuropein in the prevention of cyclophosphamide-induced hemorrhagic cystitis should be investigated by a further study.

Keywords: cyclophoshamide, hemohrragic cystitis, oleuropein, mesna

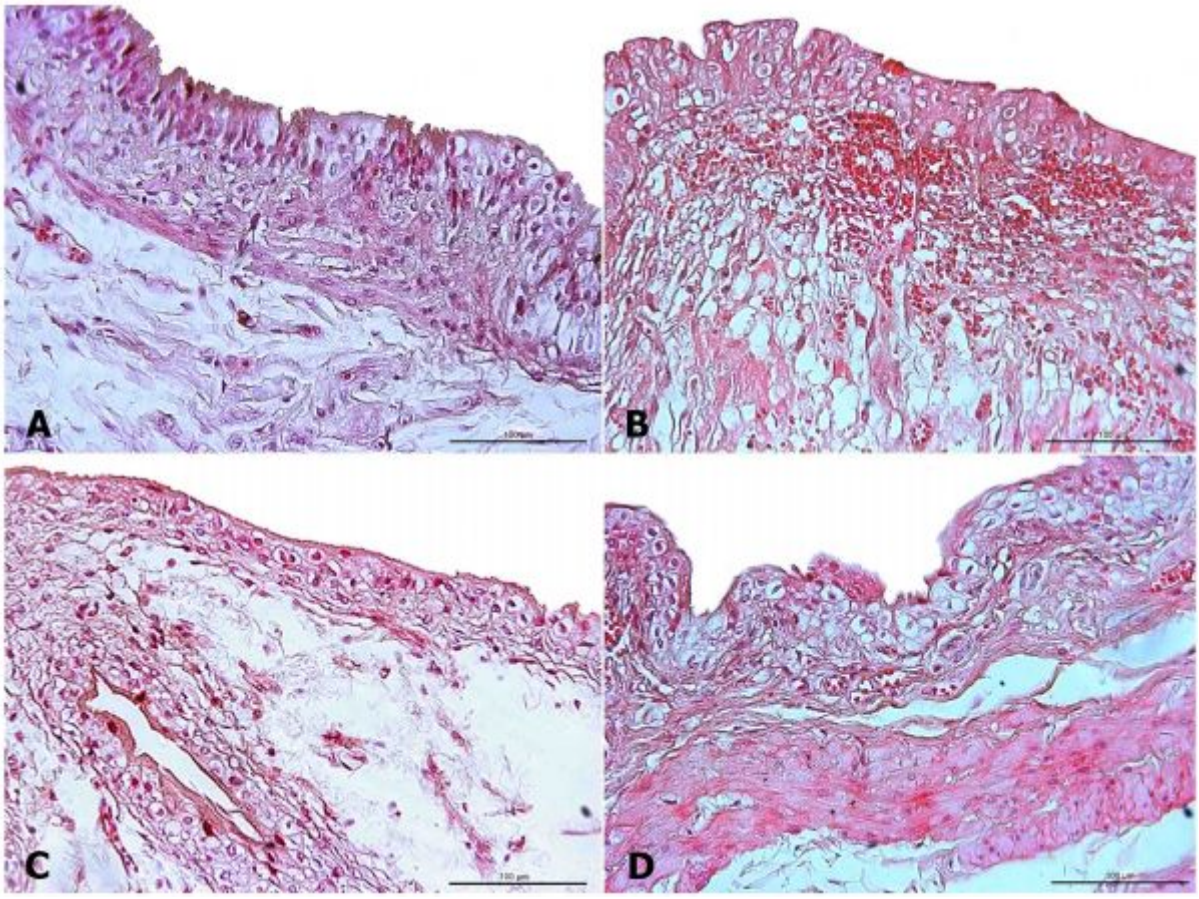

Figure 1. Illustrative figures of bladder slices treated with CP, Mesna, Mesna and Oleuropein. (A) The control group presented bladders with normal structure. (B) The CP group showed characteristic histologic findings of hemorrhagic cystitis include neutrophil infiltration, hemorrhage and edema in stromal tissue. (C-D) CP+Mesna+Ole and CP+Mesna groups showed similar histologic appearance and lower histological injury compared to CP group (H&E, Scale bar 100 µm)

P257: Rutin ameliorates chemotherapeutic induced hepatic injury in rats

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The use of chemotherapeutics is known to cause acute toxic effects in multiorgan systems. Methotrexate (Mtx) is used in the therapy of various types of malignancy and inflammatory diseases. Hepatotoxicity is one of the important problems seen after treatment with Mtx. The exact mechanisms underlying Mtx hepatotoxicity are unclear. Rutin, a bioflavonoid, has pharmacological effects including antioxidant, anti-inflammatory and anti-cancer features. It has high radical scavenging activity and antioxidant capacity that are potentially beneficial in preventing oxidative stresss related diseases. We investigated the possible protective effect of Rutin on Mtx induced hepatotoxicity in rats.

Twenty-two rats were divided into three experimental groups; Control-saline, Mtx, Mtx+Rutin. Hepatic tissue was taken for histological assessment and biochemical assays. Oxidative stress parameters malondialdehyde (MDA), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) were investigated. Liver markers aspartate aminotransferase (AST), alanine aminotransferase (ALT) were analyzed in serum.

Mtx+Rutin group showed lower histological injury compared to Mtx group, MDA and ALT levels were increased, while SOD and GSH-Px were decreased in Mtx group compared with Control-saline group. MDA and ALT levels were increased, while SOD and GSH-Px were decreased in Mtx group, compared with Mtx +Rutin group. Serum AST levels were similar among the groups.

We observed histologic changes in liver sections such as dense lymphocytic infiltration, vacuolization in hepatocytes, sinusoidal dilatation and congestion related to liver injury after Mtx treatment in our rat model. Rutin was able to improve the Mtx related histological changes in liver tissue. Rutin may be a potential adjuvant drug to reduce the hepatic side effects observed during Mtx therapy for various clinical conditions.

Keywords: chemotherapeutic, methotrexate, side effect, hepatotoxicity, rutin (Quercetin-3-rutinoside)

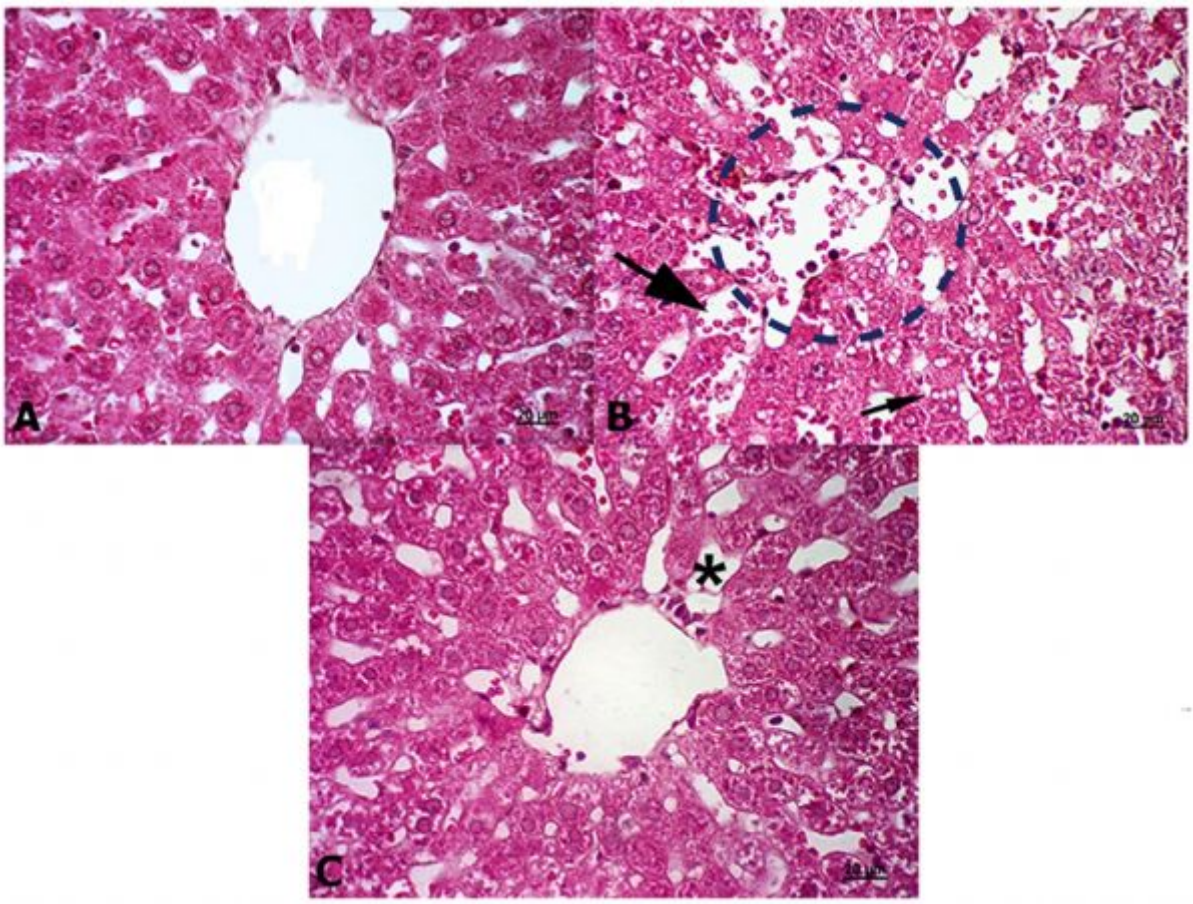

Figure 1. Hematoxylen Eosin staining of the study groups. (A) Normal histological structure of liver in Control-saline group. (B) Hepatocytes vacuolization (thin arrow), sinusoidal dilatation (thick arrow) and disruption in radial arrangement around central vein (dashed circle) in Mtx group. (C) Sinusoidal dilatation (star) and partially disruption in radial arrangement around central vein in Mtx+Rutin group (H&E, Scale bar 20 µm).

Table 1. Liver tissue malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) results in the study groups.

Oxidative stress Parameters	Control-Saline	Mtx	Mtx+Rutin
MDA (mmol/g protein)	1.16±0.53[b]	2.41±0.19[a,c]	1.45±0.63[a,b]
SOD (U/g protein)	975.93±63.64[b]	671.58±23.40[a,c]	845.78±37.68[b]
GSH-Px (U/g protein)	224.63±9.94[b,c]	164.82±2.38[a,c]	189.40±2.07[a,b]

Mtx: Methotrexate a: compared with Control-saline group. p< 0.05 b: compared with Mtx group. p< 0.05 c: compared with Mtx+Rutin group. p< 0.05

P258: Effect of CYP3A5 and 3A418B on cyclosporine levels on Egyptian renal transplanted patients

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CYP3A contribute to the low systemic bioavailability (20–30%) of Cyclosporine (CsA) after oral administration, it may contribute to interindividual variation of CsA

Ninety Egyptian populations (48 control, 42 recent renal transplant patient) were genotyped for CYP3A4 18B (rs28371759) and CYP3A5 (rs776746) by Restriction Fragment Length polymorphism (RFLP). CsA blood concentrations (ng/ml) trough (C0) and 2 hours (C2) were measured on day 7, 30, and 90 after transplantation.

Population allele's frequencies were summarized in Figure 1. Patients with 3*/3* allele had higher CsA Level compared to 1*/1* at Day-7 C0 (173.7 ±12.07, 160.5 ± 15.1, p=0.024), day-7 C2 (1049.5±92.6, 554.3±91.9, p=3*/3*=0.007), day -30 C0 (173±11.4, 115.9±23.1, p=0.033), day-90 C0 (167.3±13.9, 107.8±14.1 (p=0.038) and day -90 C2 (896.7±64, 556.3±55.5 (p=0.013) respectively.

3*/3* Subjects (non-expressor) had significantly higher CsA levels compared to both combined 1*/1* +1*/3* (expressor) at day -7 C2 (1049.5±92.6, 687.2±93.2 (p=0.009) and day -90 C2 (896.7±64.1, 635.3±75.3 (p=0.012)

As regard CYP3A41*/18B; Subject s with the 1*/1* genotype showed higher CsA levels at Day7, 30 and 90 at C0 and C2 although there is significant differences only at day -30 C0 (174.7±14.6, 105.3 ±16.5 (p=0.008),

Subject s with the 1*/18B genotype showed higher CsA levels at day7, 30 and 90 at C0 and C2 except at CsA levels at day 30 C2 although there is significant differences only at day -30 C0 (165.5 ±16.7, 105.3 ±16.5, p= 0.02).

CYP3A5 3*/3* gene polymorphism may have a pre-transplant screening prognostic value for prediction of CsA blood levels Egyptian renal transplant patients; although wide scale study may be needed to confirm this observation.

Keywords: Egyptian, transplantation, CYP3A4, CYP3A5, cyclosporine

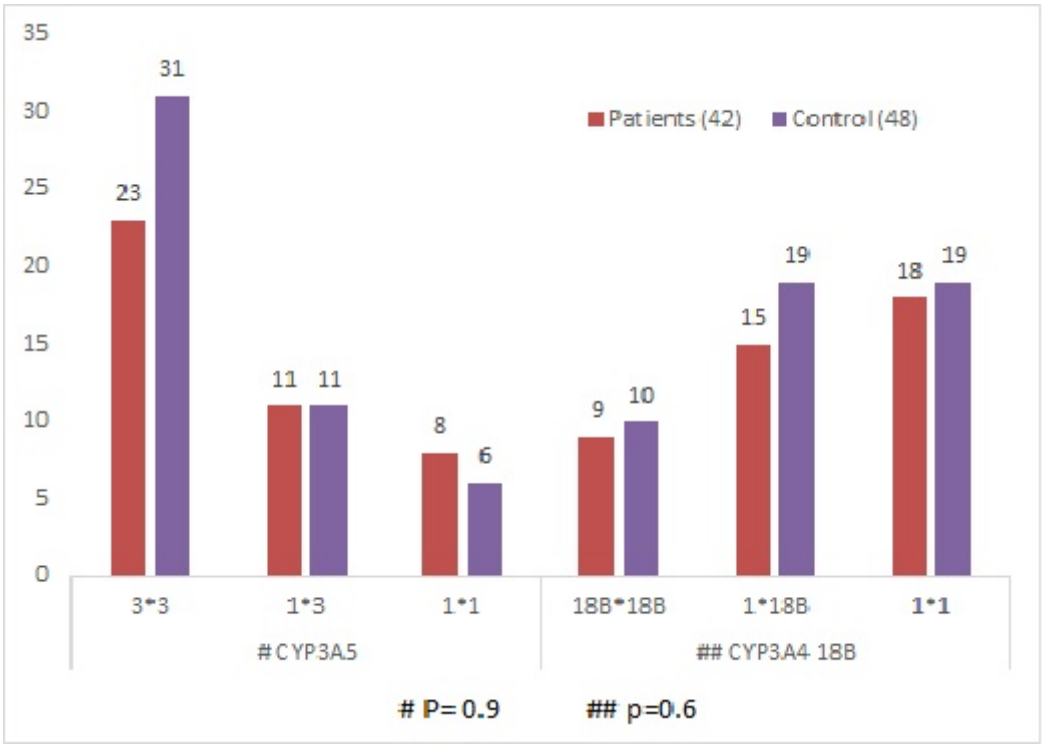

Figure 1. CYP 3A5 and CYP 3A4 gene distribution among patient and control groups.

Drug Discovery, Development and Evaluation

P259: A simple and sensitive high-performance liquid chromatographic method for the determination of mitragynine in rat plasma and tissues

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Mitragyna speciosa (Rubiaceae) is an indigenous tree growing in the Southeast Asian countries particularly in Malaysia and Thailand. Decoction of *Mitragyna speciosa* leaves (ketum drink) is regularly consumed by drug users to manage opiate withdrawal symptoms. The pharmacological effects of this plant were mainly attributed to its principal alkaloid mitragynine (MG). Several analytical methods were reported to determine the concentration of MG in biological fluids such as plasma and serum. However, these methods were costly, laborious and required the use of internal standards. Moreover, none of them was validated to determine MG in tissue samples.

The present study aimed to develop a methodology of extraction and quantification of MG in rat plasma, brain, heart, lungs, liver and kidney using HPLC-UV.

MG was extracted from rat plasma and tissue homogenates by a simple, one-step protein precipitation procedure using acetonitrile. Chromatographic separation was achieved after 12 min on Zorbax eclipse plus C8 column (250 x 4.6 mm, 5 µm). The mobile phase, consisted of 0.05% formic acid in water and acetonitrile (60:40, v/v; pH 5) was pumped at a flow rate of 1.0 mL/min. The detection was conducted at 254 nm.

The lower limits of quantification were established at 39 ng/mL in plasma and 50 ng/mL in tissue homogenates. Calibration curves were linear ($r^2 \geq 0.999$) in the range of 39-2500 ng/mL for plasma and 50-1000 ng/mL for tissue homogenates. This assay consistently yielded a good recovery (85.6-96.3%) for MG from all rat matrices. The overall data of precision and accuracy were in accordance with international guidelines for bioanalytical method validation.

A simple, selective and sensitive HPLC-UV method was developed and validated for the determination of MG in rat plasma and tissues as a first step to understand its efficiency and toxicity *in vivo*.

Keywords: *Mitragyna speciosa*, mitragynine, HPLC-UV, plasma, tissue homogenate

P260: Heijiangdan ointment relieves oxidative stresss from radiation dermatitis induced by 60Co γ-Ray in rat

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The objective of the study is to investigate the effects of Heijiangdan Ointment (HJD) on oxidative stresss in ^{60}Co γ-ray radiation-induced dermatitis in rat.

Female Wistar rat with grade 4 radiation dermatitis induced by ^{60}Co γ-rays were randomly divided into four groups (n=12 per group); the HJD-treated, recombinant human epidermal growth factor (rhEGF)-treated, Trolox-treated, and untreated groups, along with a negative control group. On the 11th and 21st days after treatment, 6 rats in each group were chosen for evaluation. The levels of SOD, MDA, and LDH were detected using spectrophotometric methods. The fibroblast mitochondria were observed by transmission electron microscopy (TEM). The expressions of fibroblast growth factor 2 (FGF-2) and transforming growth factor β1 (TGF-β1) were analyzed by western blot.

Compared with the untreated group, the levels of SOD, MDA and LDH showed significant difference ($P<0.05$). TEM analysis indicated that fibroblast mitochondria in the untreated group exhibited swelling and the cristae appeared fractured, while in the HJD group, the swelling of mitochondria was limited and the rough endoplasmic reticulum appeared more relaxed. The expressions of FGF-2 and TGF-β1 increased in the untreated group compared with the negative control group ($P<0.05$). After treatment, the expression of FGF-2, rhEGF and Trolox in the HJD group were significantly increased compared with the untreated group ($P<0.05$), or compared with the negative control group ($P<0.05$). The expression of TGF-β1 showed significant difference between untreated and negative control groups ($P<0.05$). HJD and Trolox increased the level of TGF-β1 and the difference was marked as compared with the untreated and negative control groups ($P<0.05$).

HJD relieves oxidative stresss-induced injury, increases the antioxidant activity, mitigates the fibroblast mitochondrial damage, up-regulates the expression of growth factor, and promotes mitochondrial repair in rat.

This study is supported by National Natural Science Foundation of China (No. 30973745).

Keywords: Chinese medicine, Heijiangdan ointment, radiation dermatitis

P261: Purification, biochemical characterization and proteomic identification of a fibrinogenolytic and hemorrhagic metalloproteinase from Cerastes cerastes venom

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Metalloproteinases from snake venoms (SVMPs) are often multi-domain enzymes involved in several pathological and biological effects, such as hemorrhage, inflammation, necrosis, hypotension, pro-coagulant, anticoagulant, and anti-platelet activities. Hemorrhage and tissue necrosis are common manifestations of viperid envenomation in humans. In the present study, we describe the purification and biochemical characterization of hemorrhagic metalloptroteinase, named CcMP-II, isolated from the venom of Cerastes cerastes. The proteinase was isolated by the combination of three steps chromatographic: a gel-filtration, an ion-exchange and affinity, which provided a high level of homogeneity as confirmed by reverse phase chromatography, SDS-PAGE and N-terminal amino acid sequencing. CcMP-II presents a molecular mass estimated to 35 kDa and a pI of 5.6. The amino acid sequence of CcMP-II was determined for 19 N-terminal residues (EDRHINLVSVADHRMXTKY), with high levels of homology with some other SVMPs of P-II class, which comprises together metalloproteinase and disintegrin-like domains. This proteinase displayed a fibrinogenolytic and hemorrhagic activities. The proteolytic and hemorrhagic activities of CcMP-II were inhibited by EDTA and 1,10-phenanthroline. However, these activities were not affected by aprotinine and PMSF, suggesting that CcMP-II is a zinc-dependent hemorrhagic metalloproteinase with an alpha-fibrinogenase activity. The hemorrhagic metalloproteinase CcMP-II was able to hydrolyze extracellular matrix components, such as type IV collagen and laminin, it was also able to dissolve fibrin clots. In conclusion, CcMP-II is a new PII-class metalloproteinase from Cerastes cerastes snake venom with hemorrhagic activity, that is possibly mediated by the proteolysis of blood vessel basement membrane components such as type IV collagen and laminin. Due to its capacity to promote fibrino(geno)lytic activity, CcMP-II may be an interesting tool for therapeutic approaches for the treatment of coagulation disorders.

Keywords: Cerastes cerastes, metalloproteinase, alpha-fibrinogenase, hemorrhage

P262: Screening of cytotoxic activity of selected Brazilian Northeast medicinal plants

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The large biodiversity is one of the highlights of Brazilian flora. In contrast, the therapeutic potentialities of a lot of species used in the folk medicine continuous unknown. Interestingly, several of these species are used popularly to the treatment of cancer. Herein, we investigated the cytotoxic potential of 18 plants belonging to 16 families found in Brazilian Northeast. The species studied were: Byrsonima sericea DC. (Malpighiaceae), Cupania impressinervia Acev. Rodr. var. (revoluta) Radlk (Sapindaceae), Duranta repens Linn. (Verbenaceae), Helicostylis tomentosa (Poepp. & Endl) Rusby (Moraceae), Himatanthus bracteatus (A.DC.) Woodson (Apocynaceae), Ipomoea purga (Wender.) Hayne (Convolvulaceae), Ixora coccinea Linn. (Rubiaceae), Mabea piriri Aubl. (Euphorbiaceae), Miconia minutiflora (Melastomataceae), Momordica charantia L. (Cucurbitaceae), Ocotea glomerata (Nees) Mez (Lauraceae), Ocotea longifolia Kunth (Oreodaphne opifera Mart. Nees) (Lauraceae), Pavonia fruticosa (Mill.) Fawc. & Rendle (Malvaceae), Psychotria capitata Ruiz & Pav. (Rubiaceae), Schefflera morototoni (Aubl.) Maguire, Steyerm. & Frodin (Araliaceae), Solanum paludosum Moric. (Solanaceae), Xylopia frutescens Aubl. (Annonaceae), and Zanthoxylum rhoifolium Lam. (Rutaceae). A total of 55 extracts was obteined and their cytotoxicity was tested against tumor cell lines using the Alamar Blue assay. The extracts from B. sericea, D. repens, H. bracteatus, I. purga, I. coccínea, M. piriri, O. longifolia, and P. capitata presented the most potent cytotoxic activity. Fractionation of D. repens and I. coccinea extracts led to the isolation of quercetin and the mixture of α- and β-amyrin, respectively, which quercetin showed moderate cytotoxic activity. This work contributes to understand the therapeutics potentialities of Brazilian medicinal plants.

Keywords: screening, cytotoxicity, Brazilian Northeast, medicinal plants

P263: The effects of usodeoxycholic acid on cytotoxic activity and proapoptotic potential of doxorubicin in MCF-7 cell line

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Doxorubicin (Dox) is one of the most commonly used antineoplastic agents; however, serious dose-dependent toxicity-related events limit its use. Therefore, the enhancement of Dox function along with reducing dose-dependent undesirable effects is one of the main challenges in developing novel Dox formulations. Due to an amphipathic structure, bile acids (Bas) promote transport of various substances through biological membranes, influencing their physiological and pharmacological activity. The discovery that Bas act as signaling molecules which regulate cell metabolism and fate on a systemic level, raised complexity of Bas function. The aim of this study was to determine an effect of ursodeoxycholic acid (Udca) on the cytotoxic activity and proapoptotic potential of Dox *in vitro*, in a model of human breast adenocarcinoma.

Cytotoxic activities of Dox and Udca, used as single agent or in combinations, were determined using MTT assay. The quantitative analysis of expression of genes involved in apoptosis, Bax and Bcl-2, was assessed using RT-qPCR method. Gene expression was calculated using ΔΔCt method and normalized to β-actin.

Both Dox and Udca expressed dose-dependent cytotoxic activity with IC_{50} values of 0.64 µM and 320.5 µM, respectively. Co-treatment with Dox and physiologically relevant concentration of Udca significantly increased cytotoxicity compared to Dox alone ($p<0.05$). The mRNA expression of a pro-apoptotic Bax was significantly increased ($p<0.01$) in both Dox-treated and Udca-co-treated groups compared to control, 3.22- and 2.28-fold, respectively. Bcl-2 mRNA expression was 1.42 fold higher in Dox-treated cells ($p<0.01$), whereas 1.25 fold decreased in co-treated cells ($p<0.01$). Also, Bax/Bcl-2 ratio was higher in group of co-treated cells than in Dox-treated cells.

Udca modulates cytotoxic activity of Dox and increases susceptibility of MCF-7 cells to undergo mitochondrial-mediated apoptosis, indicating that Udca may act as potential chemo-sensitizing agent.

This study is supported by the EU Horizon2020 research grant No. 690876.

Keywords: doxorubicin, ursodeoxycholic acid, apoptosis, breast cancer

P264: Evaluation of blood flow promoting effect and safety of arnica

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Muscle stiffness thought to be caused by blood circulation failure of intramuscular. So, it is important to not only reduce inflammation and kill pain but also promote blood flow. While capsaicin has been widely used as blood flow promoter of the cataplasm, it has skin irritation with redness or rash. Therefore, as a result of the search for the active ingredient, we newly found arnica. We evaluated blood flow promoting effect and safety of the cataplasm containing arnica and felbinac.

Study 1: In blood flow test, we administered transdermally (1) vehicle, (2) arnica (0.14mg/cm^2), or (3) felbinac (0.5mg/cm^2) as a cataplasm (3.0cm x 2.5cm) to the back of mice (n = 8/group). The back blood flow was measured after 30 minutes of administration. The result showed that felbinac didn't promote blood flow, whereas, arnica promoted significantly (p=0.0006). In addition, we administered transdermally (1) vehicle or (2) arnica (0.14mg/cm^2) as a cataplasm (5.0cm x 2.0cm) to the neck of 6 volunteers. The neck blood flow was measured after 30 minutes of administration. The result showed that arnica promoted significantly blood flow (p=0.0253).

Study 2: In skin irritation study, we administered transdermally the cataplasm (2.5cm x 2.5cm) containing arnica (0.14mg/cm^2) and felbinac (0.5mg/cm^2) to the back of rabbits. It was removed after 24 hours of administration, and we observed the skin after 1 hour and 24 hours of removal. The result showed that skin irritation wasn't observed.

As a result of the above, it was shown that arnica had significant blood flow effect and no skin irritation. Also in anti-inflammatory and analgesic test, cataplasm containing arnica and felbinac were confirmed to have an anti-inflammatory and analgesic effects. Therefore, transdermal administration of the combination of arnica and felbinac is effective in the treatment of muscle stiffness.

Keywords: arnica, felbinac, blood flow, skin irritation, muscle stiffness

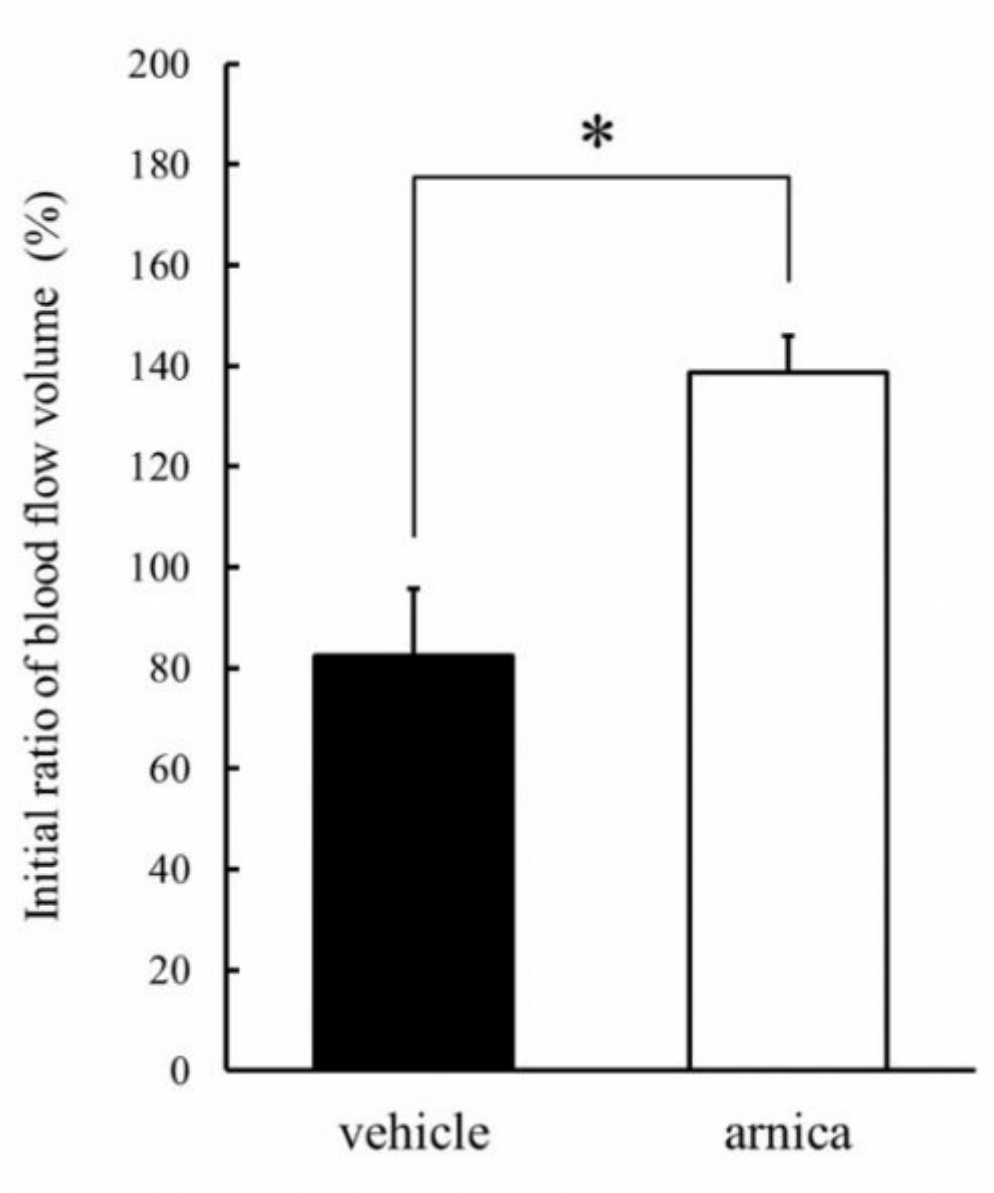

Figure 1. Initial ratio of blood flow volume after transdermal administration of the arnica cataplasm (5.0cm x 2.0cm) to the neck of volunteers. The neck blood flow was measured after 30 minutes of administration. Each value and bar represents the mean +/- SE of the results obtained in 6 volunteers. *: $P < 0.05$, significant difference from the value in the vehicle (Student t-test).

P265: Evaluation of vanadium pentoxite cytotoxicity on A549 cell line with a novel real time method: Xcelligence (RTCA)

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Vanadium pentoxide (V_2O_5) has toxic effects on human and animals. Vanadium pentoxide decreases the cell viability on A 549 cell line for 48 hours treatment. Vanadium exposure time is very important for its toxicity so our purpose is to investigate vanadium pentoxide on A549 and also normal human fibroblast cells different doses depend on real time.

Cytotoxic effect of V_2O_5 was monitored with Xcelligence Real-Time Cell Analyser (RTCA) as described by Uran et al (2010), with slight modifications. Optimal seeding concentration of A549 cells were determined in and cell proliferation, attachment and spreading were monitored every 15 min via the impedance of E-plate wells. 12.500 cells/well were seeded in the E-plate and approximately 24h post-seeding when the cells were in the log growth phase, we treated cells with V2O5 (200 µM,100 µM, 50 µM, 25 µM) and DMSO (1%) control and curcumin as a positive control (100 µM) replicated 4-times and the experiments were run for 75 hours.

The RTCA software performs cell index via the well impedance and calculates logarithmic half maximum effect of concentration

[log (IC_{50})] values at a given time point based on log concentration producing 50% reduction of cell index (CI) value relative to the control CI value (100%).

Statistical analysis were performed using GraphPad Prism software version 5.04 (GraphPad Software Inc., USA). One way ANOVA followed by Bonferrroni post-hoc test (95% confidence intervals).

After treatment of cells approximately 12 hours V_2O_5 decreased the cell index comparing to control in a dose dependent manner on A549 cells. Curcumin decreased fibroblast and A 549 cell viability at 100 µM concentration.

Cytotoxic effect of V_2O_5 on A549 cell line is around 6th hour until 13^{th} hours exposure and the most effective dose range can be limited 50-150 µM for further studies.

Keywords: vanadium pentoxide, lung cancer, xCELLigence

P266: Antifungal activity of juglone encapsulated PLGA nanoparticles

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Juglone (5-hydroxy-1,4-naphthoquinone) is an allelochemical which is obtained from walnut (*Juglans sp.*) and have pharmacological properties such as antimicrobial, antifungal, antiviral, anti-cancer, antitumor etc. It is used against various diseases as an alternative medicine. However, its poor water solubility because of its hydrophobic structure and its toxic effect limits the use of juglone in biological systems. In recent years; with nanoparticulate systems that are used in nanotechnology, biotechnology and medicine, it is aimed to increase biocompatibility and bioavailability of suchlike chemicals. Furthermore; through sustained release provided by nanoparticulate systems, long time effect with use of less active substance is possible. To achieve the use of nanoparticulate systems in living organisms; polymeric nanoparticles that are biodegradable and biocompatible, and reach easily to target area due to their nanosized structures, are frequently preferred. Especially nanoparticles that are synthesized by loading antimicrobial agents into PLGA (poly-D, L-lactic-co-glycolic acid) increases biocompatibility, therapeutical index and pharmacokinetic properties when compared to free form.

In this research, nanoparticulate systems were synthesized by encapsulating juglone into PLGA and then biological activity of nanoparticles was evaluated comparatively with free form of juglone. Juglone loaded PLGA nanoparticles that were prepared by single emulsion method and equivalent amount of free juglone according to the encapsulation efficiency were used. Nanoparticle and free juglone solutions, *Fusarium spp.* cells and top agar were mixed in separate tubes for each concentration (125 µg, 62.5 µg, 31.25 µg) and poured onto 2% sabouraud glucose agar plates. After 5 days incubation at room temperature, MIC (minimum inhibitory concentration) values were determined by comparing cell growth visually. MIC of nanoparticles was found as 31.25 µg, whereas for free juglone it was 62.5 µg. According to the results, juglone nanoparticles were increased antifungal effect when compared to free form.

This study is supported by TUBITAK. Project No: 114Z093.

Keywords: juglone, nanoparticle, PLGA, antifungal

P267: Bioflavonoid encapsulated PLGA nanoparticles against *Listeria monocytogenes*

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Flavonoids are synthesized from phenylalanine and ubiquitous in photosynthesizing cells. Besides giving color to flower petals; they are also found in seeds, stems, fruits, vegetables and propolis and they play role in plant growth regulation. Ever since ancient times, it is known that bioflavonoids have some useful properties such as anti-inflammatory, antiallergic, antioxidant and antimicrobial activities. Hence, they are used in many herbal preparations especially against infections.

Because of emerging antimicrobial resistance which is a major threat to public health, it is required to develop new therapeutic strategies and the use of bioflavonoids is one of them. Yet, some properties of flavonoids such as poor water solubility and instability limit their applications. In recent years; the use of nanoparticle delivery systems to solve these problems is noteworthy. Especially polymeric nanoparticles such as PLGA (poly-D, L-lactic-co-glycolic acid) provides biocompatibility, biodegradability and sustained release and in this way, they increase the activity of antimicrobial agents when compared to free forms.

In this study, quercetin as bioflavonoid was loaded into PLGA (drug/polymer ratio = 50/100) and then its antimicrobial activity against *Listeria monocytogenes* was evaluated comparing with free quercetin by performing broth dilution and time kill methods. According to the results, reduction percentage reached to 96.48% when compared to free quercetin after first day. Moreover, MIC (minimum inhibitory concentration) values were found as 100 µg/ml and 200 µg/ml for nanoparticles and free form respectively.

This study is supported by Yildiz Technical University Scientific Research Projects Coordination Department. Project No: 2015-01-07-KAP02.

Keywords: bioflavonoid, nanoparticle, PLGA, antimicrobial

P268: Hepatoprotective Effects of *Scorzonera* L. Species on Carbon Tetrachloride-induced Liver Toxicity in Rats

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The aim of this study is to evaluate hepatoprotective effect of *Scorzonera* L. metanol:water extract in rat model of carbon tetrachloride (CCl_4) induced liver toxicity.

Thirty five Sprague Dawley rats were divided seven equal groups.
Group 1: Control (isotonic saline solution, ISS), 0.1 mL,
Group 2: Carbon tetrachloride (CCl_4) (0.8 mL/kg),
Group 3: *Scorzonera latifolia* (100 mg/kg) + CCl_4 (0.8 mL/kg),
Group 4: *Scorzonera paraiflora* (100 mg/kg) + CCl_4 (0.8 mL/kg),
Group 5: *Scorzonera mollis* (100 mg/kg) + CCl_4 (0.8 mL/kg).
Group 6: *Scorzonera tomentosa* (100 mg/kg) + CCl_4 (0.8 mL/kg).
Group 7: *Scorzonera cana var. jacquiniana* (100 mg/kg) + CCl_4 (0.8 mL/kg).

Treatment groups were allocated for 2 days with CCl_4 and for 5 days with plant extract via intraperitoneally. On the sixth day, animals were sacrificed, their blood and liver tissues were collected. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured from blood serum. Liver tissues were stained with hematoxylin-eosin and they were evaluated histopathologically.
There were no significant differences in AST levels in blood serum of groups. Also in terms of ALT levels, treatment groups had no differences or increased enzyme levels with CCl_4 groups. According to the histopathological results, treatment groups were significantly less damaged.

This study can provide evidence that *Scorzonera* L. methanol:water extract has significant hepatoprotective effect on carbon tetrachloride induced liver toxicity.

Keywords: *Scorzonera* L. species, hepatoprotective effect, CCl_4

P269: Real-time xCELLigence cell analysis of RL95-2 human endometrial adenocarcinoma cells and the cytotoxicity of alpha-chaconine and alpha-solanine compunds on RL95-2 cells

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In this study, to investigate continuous monitoring of human endometrial adenocarcinoma RL95-2 cancer cells with different cell number using xCELLigence system and cytotoxicity activity of α-chaconine and α-solanine on RL95-2 cells with Sulphorodamine B (SRB) assay are aimed.

Xcelligence is a device that calculates cell index based on current alterations and analyzing cells in real time.

RL95-2 cells were suspended in medium and adjusted to 40.000, 20.000, 10.000, 5000, 2500 and 1250 cells/well of the E-plate. After seeding 100µL of the cell suspensions in E-plate 96 wells, RL95-2 cells were monitored every 15min for a period of 120 hours with the xCELLigence system.

SRB assay is for the toxicity screening of compounds to adherent cells in a 96-well plate format.

Cytotoxicity of α-chaconine and α-solanine on RL95-2 cells was performed with SRB assay.

Statistical analysis were performed using GraphPad Prism software version 5.04 (GraphPad Software Inc., USA). One way ANOVA followed by Bonferrroni post-hoc test (95% confidence intervals).

According to Xcelligence profiling it is determined that the optimal cell number is 20.000 cells/well for cell proliferation and viability measurements. Half inihibitory concentrations (IC_{50}) of alpha-chaconine and alpha-solanine on RL95-2 cells were determined with SRB assay and calculated as 5 µM and 30 µM, respectively. Futhermore, alpha-chaconine and alpha-solanine revealed dose dependent cytotoxic effect on RL95-2 cells.

In this study, profiled RL95-2 cells with real-time cell analysis method, determined IC_{50} values of α-chaconine and α-solanine on RL95-2 cells for the first time and it has been found valuable to further investigation.

Keywords: RL95-2, alpha-chaconine, alpha-solanine, xCELLigence, endometrium cancer

P270: A novel naphthoquinone derivative inhibits viability, blocks cell cycle progression and induces apoptosis in human chronic myelogenous leukemia cells

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Human Chronic Myelogenous Leukemia (CML) is a hematological stem cell disorder characterized by excessive proliferation of cells of the myeloid lineage associated with activation of Bcr-Abl-Stat5 oncogenic pathway. Direct Bcr-Abl inhibitors are initially very successful for the treatment of CML but over time many patients develop drug resistance. A number of naphthoquinone (NPQ) derivatives have been found to possess significant pharmacological effects associated with antitumor activities.

Evaluate a novel NPQ derivative with possible therapeutic properties for Bcr-Abl- and Stat5-related malignancies.

Effects of CM363, a novel NPQ derivative, were evaluated on CML-derived K562 cells. Mitochondrial metabolization of the tetrazolium salt 3-(4,5-methyltiazol-2yl-)-2,5diphenyl-tetrazolium bromide] (MTT) was used as indicator of cell viability. Whole or cytosolic (Cyt-C) lysis extract were separated by SDS-PAGE and transfered onto nitrocellulose membranes for immunoblotting to detect proteins of interest. K562 cells were stained with Hoechst 33258 to evaluate nuclear chromatin condensation. Fluorescent microscopy and flow cytometric analysis of PI-stained nuclei were used to evaluate cell cycle and apoptosis.

CM363 revealed significant antitumor activity with IC_{50} of 0.7 ± 0.5 µM, with higher efficacy than on other hematological or non-hematological cancer cells. Constitutive activation of pTyr694-Stat5 and pTyr177-Bcrl-Abl, critical for K562 cell survival, were significantly inhibited after exposure to 1-5 µM CM363 for 6h. CM363 exhibited a time-dependent cell cycle arrest and apoptosis on K562 cells. CM363 caused a reduction of K562 cells in G0/G1 and G2/M phases. Reduced viability of K562 cells was associated with a time-dependent increase of annexin V-positive cells and increased number of apoptotic nuclei. CM363 caused a time dependent release of cytochrome C from mitochondria and induction of cleavage of caspase-3, -9, and PARP.

These results indicate that CM363 is a potential therapeutic agent for hematologic malignancies dependent on Stat5 signaling triggering the mitochondrial apoptotic pathway.

Keywords: naphthoquinone-based compounds, oncology, leukemia

P271: Hepatoprotective effect of Opuntia robusta and Opuntia streptacantha fruits against acetaminophen-induced acute liver damage

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Acetaminophen (APAP) hepatotoxicity is the leading cause of drug-induced liver failure. The aim of this study was to analyze the antioxidant activity in vitro and the hepatoprotective effect of the fruits of Opuntia robusta and Opuntia streptacantha, against acetaminophen toxicity.

Fruits were selected from Aguascalientes, Mexico and extracted. A single dose of the extracts (800 mg/kg/day, orally) was given to male Wistar rats during five days, before intoxication with APAP (500 mg/kg). Blood and hepatic tissue samples were collected after 5 hrs post-intoxication for biochemical tests and 24 hrs for histology. Hepatocytes were isolated from normal Wistar rats. Hepatocyte cultures were exposed to 20 mmol/L APAP to induce necrosis or the toxic bile acid glycochenodeoxycholic acid (GCDCA) at 50 umol/L to induce apoptotis. Necrosis was assessed by LDH (lactate dehydrogenase) leakage into medium, apoptosis was determined using caspase-3 activity assay.

In vivo: APAP significantly increased levels of hepatic transaminases (ALT 217%, ALT 402% of control group) and decreased levels of reduced glutathione (GSH) (11% of control group). Histopathological examination confirmed that APAP induced cytoplasmic vacuolation of hepatocytes and reduced glycogen content. The Opuntia extracts reduced liver damage (ALT 39% and AST 56% of APAP group), improved histology, restored glycogen and GSH content.

In vitro: The extracts used as a prophylactic and even up to 8 hours after intoxication with APAP had the capacity to reduce the leakage of LDH to 40% compared to APAP group. In addition, the extracts reduced GCDCA-induced apoptotic hepatotoxicity.

The extracts contained high levels of bioactive compounds and showed significant anti-oxidant activity.

Opuntia extracts might scavenge free radicals generated by APAP metabolism (N-acetyl-p-benzoquinoneimine, NAPQI) and protect prophylactically against APAP-induced necrotic and GCDCA-induced apoptotic cell death. Hence, we suggest that these extracts can be used in oxidative stresss-induced acute liver failure.

Keywords: acetaminophen, acute liver failure, antioxidant, Opuntia extracts, oxidative stresss

Biopharmaceuticals and Biosimilars

P272: Biochemical composition, antioxidant, analgesic and anti-inflammatory activities of *Opuntia microdasys* cladodes

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Opuntia sp. has been traditionally used to reduce inflammation. This plant possesses a varied composition of phytochemicals.Accordingly, this research work was conducted to characterize the cladode of *Opuntia microdasys*regarding their chemical composition and to evaluate the analgesic and anti-inflammatory activities.

Analysis of cladode showed their wealth in anthocyanins (2.30± 0.58 mg / g DM), dietary fiber (60 ± 1 g /100 g DW) and protein (0.34 ± 0.02 % FW) with lower levels of β-carotene (0.189 ± 0.89 mg / g DM) and lycopene (0.16± 0.005 mg /g DM). Total polyphenols contents from cladodes were 29.30 ± 1.97 mg of AGE / g of extract, flavonoids 19.97 ± 1.92 mg CE / g of extract and tannins 4.98± 0.87 mg CE/ g of extract using ethanol as solvent. Ethanolic extract of *Opuntia microdasys* cladodes was also investigated for its free radical scavenging capacity determined by ABTS and DPPH assays. The cladode extract showed interesting free radical scavenging properties: (88.97 ±1.07% inhibition of DPPH., and 80.10 ± 0.78% inhibition of ABTS.+).The anti-inflammatory activity of cladode ethanolic extract was studied by using the model of carragenin-induced oedema. The ethanolic extract of Opuntia microdasys cladodes was observed to have exerted significant effect at the early stage of inflammation (1–2 h) indicating effect probably on histamine, serotonin and kinnins that are involved in the early stage of carragenin induced oedema. On the analgesic property acetic acid induce writhing was significantly reduced (65.30%) compared to control group.

In summary, this paper provides further information on the chemical composition of the *Opuntia microdasys* cladodes, which forms the basis for its pharmacological properties.

Keywords: *Opuntia microdasys*, anti-inflammatory activity, analgesic property

P273: Biochemical composition, analgesic and anti-inflammatory activities of *Opuntia microdasys* seeds

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There are nearly 1500 species of cactus belonging to the Opuntia genus (Cactaceae), which are mainly distributed in Africa and Mediterranean countries. The seeds of Opuntia spp. contain high amounts of polyunsaturated fatty acids, especially linoleic (C18:2n6) and linolenic (C18:3n3) acids. Accordingly, this research work was conducted to characterize the seeds of Opuntia microdasys, focusing on their chemical composition and their analgesic and anti-inflammatory activities. Opuntia seeds showed high contents in tocopherols, with γ-tocopherol standing out as the isoform quantified in highest amount (7.4 mg/100 g DW). The detected quantities are similar to those observed in O. joconostle and O. matudae. Regarding fatty acids composition, linoleic acid was the major fatty acid (71%) followed by palmitic acid (C16:0, 13.9%) and oleic acid (C18:1n9 10.0%). These findings might be understood as an incentive to use the seeds of O. microdasys in different applications, since polyunsaturated fatty acids are documented as having a wide variety of health-promoting properties.

The anti-inflammatory activity was studied using the model of carrageenan induced edema. The oil obtained from the seeds of O. microdasys exerted a significant effect at the early stages of inflammation (1-2 h), being probably correlated to histamine, serotonin and kinins, which are involved in the initial stages of carrageen an induced edema. Concerning the analgesic properties, acetic acid induced writhing was significantly reduced (80 %) when compared to the control group.

In conclusion, the results of this study demonstrated that O. microdasys possesses interesting anti-inflammatory and analgesic properties, which might be related to some of the profiled chemical components. Nevertheless, further investigation is needed, particularly in elucidating the cellular mechanisms and establishing structural-activity relationships, aiming to standardize the active ingredients formulations in any derived products.

Keywords: *Opuntia microdasys*, tocopherol, anti-inflammatory activity, analgesic property

P274: Investigation of norharmane presence in *Chroococcus minutus* and *Geitlerinema carotinosum*

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The purpose of this study is to investigate the presence of Norharmane metabolite, which has a pharmaceutical impact as a anticancer agent on some cyanobacteria
Cyanobacteria obtained from the water sample taken out of the Tokat Yeşilırmak River were mechanically isolated under an inverted microscope. *Chroococcus minutus* (Kützing) Nageli and *Geitlerinema carotinosum (*Geitler) Anagnostidis species were morphologically defined from the identification of species guides. Presence of metabolite in the species purely cultivated and reproduced in the BG-11 medium was conducted by HPLC analysis. Norharmane metabolite was fragmented in the C18 reverse phase under 40 0C temperature and flow rate of 1 ml/min.

Amounts of Norharmane metabolite generated by each species during the logarithmic increase phase (µg/g) were calculated according to the Gauss method by means of drawing a calibration curve over the absorbance value in the 247 nm wave length of the standard. Based on the results, the amount of Norharmane was found to be 8,816 µg/g and 1,055 µg/g in *Chroococcus minutus* and in *Geitlerinema carotinosum*, respectively.

Presence of aromatic compound Norharmane was significantly determined in two species of cyanobacteria in this study. This condition signifies that cyanobacteria can be models for containing very useful pharmaceutical raw materials.

Keywords: norharmane, HPLC, cyanobacteria

P275: Antiproliferative activity of *Cladophora fracta* (Müller ex Vahl) Kützing on HeLa and C6 cells lines

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The objective of this study is to determine antiproliferative activities of various extracts of *Cladaophora fracta*.

Cladophora fracta was collected and isolated from Tokat GOU campus pool then was cultured. Antiproliferative effects of methanol extract and hexane extract were executed on HeLa (human cervix carcinoma) and C6 (rat brain tumor) cell lines using BrdU Cell Proliferation ELISA kit.

The hexane extract revealed the good activity on HeLa cell lines at high concentrations. However, methanol extract exhibited excellent activity on HeLa cell lines at all concentrations. The hexane extract showed the same activity with 5-FU but methanol extract exhibited the better activity than 5-FU at all concentrations (Fig.1) The methanol extract revealed the excellent antiproliferative activity on both HeLa and C6 cell lines.

Cladophora fracta is a potential source of biologically active compounds that may be useful as an anticancer agent.

Keywords: *Cladophora fracta*, antiproliferative activity, HeLa, C6

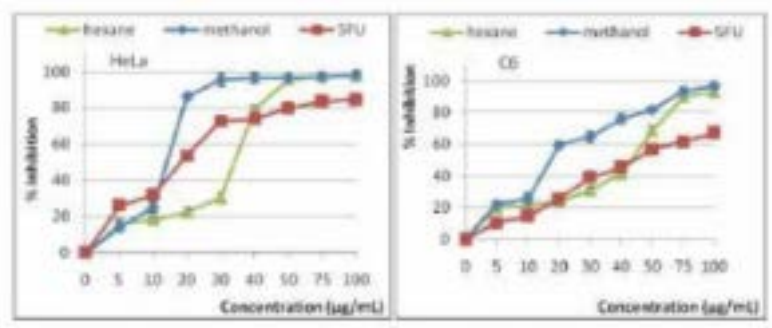

Figure 1. Antiproliferative effect of hexane and methanol extract of Cladophora fracta on HeLa and C6 cell lines

P276: Osteoprotective properties of RNA-containing drug Osteochondrin S on osteoporotic rat model

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The aim of this study is to evaluate osteoprotective properties of Osteochondrin S on the osteoporotic rat model.

Experiments were conducted on 18 outbred female rats weighing 200-240 g. In order to develop osteoporosis in rats, bilateral ovariectomy was used. Animals were divided into 3 groups: group 1 - seven days after ovariectomy animals received Osteochondrin S drug intraperitoneally in an amount of 0.2 ml per rat 3 times a week for 12 weeks; group 2 (control) received simultaneously an equivolume of sterile saline solution in a similar way; group 3 - intact animals (without ovariectomy). To evaluate the osteoprotective properties of Osteochondrin S, histological examination of the femur and bone density was examined after 3 months.

Bone tissue of intact animals shows compact bone (cortical layer) and trabecular (spongy) bone. The morphological structure of the bone tissue represents major cellular elements (osteoblasts, osteocytes, osteoclasts), matrix, mineral components. After ovariectomy the development of clear signs of osteoporotic bone changes occurred, characterized by thinning of the trabecular bone, decreasing its mechanical strength and appearance of microfractures. The compact layer is thinned; most of it is spongy with fat vacuoles and cellular components. In the group of animals treated with Osteochondrin S, the ratio of compact and trabecular layers is normal. Also there is normal ratio between bone tissue and spongy substance. Bone tissue consists of a basic cell elements, matrix and mineral components.

The results obtained in the course of determining bone density, fully confirm the data of morphological studies. In ovariectomized animals bone density is lower compared to intact rats. In the group of animals treated Osteochondrin S, bone density is higher than in the control, and reaches values in intact animals.

The morphology and densitometry data confirm that Osteochondrin S has expressed osteoprotective activity, preventing the destruction of the bone tissue.

Keywords: osteoporosis, RNA-containing substances, oseochondrin S

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