## Introduction

Pharmaceutical science is directly related to the study of the laws of biochemical processes and the creation of new methods for studying the functioning of the body, as well as processes arising under the influence of drugs on it.

All medicines are divided depending on their relation to the human body into autobiogenic (natural) and xenobiotics (heterogeneous for the body, synthetic). Natural ones are natural products of the body and directly participate in the implementation of biochemical processes. Normally, xenobiotics are absent in the human body or are present in very small quantities [2]. They are synthetic compounds or substances that are not used in the body as a basis for energy and structural parts of cells and tissues. Many medicinal substances are classified as xenobiotics, since most medicinal compounds of synthetic, plant and mineral origin are foreign to the body.

Our course will consist of three modules.

The objectives of the first module are to study the biochemistry of biogenic drugs.

The objectives of the second module are to study drug metabolism.

The objectives of the third module are to apply bioinformatics methods to study drug metabolism.

## Module 1

### Lecture 1. Biophysical and Biochemical Characteristics of Therapeutic Proteins

Protein-based therapeutics are highly successful in clinic and currently enjoy unprecedented recognition of their potential.

More than 100 genuine and similar number of modified therapeutic proteins are approved for clinical use.

Based on their pharmacological activity, they can be divided into five groups:

- (a) replacing a protein that is deficient or abnormal;
- (b) augmenting an existing pathway;
- (c) providing a novel function or activity;
- (d) interfering with a molecule or organism;
- (e) delivering other compounds or proteins, such as a radionuclide, cytotoxic drug, or effector proteins.

Therapeutic proteins can also be grouped based on their molecular types that include: antibody-based drugs, Fc fusion proteins, anticoagulants, blood factors, bone morphogenetic proteins, engineered protein scaffolds, enzymes, growth factors, hormones, interferons, interleukins, thrombolytics.

PROTEIN STRUCTURE

Post-translational Modifications

PROTEIN FOLDING

### Lecture 2. Biologically active peptides

Bioactive peptides are specific protein fragments which, above and beyond their nutritional capabilities, have a positive impact on the body’s function or condition which may ultimately influence health.

Effects: Antimicrobial, blood pressure-lowering (ACE inhibitory), cholesterol-lowering ability, antithrombotic, antioxidant activities, opioid activities, enhancement of mineral absorption and/or bioavailability, cytomodulatory, immunomodulatory effects, antiobesity, anti-genotoxic activity.

Bioactive peptides characteristics

Suitability of bioactive peptides as pharmaceutical ingredients

Production of bioactive peptides

Functional properties of bioactive peptides

The search of bioactive peptides through microbial fermentation will remain a promising and a cheap strategy for generating bioactive peptides in foods as generally regarded as safe microbial proteolytic systems yield several peptides of diverse potentials during fermentation.

In the near future, development and use of genetically improved strains will become important as they would release large amounts of proteolytic enzymes to hydrolyze food proteins.

Also, pure food derived bioactive peptides would soon be abundant on the market and sold as nutraceuticals.

Such peptides could be regulated as drugs since they would be well characterized and their properties and mechanisms of action established.

**Lecture 3. Oligonucleotides**

Oligonucleotide therapeutics - a general term for state-of-the-art, molecular-target agents that employ chemically synthesized oligonucleotides with a single-stranded deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) backbone with potential specificity. Antisense oligonucleotides (ASOs), small interfering RNA (siRNA), microRNAs (miRNAs), aptamers, and decoys.

History of Oligonucleotide Therapeutics: From Pioneer Works to Current Diversity

Antisense Oligonucleotides

Ribozymes and DNAzymes

Triple Helix-Forming Oligonucleotides

SiRNA

MicroRNA

Decoys

Aptamers

**Lecture 4. Antibiotics**

Historical development

The most widely accepted definition of an ‘antibiotic’ promulgated by the scientific jargons is — ‘a chemical substance produced by a microorgansims, that has the capacity, in low concentration, to inhibit or kill, selectively, other microorganisms’. In general, the ‘antibiotics’ are produced on a large scale by three well-known and defined methodologies: fermentation process; semi-synthetic process; synthetic process.

Antibiotic Development

PRODUCTION OF ANTIBIOTICS

Antibiotic-Microorganisms-Therapeutic Usage

The Penicillins

Streptomycin

Tetracycline

**Lecture 5. Vitamins**

Vitamins: general characteristic

The importance of vitamins

Biosynthesis

Antioxidant function

The functional role of Coenzymes

**Module 2**

## Lecture 6. An Introduction into Drug Metabolism

Metabolic reactions can lead to metabolites that differ substantially from their parent compounds in their physicochemical and pharmacological properties. They are traditionally classified into phase I and phase II reactions. Phase I reactions are carried out by a variety of metabolic enzymes (most notably the cytochrome P450 (CYP) enzyme family) that catalyze chemical modifications of small organic molecules, including oxidation, reduction, hydrolysis, and ring opening and closing. Carboxyl, hydroxyl, amino, and sulfhydryl groups are often introduced by phase I reactions, which generally result in the formation of more hydrophilic and sometimes reactive metabolites. Phase I reactions are often followed by phase II metabolism, in which usually a functional group (either existing in a molecule or generated by phase I metabolism) is conjugated with an endogenous hydrophilic compound. The most important enzymes catalyzing phase II reactions are UDP-glucuronosyltransferases (UGTs), glutathione Stransferases (GSTs), N-acetyltransferases (NATs), and sulfotransferases (SULTs).

## Lecture 7. Experimental Approaches to Study Metabolism

Drug metabolism as a multidisciplinary science was born in the first half of the 19th century, when hippuric acid was discovered in horse urine. Subsequent progress was impressive, but it remained restricted to a narrow circle of biochemists. It was only in the 1950s that drug metabolism really took off due to a convergence of factors including a) the progressive awareness among pharmaceutical scientists of the variety and significance of metabolic reactions, and the involvement of metabolites in unwanted drug effects; b) the groundbreaking studies of distinguished pioneers; c) the explosive development of analytic instrumentation; and d) the acknowledged scientific and didactic impact of a few books. Drug metabolism research has grown from a desire to understand the workings of the human body in chemical terms to a major force in the effort to develop drugs tailored to the individual.

## Lecture 8. Redox Reactions

Redox reactions (oxidations and reductions) are clearly the most important ones in drug and xenobiotic metabolism. First, the biotransformation of a xenobiotic often begins with redox reactions, and particularly reactions catalyzed by cytochromes P450 (abbreviated as CYPs). Second, a vast majority of drugs (and of other xenobiotics, as far as this information is available) are substrates of CYPs. Although any attempt to quantify the total number of marketed drugs, drug candidates, and preclinical candidates that are substrates of human CYPs is but a ‘guess-estimate', a figure of ca. 90% is generally accepted. This percentage is certainly higher when all drug-metabolizing oxidoreductases are taken into account. The third reason for the predominance of redox reactions in drug metabolism is the large diversity of metabolites that may be produced from a single substrate. This diversity involves differences in the chemical nature of the resulting functional groups, as well as positional or stereochemical differences in the creation of a single type of functional group.

## Lecture 9. Reactions of Hydrolysis

Reactions of hydrolysis are a major focus of interest in the metabolism of drugs, prodrugs, and other xenobiotics. The metabolism of drugs and other xenobiotics is often a *biphasic process* in which the compound may first undergo a functionalization reaction ( phase-I reaction). This introduces or unveils a functional group such as a hydroxy or amino group suitable for coupling with an endogenous molecule or moiety in a second metabolic step known as a conjugation reaction (phase- II reaction). The $H_2O$ molecule, either as a reactant or a product, plays an important role in the metabolism of innumerable endogenous and exogenous compounds. Water is of course a by-product of some metabolic redox reactions, *e.g.*, reactions catalyzed by monooxygenases. A complicating characteristic of metabolic reactions of hydrolysis is the fact that they may be partly non-enzymatic. Indeed, the nucleophilicity of H2O makes it an active reagent, especially in synergy with catalysts such as H+, HO—, or a base. As a result, the metabolic hydrolysis of some labile esters such as acetates may contain a nonenzymatic component which should not be neglected, especially in prodrug design.

## Lecture 10. Reactions of Conjugation

For a reaction of conjugation to occur, a suitable functional group must be present in the substrate, which will serve as the anchoring site for an endogenous mole- cule or moiety such as CH3, sulfate, glucuronic acid, or glutathione. Conjugation reactions are thus synthetic (i.e., anabolic) reactions whose products are of modestly to markedly higher molecular weight than the corresponding substrate. As for the anchoring group, it can either be present in a xenobiotic or be created by a functionalization reaction. Reactions of conjugation act on exogenous substrates (i.e., xenobiotics [1]) as well as endogenous substrates (i.e., endobiotics). This dual functionality may create a potential for metabolic interaction between a drug and an endogenous substrate, a frequently overlooked mechanism of toxicity. Thus, there may be competitive affinity for the catalytic site of an endobiotic-metabolizing enzyme, or there may be competition for the limited supply of a cofactor.

## Lecture 11. Metabolism and Bioactivity

When it comes to drug metabolism, its consequences are of utmost relevance in fields such as drug discovery and develop- ment, clinical pharmacology and toxicology, and therapeutics. The same relevance is now recognized to xenobiotic metabolism in, e.g., agrochemistry and food chemistry, industrial hygiene, workplace safety, and environmental welfare. Presenting the pharmacological and toxicological consequences of drug and xenobiotic metabolism can be achieved by focusing on rules and general principles

## Lecture 12. Factors Affecting Drug Metabolism

Inter-individual factors are factors which differ between individuals but are constant throughout the life of an organism. This applies to factors which are based on the genetic code of an individual. We call them intra-individual factors; they include enzyme inhibition and induction due to physiological or pathophysiological conditions and to environmental influences. Genetic variations in metabolizing enzymes can lead to differences in enzyme expression levels, in substrate and product selectivity, and in activity, respectively. Carriers of variant alleles can be at risk to encounter toxic effects or therapy failure when treated with a substrate of the respective enzyme.

Focuses on the various mechanisms regulating enzyme expression and activity, starting from epigenetic and transcriptional control, and concluding with enzyme inhibition and activation on the protein level. DNA-Binding transcription factors are given particular attention, as they regulate the expression levels of many enzymes and, hence, their metabolic activity.

Discussed tissue-specific and age-related expression patterns of drug- metabolizing enzymes. Major age-dependent changes in drug metabolism occur at the very early stage of life, namely within the first months after birth. Drug-metabolizing enzymes follow distinct developmental patterns, resulting in significant differences in drug metabolism and pharmacokinetics between adult and pediatric patients.

## Lecture 13. Pharmacogenetics and Pharmacogenomics

The fields of pharmacogenetics, genomics, and drug transporters have profoundly impacted drug metabolism research by providing plausible mechanisms for interindividual variability in drug response and metabolism - related toxicity. They have provided tools with which to understand enzyme regulation, identify factors that affect drug exposure, the potential for drug – drug interactions, and species differences in drug disposition. Knowledge from these fields is being used to form the scientific basis for designing appropriate clinical studies and data interpretation, leading to the development and use of safer and more efficacious drugs.

Pharmacogenomics is a more recent term, coined to define a more holistic or global approach, in which the expression levels, regulation, functions, and interactions of multiple genes are simultaneously studied, and their effects on overall variability in drug response determined. It is sometimes used interchangeably with pharmacogenetics, despite these subtle differences. These fields are continually being integrated in various aspects of the life sciences, including drug discovery and development, with the expectations that they would lead to the development of safer drugs that can be tailored to subsets of patients based on their genetic makeup, the so - called personalized medicines.

# Module 3

## Lecture 14. Omics technologies

“Omics” technologies: genomics, transcriptomics, proteomics, metabolomics. Genomics had revealed the static sequences of genes and proteins and focus has now been shifted to their dynamic functions and interactions. Transcriptomics, proteomics and metabolomics reveal the biological function of the gene product. The aim of omic technologies - the nontargeted identification of all gene products (transcripts, proteins, and metabolites) present in a specific biological sample. Opened new avenues towards biomarker discovery, identification of signaling molecules associated with cell growth, cell death, cellular metabolism and early detection of cancer.

## Lecture 15. Software, Web Servers and Data Resources to Study Drug Metabolism

With increasing awareness and understanding of metabolism as a key factor for the safety and efficacy of drugs and chemicals in general, the development of methods for the prediction of xenobiotic metabolism has become a highly active field of research in recent years. We aim to provide an overview of software tools available to scientists working in drug metabolism research. We will discuss the scope and limitations of current approaches for (i) site of metabolism (SoM) prediction, (ii) metabolite structure prediction, and (iii) prediction of the interaction of metabolites with metabolic enzymes.