In vivo monitoring of oxygen saturation in murine carcinoma during PDT by diode laser light diffuse reflectance

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ABSTRACT

In the paper we explored the possibility of monitoring oxygenation of the tumor tissue through the registration of light diffuse reflectance. This method can also be helpful for assessment the effectiveness of PDT, defining the level of vascular damage and the degree of the tumor oxygenation. We also propose the modification of PDT procedure by using a modulated laser that enables to better maintain the necessary parameters for the PS activation and oxygen generation in irradiated tissues simultaneously allowing to reduce the light dose required for tumor treatment.

Keywords: photodynamic therapy, photosensitizer, oxygen saturation, CCD spectrometer, diffuse reflectance spectroscopy, modulated laser irradiation, tumor

1. INTRODUCTION

Photodynamic therapy (PDT) used in cancer treatment as an alternative or combined with conventional treatment of tumors[1-3]. PDT involves three key components: a photosensitizer, a light source and tissue oxygen. The combination of these three components leads to the chemical destruction of any tissues which have both selectively taken up the photosensitizer (PS) and have been locally exposed to light.

After the light exposure the concentration of molecular oxygen in the tumor rapidly drops because of its interaction with PS and singlet oxygen formation [4-8]. Restoring the concentration of molecular oxygen (and PS) in malignant tissue depends on the blood circulation in this area and requires some time during which further tumor irradiation is meaningless and leads to PS photodestruction without achieving the desired effect [9-12]. Hence, there is an urgent need to develop such schemes of tumor photoirradiation which would allow constant renewing of critical concentrations of the active PDT agents in the process of tumor elimination.

There are various methods of PDT procedure such as using a laser scanning device that enables to better maintain the necessary parameters of the PS and oxygen in irradiated tissues simultaneously allowing to reduce the light dose required for tumor treatment[13]. The method consists in tumor irradiation with a beam of low power and small cross section, which moves through the selected area with a certain delay at each point. The scanning is performed only in the tumor zones with detected fluorescence. To register tumor fluorescence we use CCD-camera, connected to a PC on Windows 7.

In the paper we propose the modification of PDT procedure by using a modulated laser. We compare the effectiveness of photodynamic therapy with the use of conventional continuous laser irradiation of tumors and modulated method. Utilizing a feedback from the treated area to guide the laser beam makes it possible to stop laser irradiation when the oxygen concentration drops and turn laser on automatically upon its accumulation in the zone of exposure[6]. Monitoring of oxygen level in the tissues and corresponding correction of irradiation will be able to increase the PDT effectiveness.

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2. MATERIALS AND METHODS

To render the modification of modulated tumor treatment method , the semiconductor laser "Lika-Khirurg M" ("Fotonika Plus", Ukraine) was applied. Tumors were irradiated in continuous regime, (wavelength $\lambda = 660$ nm, power of light P = 0,1-3 W). The device can modulate the laser radiation with different duty cycle signal, thereby adjusting intervals of the pulse pause (0,05-1s) depending on the level of molecular oxygen and FS. As a main drawback of the proposed method the increased time of the procedure should be mentioned.

To evaluate the hemoglobin oxygen saturation and relative hemoglobin concentration in tissue we used the method which is based on the diffuse reflectance of light of the red and infrared wavelength. The method is applied in the range, where the spectral difference between oxygenated and deoxygenated hemoglobin is rather high. In this method utilization of the fiber-optics probes makes possible to monitor the oxygen saturation on a surface and inside a tumors. We also applied CCD spectrometer with accessible interface, high speed and sensitivity for detection of reflected signal, which allowed real-time estimation of tumor oxygenation dynamics.

Under low oxygen saturation there is a highly significant mismatch between the absorption and scattering coefficients of the 660 nm near red and 890 nm infrared light, with the near red light being more strongly absorbed and scattered[14-15]. This absorption and scattering mismatch results of the near red and infrared light are vary in different tissues which significantly deminishes the accuracy of the arterial oxygen saturation calculation[16,17].

To determine the level of saturation in a model tumor laser diodes (LDs) with wavelengths of 635 and 990 nm were selected. These wavelengthes also allowed us to diagnose oxygen saturation during the process of primary tumor irradiation with a wavelengthes of 405 or 660 nm[18,19]. As a result, we get two spectral lines in feedback loop signals that were not overlapping. The use of LDs was justified by low power requirements of diagnostic radiation - from 1 to 0.8 mW. Radiation from LDs was focused in the distal of optical fiber which guided light to biological object. The radiation of LDs with vawelength of 635 and 990 nm was directed at the tumor. Light emmited from the LD passes through the tissue, undergo reflection and scattering, and then reaches the receiving fiber of spectrometer[20-24].

Optical fibers are made of quartz 400 microns in diameter. Transmitting optical fiber ensures radiation transmission from the laser to the object under investigation. Receiving optical fiber is responsible for receiving fluorescence from the particular region and its transmission to the spectrometer's input connector. Input connector of spectrometers in use is of SMA-905 standard (tip diameter 3,17mm). In the course of the experiment we found that the change in angle and the distance of receiving fiber of spectrometer and optical fiber transmitting laser radiation significantly influence the obtained fluorescence signal and, when the location is constantly changed, might distort the results.

Receiving and irradiating fibers were positioned at a short distance from surface of treated area (1 mm) to eliminate interferences with its optical properties. However, since the distance between the optical fiber is more than 3 mm (in our case, from 4 to 8 mm) frenel light reflection from tissues not recorded (Figure 1) [25].

The separation between the fibers (L, Figure 3) can also prevent direct (without going through the fabric) getting radiation from transmission to reception fibers.

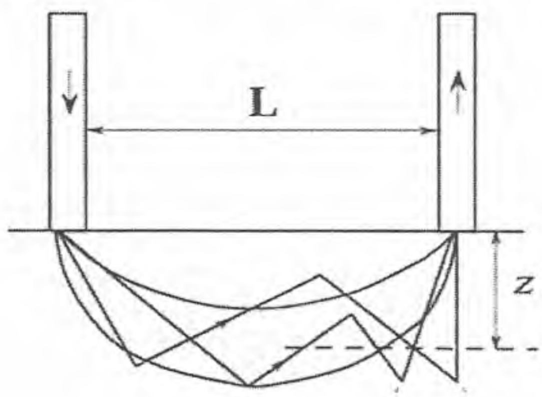

Figure 1. Scheme of the optical fibers in the object[25]

The receiving fiber-optics probes are send a light directly on the spectrometer («Ocean Optics USB 4000», USA), which was guided by computer via USB-interface.

Charge-coupled device (CCD) spectrometers were used to measure intensity of the PS fluorescence and oxygen saturation signals. monitoring of PS fluorescence level by spectrometric technique allowed us to register the dynamics of PS accumulation in the tissue.

The main component of the spectrometer is CCD matrix consisting of separate light-sensitive elements that can carry electric charge. Light falling on the line of these elements causes charge distribution that correspond by intencity to the given signal. The signal is then digitally displayed on computer monitor. Comparing to the monochromator, the device gives instantaneous response throughout the whole spectral range, so there is no need to scan the spectrum to measure intensity at certain wavelengths.

Advantages of CDD spectrometer include:

- measurement of signal intensity simultaneously in a wide wavelength range,
- the process of obtaining and analog-to-digital signal processing take place within one CCD structure,
- software and high resolution helps to further process the results,
- fiber optic connector SMA- 905, which conforms to international standards.

After a series of measurements, spectrometer Ocean Optics USB4000 (USA) was acknowledged as the best choice because of its sensitivity, wide range of wavelengths, compact design and user friendly software.

3. EXPERIMENTS WITH A TUMOR MODEL

As an animal model we used the C57Bl/6 mice bearing Lewis lung carcinoma transplanted into the animal posterior limb. One hour before the laser irradiation, photosensitizer "Fotolon" (Belmedpreparaty, Belarus) (chlorin e6) was intravenously injected into the mice at a dose of 10 mg/kg. To perform irradiation, mice were anesthetized and harmlessly fixed in warm-keeping holders with a large opening for free breathing.

4. RESULTS AND DISCUSSION

To determine whether the chosen method reflects the level of oxygen saturation in the biological tissue we studied relative degree of oxygenation and hemoglobin concentration in the tumor. The Figures 2 and 3 are experimentally measured laser diffuse reflectance spectra on wavelength 990 and 635 nm, respectively. Diagnostic laser wavelength λ = 990 nm is well absorbed by oxyhemoglobin of blood and as it is seen from Fig.2 the signal is falling after PDT. This may indicate a slow increase of oxyhemoglobin level in tissues. Photoirradiation was performed at a wavelength of 660 nm, using a laser power density PD_s= 85 mW/cm^2.

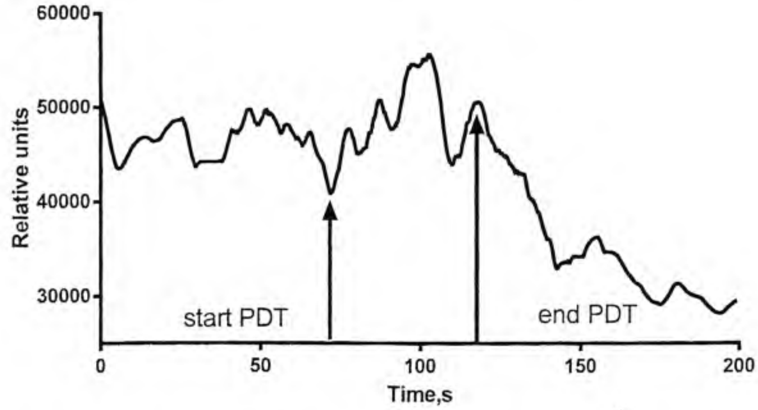

Figure 2. The dynamics of the degree of oxygenation before and after PDT, wavelength of laser irradiation 990 nm

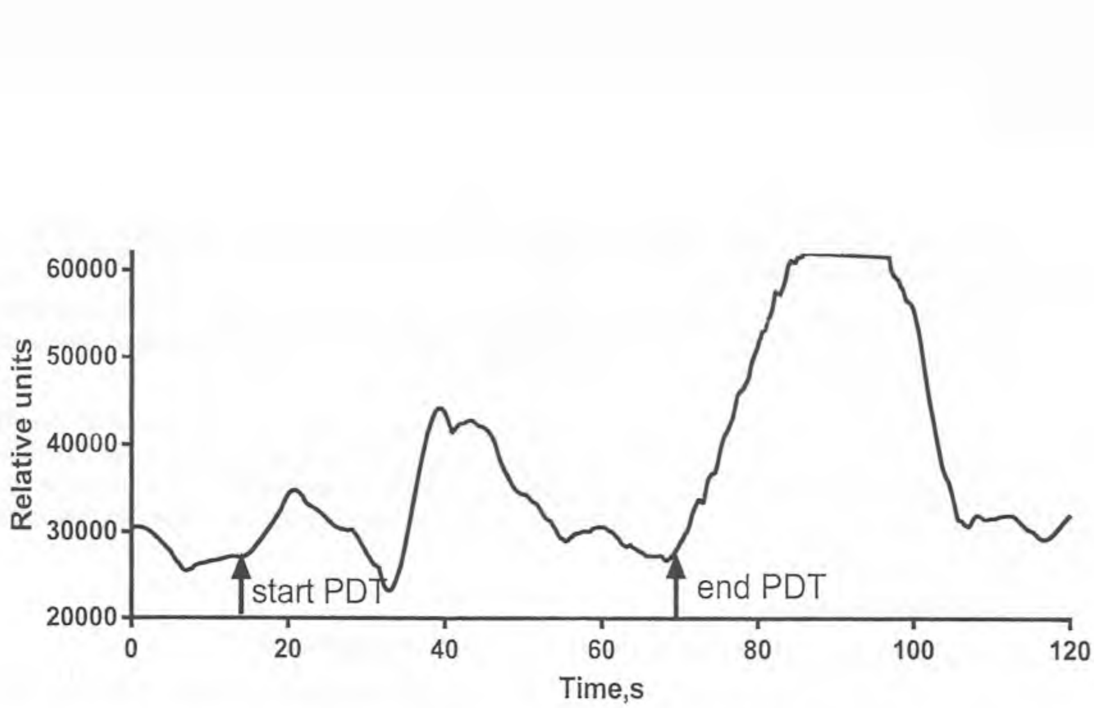

Figure 3. The dynamics of the degree of oxygenation before and after PDT, wavelength of laser irradiation 635 nm

Also, for additional monitoring may be used a laser with a wavelength of 635 nm. This wavelength has the opposite characteristics to laser of 990 nm and relatively well absorbed by hemoglobin. As It is seen t on the Fig.3, the spectral curve depends on the degree of oxygenation and increases after PDT. Photoirradiation was performed at a wavelength of 405 nm, using a laser power density $PD_s = 30$ mW/cm^2.

We conducted a number of experiments considering various angles of positioning of the receiving optical fiber of spectrometer and optical fiber that comes from the laser. 45° angle seems to give better results, but advantage is not significant at the same it would complicate the design of the system for noninvasive PDT. Having examined spectrometer's CCD design, namely the slit through which light passes, we came to the conclusion that it is inappropriate to use cord as receiving fiber. Since the size of the slit in spectrometer is constant the signal obtained by increasing the area of optical fiber is cut off by the size of the slit.

We also conducted experiments on polymer duplex optic fiber where one fiber comes from spectrometer and another one from the laser. Both distal ends are glued into metal tubes and connected. The results obtained allowed us to suggest the possibility of polymer fiber use in diagnostics, which would allow significantly save on cost of optical fiber making.

The similarity of the experimental results allow us to confirm the utilization of this algorithm to determine the level of oxygenation in the selected area. Using this method it is planned to evaluate the recovery rate of molecular oxygen in the tumor and to determine the optimal time for re-irradiation of tumor zones. This method are also able to help controlling the effectiveness of PDT, the level of vascular damage and the degree of the tumor oxygenation.

To verify the effectiveness of the modulated laser of tumor treatment, we conducted a series of PDT experiments to compare it with the conventional method of continuous laser irradiation. Tumors were irradiated using modulated (with intervals of the pulse - 1s and pause - 1s) or continuous regimen. Both regimens were similar by wavelength ($\lambda = 660$ nm), power density of light ($PD_s = 50$ mW/cm^2) and by general exposure time (t = 30 min). The radiation dose received by a tumor under continuous mode was 150 J/cm^2 and modulation mode was 45 J/cm^2.

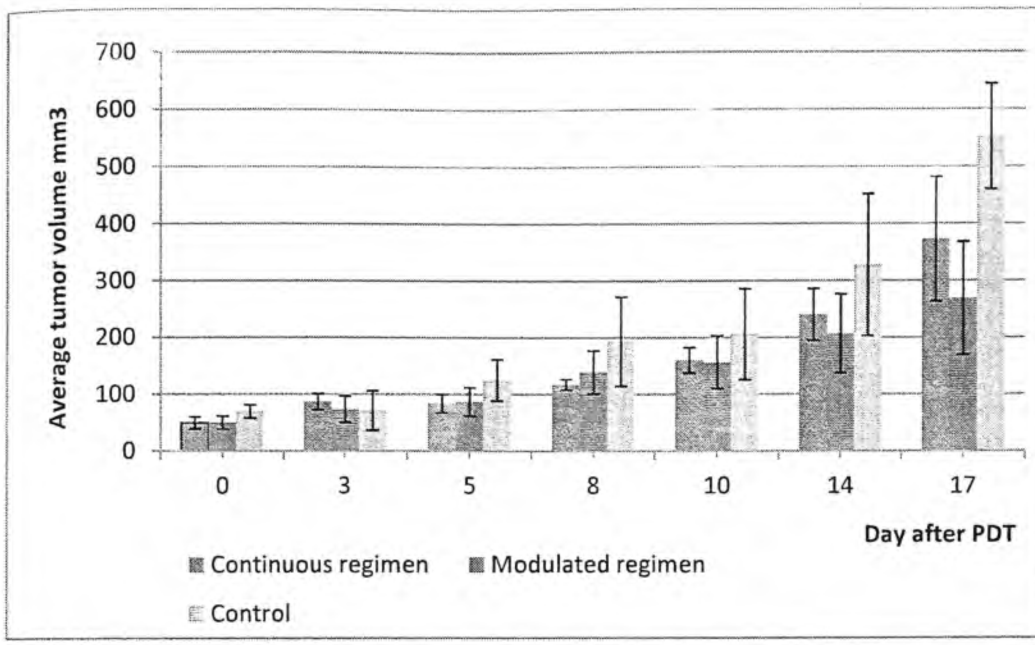

Figure 4. Dynamics of Lewis lung carcinoma growth after PDT with different irradiation regimens.

After the PDT, the tumors were measured every 2-3 days. results of the treatment are presented on the fig. 4. Each point in the curve is an mean tumor volume (calculated as the volume of an ellipsoid) in animal of experimental and control groups (n=5).

As it is seen from Fig. 4, both treatment regimens resulted in growth inhibition of experimental tumors. As it is seen from Fig. 4, both treatment regimens resulted in growth inhibition of experimental tumors. Thus, on the eighth day after the treatment, the treated tumor size already significantly differed from the control group. On the 17^{th} day, tumor growth inhibition rates in the groups, subjected to the modulated or continuous irradiation regimens, were 51% and 33%, respectively. Therefore, the obtained results were better, although the light dose with modulated regimen was twice lower.

5. CONCLUSIONS

Experimental verification of the modulated laser method efficiency *in vivo* on murine tumor model showed that tumor growth inhibition rate after PDT with this mode of irradiation yielded to the continuous treatment method by 18%, while the applied light dose was reduced by 2 times. We also considered the possibility of monitoring oxygenation of the tumor tissue through the registration LDs light scattering with CCD-spectrometer during photodynamic therapy. Obtained results witness that the proposed version of PDT technique deserves further elaboration and broader probation.

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