Front. Optoelectron. 2017, 10(3): 317–322
DOI 10.1007/s12200-017-0725-5

RESEARCH ARTICLE

Dynamic influence of pentoxifylline on the oxygen status of Pliss's lymph sarcoma in rat

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Abstract Tumor oxygenation is one of the key factors influencing disease prognosis and the effectiveness of treatment. Assessment of tumor oxygenation levels facilitates the selection of optimum conditions for radiation therapy, and plays an important role in creating alternative regimes of irradiation. Treating tumors with agents capable of increasing tumor oxygenation in order to increase radiosensitivity is a promising avenue of enquiry. Diffuse optical spectroscopy (DOS) allows a noninvasive determination of tissue oxygen levels based on information about the local changes in optical parameters, and the visualization of metabolic processes in the region of interest. DOS allows reconstruction of the two-dimensional distribution of main tissue chromophores that characterize the processes of oxygen supply (oxygenated hemoglobin) and oxygen consumption (deoxygenated hemoglobin), as well as the blood oxygen saturation levels, which indirectly reflect the tissue oxygenation levels. In the present study, a hemorheologic drug, pentoxifylline, which can improve microcirculation in regions with circulatory disturbances, was used for modifying tumor tissue oxygenation. Pliss's lymph sarcoma (PLS), which is characterized by rapid growth and early occurrence of necrotic areas, was chosen as a tumor model. Tumor oxygenation was monitored by DOS with parallel plane geometry. Pentoxifylline could improve tumor oxygenation by increasing the concentration of oxyhemoglobin. The increased blood oxygen saturation persisted from 30 to 120 min after drug administration. Normal healthy tissue (muscle) and tumor tissue responded differently to the drug. DOS can be used for testing new agents that influence tissue oxygen status and blood-filling rate.

Received April 5, 2017; accepted June 30, 2017

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Keywords tumor oxygenation, diffuse optical spectroscopy (DOS), pentoxifylline, Pliss's lymph sarcoma (PLS)

1 Introduction

Tumor oxygenation is one of the key factors influencing disease prognosis and the effectiveness of treatment [1]. The relationship between tissue oxygenation and the efficiency of radiotherapy was demonstrated in the middle of the 21 century [2]. Whereas hypoxic tumor cells are resistant to ionizing radiation, sensitivity to radiation is gained by applying methods that enhance tumor oxygenation during radiotherapy. The effect of oxygen applied to tumors during radiotherapy occurs immediately during exposure [3,4]. Based on this knowledge, it is urgent to understand the temporal action parameters of agents capable of influencing tumor tissue oxygenation.

Pentoxifylline is a promising agent that is capable of enhancing the neoplastic tissue oxygenation level by influencing the microcirculatory perfusion of the tissue. Pentoxifylline injection enhances collateral blood circulation, increasing the blood volume flowing through the tissue cross-section unit, and causing microcirculatory enhancement in areas of blood supply impairment due to vasoactive influence, increasing the blood flow velocity, and the elasticity of blood cells. Pentoxifylline is used for the enhancement of microcirculation in areas with serious impairment, e.g., a cerebral circulation impairment, chronic heart disease, diabetes, atherosclerosis, occlusive lesions of peripheral arteries in pulmonary circulation, and circulation in the retina or in the choroid [5]. Pentoxifylline can modulate tumor oxygen levels and is capable of increasing the efficiency of subsequent radiotherapy [6–8]. In the present study, we investigated the dynamics of the oxygen state of an experimental tumor and a normal tissue

area under the influence of pentoxifylline, for development of optimal modes of clinical administration as an oxygen-modifying agent for radiotherapy. Diffuse optical spectroscopy (DOS) was used for monitoring changes in the oxygen status of the tumor and the normal tissue [9].

2 Materials and methods

2.1 Tumor model

Experiments were conducted on six wild type outbred male rats with weights ranging from 200 to 230 g, provided by Stolbovaya breeding nursery in accordance with the requirements of codes and enactments ruling research works as to the safety and efficiency of pharmaceuticals (Order by MH and SD RF No. 708-n from 23.08.2010), and international legal and ethical codes of experimental use of animals (NIH Publications No. 8023, revised, 1978). Pliss's lymph sarcoma (PLS) was chosen as a tumor model [9]. For tumor model generation, 1.0 mL of 50% of PLS tumor tissue suspension in Hanks' solution was injected subcutaneously into the right internal femoral surface. At the time of the experiment, the tumor volumes were (3.8 ± 1.8) cm^3.

2.2 DOS setup

The experiment was performed using DOS with plane scanning geometry (Institute of Applied Physics RAS, Nizhny Novgorod), with light source and detector facing each other and stepped object scanning (1 – 2 mm) (Fig. 1). The DOS laser setup included red and near infrared-range lasers with a wavelength of 684 nm (close to the absorption maximum of deoxyhemoglobin (HHb)), 850 nm (close to the absorption maximum of oxyhemoglobin (HbO_2)) and 794 nm, at which the absorption coefficients of these two forms of hemoglobin coincide. For each relative spatial position of the source and the detector, the attenuation index of the biological tissue was measured at all three wavelengths. As a result, for each of the source wavelengths, 2D images were obtained for the attenuation indices of the diffuse light component, which were subsequently processed to obtain 2D images of concentration of oxy- and deoxyhemoglobin, and the blood oxygen saturation level (StO_2) [10].

2.3 Experimental design

Prior to starting the experiment, the rats were anaesthetized with intravenous injection of 50 mg/kg of zolethyl, then fixed on a support plate for fixation during scanning. The animals were placed in a 35 mm in thickness cuvette containing immersion liquid optically similar to the animals' tissue to compensate for irregular thickness and obtain relatively uniform parameters in the scanning medium [11]. The scanning area included both the tumor and the symmetrically contralaterally positioned femoral muscle tissue. During scanning, the temperature of the immersion liquid was maintained at 28°C.

A single 10 mg/kg dose of pentoxifylline was administered by intraperitoneal injection. The first DOS measurement was obtained immediately before the agent was injected. Subsequent measurements were obtained at 15, 30, 60, 120, 180, and 240 min after pentoxifylline administration. A 2D distribution of the total hemoglobin

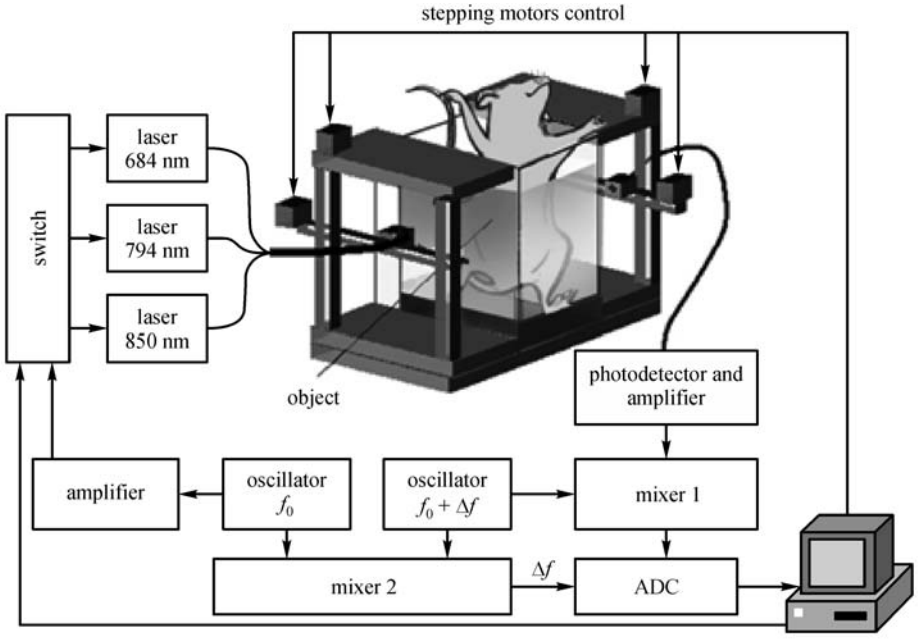

Fig. 1 Schematic (block diagram) of experimental DOS-setup

(tHb), oxy- and deoxyhemoglobin concentrations was generated, and the formula $StO_2 = [HbO_2]/[HbO_2 + HHb] \times 100\%$ was used to calculate blood oxygen saturation [3].

The average and standard deviation were calculated for each parameter in the tumor and in the contralateral normal muscle tissue, and compared by Student's *t*-test for independent sample groups. For comparison with the initial levels, Student's *t*-test for dependent sample groups was used. Differences between groups were considered to be statistically significant when $p < 0.05$. The images are represented in a pseudo-color palette with shades from blue (minimum value) to red (maximum value) for each measured parameter.

3 Results and discussion

Prior to administration of pentoxifylline, PLS tumors were distinguished by increased levels of deoxyhemoglobin and reduced levels of oxyhemoglobin compared to that of the normal tissue, leading to reduced oxygenation in the tumor model (Figs. 2 and 3). The blood oxygen saturation level in the tumor was significantly lower than that of the normal tissue ($p < 0.01$). Both HHb and HbO_2 levels were significantly different in the tumor and normal tissues ($p = 0.01$ and $p = 0.03$, respectively). These indices demonstrate an impaired balance between the delivery and demand of oxygen in the tumor tissue, in accordance with the special biological features of PLS, and contribute to its low oxygenation level and the presence of vast hypoxic areas [11]. In normal tissue (marrow and cross-stripe muscle), the total hemoglobin level indicates the level of blood-filling of the tissue, while the oxyhemoglobin level indicates the oxygen supply, and the deoxyhemoglobin level indicates the tissue oxygen consumption [12,13]. Thus, in tumor tissue, increased concentration of deoxyhemoglobin may reflect disproportionate oxygen supply and demand, which is typical of an actively proliferating tumor such as PLS.

After pentoxifylline injection, the concentration of oxyhemoglobin (Fig. 2(c)) and total hemoglobin (Fig. 2(d)) increased, and, accordingly, the total oxygenation level in the tumor increased (Fig. 2(a)). Statistically significant differences in the oxyhemoglobin concentration were observed at 60 ($p = 0.04$) and 120 min ($p = 0.04$), and in the total hemoglobin level at 60 min ($p < 0.01$) and 120 min ($p = 0.02$), compared to that of the initial values. Statistically significant differences in the tumor StO_2 were observed at 30 min ($p = 0.03$), 60 min ($p = 0.01$), 120 min ($p = 0.04$), and 240 min ($p = 0.02$), compared to that of the initial values. The concentration of deoxyhemoglobin in the tumor following pentoxifylline administration (Fig. 2(b)), reflecting the oxygen consumption rate of the tissue, was not significantly different to the initial value ($p > 0.05$) for the duration of the experiment.

It is noteworthy that pentoxifylline administration

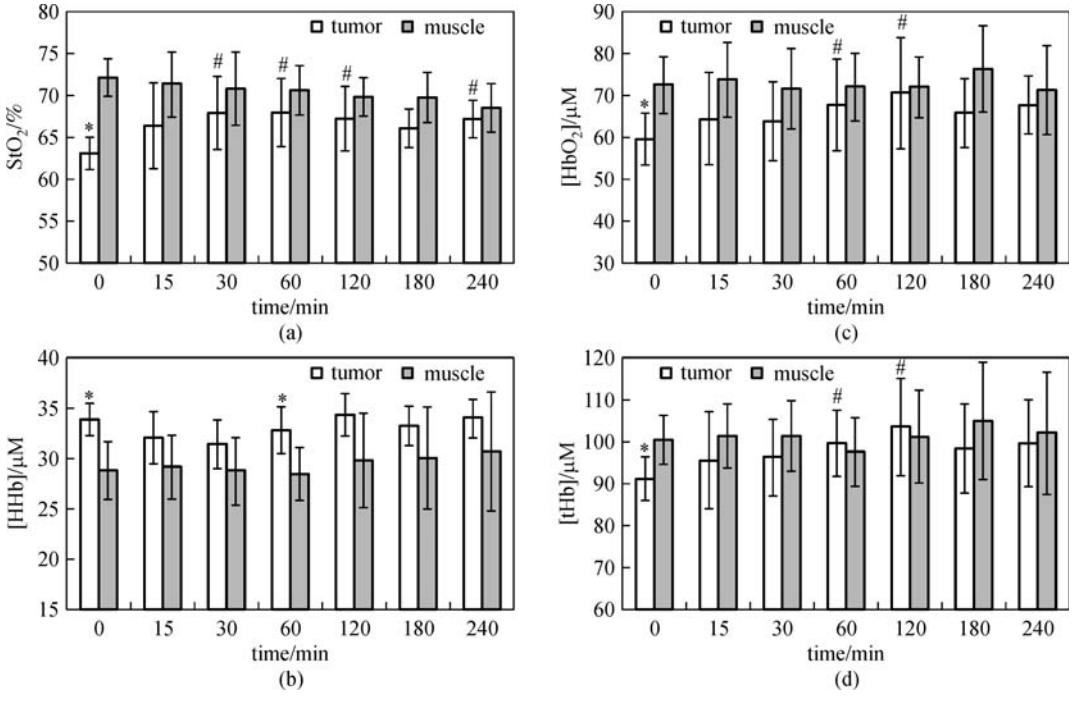

Fig. 2 Values for (a) StO_2, (b) HHb, (c) HbO_2 and (d) tHb, determined by DOS in tumor and muscle tissue after Pentoxifylline administration. * - statistically significant differences compared with muscle tissue; # - statistically significant differences compared with initial values

decreases the differences between the values of all measured parameters in the normal and the tumor tissue, suggesting that the drug is capable of returning the oxygenation of the tumor tissue to 'normal' levels.

The dynamics of tumor oxygenation levels after pentoxifylline administration have been described in a number of studies using polarographic measurement of partial pressure of oxygen (pO_2) [7,14]. However, the mechanisms by which the oxygen status of the tumors was altered were not described in these studies. In the present study, DOS identified increased blood-filling in the tumor tissue (increase of total hemoglobin) and increased oxygen supply rate (increase of oxyhemoglobin), which explain the observed StO_2 dynamics. The observed changes are supported by the known therapeutic action of pentoxifylline in relation to the pathologic vascular bed [5–7], whereby the alteration of the oxygen consumption rate by the tissue does not participate in the reaction. This assumption is confirmed by the stable concentration of deoxyhemoglobin observed in the tumor area for the duration of the experiment. A summary of the mechanisms by which pentoxifylline improves the blood flow of tissues, such as decreasing blood and plasma viscosity, lowering plasma fibrinogen levels, and improving blood filterability, are presented in Ref. [15]. Rheological properties of the drug enhance the capillary blood-filling and accordingly, increase the oxygen supply to tissues.

No changes were detected in the muscle tissue oxygenation parameters (Figs. 2(a), (b), (c), and (d)), with the background pentoxifylline effects. The levels of oxyhemoglobin, deoxyhemoglobin, and the total hemoglobin did not change significantly after pentoxifylline administration for the duration of the experiment. Accordingly, the oxygen saturation did not change either.

Representative DOS images of PLS before pentoxifylline administration and at 30, 120, and 240 min after administration are shown in Fig. 3. After administration of pentoxifylline, an increase in the total hemoglobin and oxyhemoglobin concentrations were observed in the tumor. As a result, the degree of tumor oxygen saturation increased. The oxygen consumption rate remained unchanged.

In the present study, we demonstrated the application of DOS for non-invasive monitoring of the oxygen status of an experimental tumor under direct action on microcirculatory perfusion. DOS is advantageous because it is possible to simultaneously obtain information about the level of tissue oxygenation and the source of changes in oxygenation, by taking into account the parameters that reflect the oxygen consumption and supply to the tissue. Thus, it is possible to indirectly evaluate the mechanisms by which both the tumor and normal tissue respond to the administration of pentoxifylline simultaneously.

We have demonstrated the differential reaction of tumor and muscle tissue to the administration of pentoxifylline, which influences microcirculatory perfusion. Based on this data, we can assume that the reasons for this differential reaction are structural or functional features of the vascular bed in the tumor and normal tissue. The blood vessels in the tumor have a number of serious anomalies, including

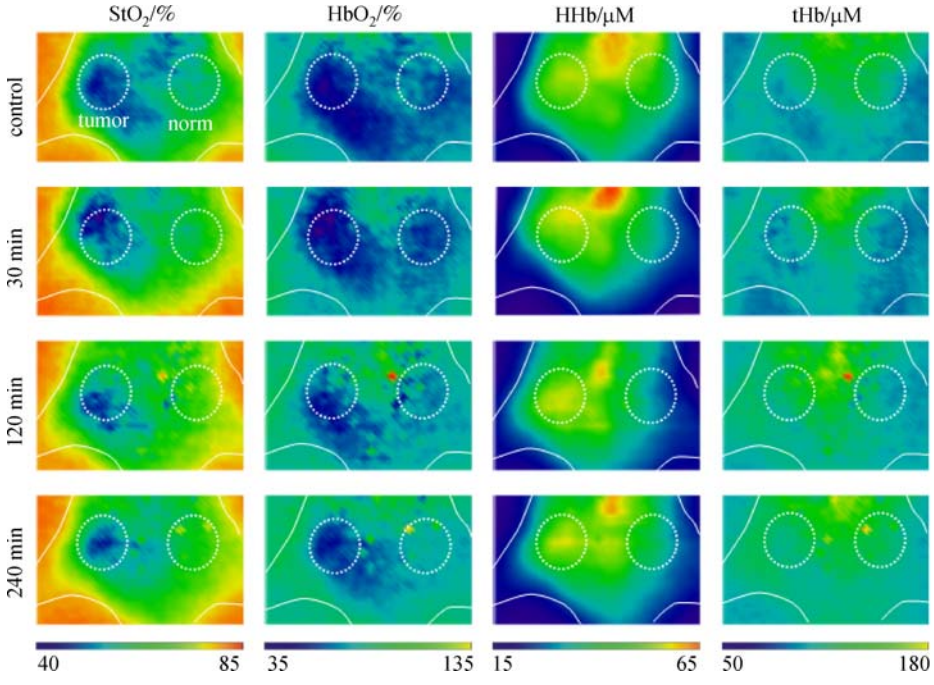

Fig. 3 Representative DOS images of PLS before and 30, 120, 240 min after pentoxifylline (Ptx) administration. The solid lines contour the animal body within the scanning region and the dotted lines designate the tumor and contralateral normal area used for analysis. The image size is 80 mm × 50 mm

the absence of endothelial lining, engorged or constricted sections, tortuosity, and the formation of shunts and blind ends causing impairment to blood flow [16,17]. Tumor and normal vessels also have different permeability for blood cells. Pentoxifylline increases the extensibility of these blood cells and reduces their ability to aggregate, thereby increasing the flow of blood into tissue sites with previously impassable microvessels. In normal tissues, changes in the properties of red blood cells do not affect blood-filling [18,19]. Due to its pharmacokinetic action, pentoxifylline outbalances the consequences of such vascular anomalies, and, due to perfusion enhancement, stimulates the tumor oxygenation level for a certain period of time [7]. The latter is essentially significant in those cases where it is important to selectively increase the tumor sensitivity to irradiation without influence on the healthy tissue environment.

Nevertheless, it should be taken into account that DOS makes it possible to detect differences only in the oxygen levels of tissues, and for the accurate assessment of blood-filling of the capillary network, higher resolution methods such as magnetic resonance tomography, optoacoustics, or speckle-OCT are required [18].

4 Conclusions

In the present study, we report on the use of DOS for the detection of oxygenation changes in PLS and muscle under the influence of pentoxifylline. Improvement of oxygenation was registered in response to drug administration in tumors but not in normal tissue. The tumor oxygenation enhancement persists within 30 – 120 min after the pentoxifylline injection. The enhancement of tumor oxygenation can be attributed to an increase of oxygen supply to the tumor tissue. This is confirmed by the observed increase in oxyhemoglobin and total hemoglobin content. DOS can be used to test new agents that affect the oxygen status and blood-filling rate of tissues.

Acknowledgements the authors extend their gratitude to I. P. Ivanova for creation of tumor models, to V. I. Plekhanov, V. A. Vorobyov for technical support of the studies, to A. S. Plekhanova, A. B. Volovetsky for assistance during the experiments. This work was supported by Russian Foundation for Basic Research (project No. 15-42-02528) for *in vivo* DOS experiments and statistical analysis. The study is supported by the Governmental project of the Institute of Applied Physics RAS (project No 0035-2014-0008) for DOS data reconstruction. The authors declare no conflict of interests.

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