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Letter

The study of oxygenation dynamics during experimental tumor growth using frequency-domain diffuse optical spectroscopy

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Abstract

The objective of this work was to study the dynamics and mechanisms of oxygenation changes of an experimental rat tumor *Plyss lymphosarcoma* in the process of its growth using diffuse optical spectroscopy (DOS). DOS in trans-illumination configuration utilizing high-frequency (140 MHz) modulation of light intensity is used to assess absorption and scattering coefficients averaged over tumor tissue at several wavelengths 684, 794, and 850 nm. Those values were used to assess concentrations of tissue oxyhemoglobin and deoxyhemoglobin and oxygen saturation. During the observation period (from the 5th to the 15th day after transplantation) the tumor volume increased up to 25 times. In the process of tumor growth, a gradual increase in the content of deoxyhemoglobin, a decrease of oxyhemoglobin level, and a decrease of blood oxygen saturation without changes in the level of total tissue hemoglobin concentration were observed. Thus, the main mechanisms of oxygen saturation decrease in the process of tumor growth are associated with two effects: the increase of oxygen consumption rate (demonstrated by the increase of deoxyhemoglobin) and the decrease of oxygen supply (demonstrated by the decrease of oxyhemoglobin).

Keywords: frequency-domain diffuse optical spectroscopy, diffuse optical imaging, tumor oxygen state, tumor growth, *in vivo* monitoring, Pliss lymphosarcoma

(Some figures may appear in colour only in the online journal)

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1. Introduction

Tissue hypoxia is a powerful and independent adverse prognostic factor in solid malignancies resulting in tumor progression and resistance to therapy [1]. It is one of the most important problems in modern clinical oncology that is considered to be both a consequence of inadequate tumor blood flow and a powerful incentive of tumor neoangiogenesis. Hypoxia that results from an imbalance between the tissue oxygen consumption and delivery is a characteristic feature of many solid tumors. Tumor hypoxia affects various metabolic, molecular-genetic, pathophysiological processes, including proliferation, apoptosis, angiogenesis, and metastasis [2]. Most tumors have a lower median partial pressure of oxygen (pO_2) in comparison with the corresponding normal tissues; many tumors contain areas of reduced pO_2, that cannot be predicted based on clinical characteristics (stage, size, location, histological structure, etc); there is a variability of the oxygen status from the tumor to tumor; recurrent tumors appear to be worse oxygenated in comparison with the corresponding primary tumors [3]. The increasing understanding of the role of tumor hypoxia in tumor prognosis and treatment outcome gave an impetus to studies on its diagnostics and correction.

Methods allowing evaluation of tumor metabolic properties, including the oxygen state, are needed for effective assessment of tumor hypoxia. Currently, this approach has been applied involving several imaging techniques such as magnetic resonance tomography, positron emission tomography, and single-photon emission computed tomography, as well as diffuse optical spectroscopy (DOS) and tomography [4, 5].

Diffuse optical methods are based on differences in the absorption spectra of different tissue chromophores, in particular, oxygenated (HbO_2) and deoxygenated (HHb) hemoglobin in visible or near-infrared wavelength ranges [6]. Reconstruction of HbO_2 and HHb concentrations is performed using data on light attenuation in tissue at several wavelengths and an appropriate light transport model accounting for light scattering and absorption. Different geometries of source and detector configurations are applied to obtain the best results for the specific biomedical task. Diffuse reflectance spectroscopy, based on probing and detecting light in reflectance mode (source and detector reside in the same plane, i.e. at the same tissue surface), has good sensitivity to superficial tissues, while trans-illumination (or transmission) technique has better performance for deep tissue analysis. In this regard, trans-illumination configuration is beneficial for assessing average hemoglobin concentrations in a tumor located deeply in tissue, because this geometry allows light interacts more with deep tissues, and reconstructed hemoglobin concentrations are more relevant to tumor tissue rather than to surrounding [7].

Steady-state DOS techniques use reduced scattering coefficients from literature data, which does not allow for accurate measurements of chromophore concentrations, because of high tissue-to-tissue variations in this value [8]. Time-domain [9, 10] or frequency-domain [11] approaches are applied in diffuse optical methods to increase the precision of chromophores concentration reconstruction by assessing tissue absorption and scattering coefficients independently.

In this paper, we apply frequency-domain DOS setup with trans-illumination configuration allowing a noninvasive and accurate assessment of the oxygenation state of tumor tissue. The work objective was to study the dynamics and mechanisms of oxygenation changes in an experimental rat tumor *Plyss lymphosarcoma* (PLS) in the process of its growth.

2. Materials and methods

The experiments were carried out on nine white outbreed rats (Stolbovaja nursery) (settlement Stolbovaja, Russia). PLS was chosen as a tumor model. Tumor strains were purchased from the N.N. Blokhin Russian Oncological Scientific Center (Moscow, Russia). PLS is a metastasizing connective-tissue tumor [12], consisting of compact small and large irregularly shaped cellular elements with nuclei varying in shape and size. The tumor is characterized by high mitotic activity, high cellularity, and a sufficient number of blood vessels. Rapid growth and early occurrence of necrotic areas are the distinctive features of this model. At the time of the experiment, the rats weighed 200–230 g. The tumor was transplanted subcutaneously into the inner side of the right thigh. The first passage was carried out on two rats in 0.5 ml of 30% suspension of cells from the capsule. To maintain the strain, the following passages were carried out on rats to 1.0 ml of 50% suspension of tumor tissue in a Hanks Solution. Before each DOS-study tumor size was measured by the caliper in three mutually perpendicular directions and the tumor volume was determined by multiplying these data.

To detect the changes in the PLS oxygen state a series of consecutive DOS investigations on the 5th, 7th, 9th, 11th, and 15th day after strain transplantation was performed. Experiments were carried out in accordance with the requirements of the regulations governing the implementation of research on the safety and efficacy of pharmacological agents in the Russian Federation (Order of the Ministry of Health of Russian Federation 'On approval of rules of good laboratory practice' № 267 from 19 July 2003), and the international rules of legal and ethical use of animals. The animal studies were approved by the Ethics Committee of N.I. Lobachevsky State University of Nizhny Novgorod (Protocol #48, 09 November 2020). Experimental animals were anaesthetized with intraperitoneal injection of 50 mg kg^{-1} Zoletil 100 (Valdepharm, France) and immobilized on a transparent plastic plate; the tumor region and contralateral zone of normal muscle were positioned into the scanning area. During scanning, animals were placed into a 35–40 mm thick cuvette containing the immersion liquid with absorption and scattering parameters close to biotissues [13].

Experiments on DOS were performed on the experimental setup with parallel plane geometry created at the Institute of Applied Physics of the Russian Academy of Science (Nizhny Novgorod, Russia) [14]. Three lasers at wavelengths

of 684 nm corresponding to the high absorption of deoxygenated hemoglobin, 850 nm corresponding to the high absorption of oxyhemoglobin, and 794 nm, at which absorption coefficients of oxygenated and deoxygenated hemoglobin are identical, have been used as light sources. High-frequency modulation of light intensity, 140 MHz, is applied to separate absorption and scattering coefficients from the registered amplitude and phase shift of light intensity propagated through investigated tissue. DOS data were obtained by a synchronous «step by step» movement of the source and the detector located along the coronal plane (corresponding to the cuvette plane) from the opposite sides of the studied subject with scanning steps of 1–2 mm. The concentrations of oxygenated and deoxygenated hemoglobin in each spatial source and detector position were calculated using absorption coefficients calculated at three wavelengths. Total hemoglobin (tHb) was calculated as HbO_2 + HHb, and blood oxygen saturation level $StO_2 = [HbO_2]/[tHb]$. As a result of DOS measurements, 2D plots (DOS images) of StO_2, tHb, HbO_2, and HHb were obtained.

Ratios of concentrations of tHb, HHb, HbO_2, and ratio of StO_2 levels in the tumor zone and contralateral region of the rat body were used for the processing of DOS results [15]. All statistical calculations were performed with Statistica 6.0 (StatSoft) software. The data are presented as mean and standard deviations. The *t*-test was performed to determine the statistical—significance difference between correspondent parameters. *P* values below 0.05 were considered to be statistically significant.

3. Results

The tumor volume increased from 2.1 ± 1.9 cm^3 on the 5th day after transplantation to 53.6 ± 16.5 cm^3 on the 15th day after transplantation. The dynamics of tumor growth of PLS was in accordance with the data obtained by Vasiliev *et al* [16], who investigated this model growth under various conditions of inoculation. Figure 1 shows tumor volume changes of PLS in the process of its growth.

Typical examples of DOS images (2D plots of StO_2, tHb, HbO_2, and HHb) obtained on 5th and 15th days after tumor transplantation are represented in figure 2 in the pseudocolor palette, where the blue regions correspond to the minimum value of the determined parameters, and the red is the maximum value. On the 5th day after transplantation (the 1st day of monitoring) DOS images demonstrated an insignificantly increased concentration of deoxyhemoglobin in the tumor tissue (figure 2 left) and a moderately decreased concentration of oxyhemoglobin compared to the surrounding normal tissues (figure 2). Blood oxygen saturation and tHb concentration of PLS appear to be comparable with surrounding tissues. An increase in the HHb content and a decrease in the HbO_2 content, along with a decrease in blood oxygen saturation are clearly defined as the size of the tumor increases by the 15th day of growth (figure 2 right). By this day of neoplasm growth,

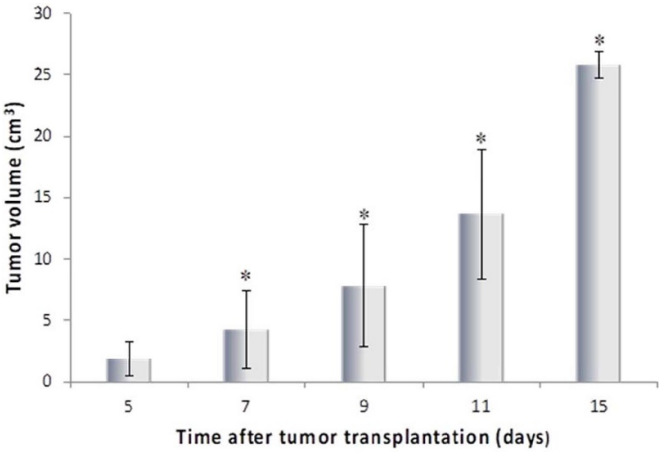

Figure 1. Changes in the volume of Pliss lymphosarcoma in the process of its growth. Results show mean ± SD (*: statistically significant differences by comparison with the 5th day, $p < 0.05$).

the content of chromophores in the tumor differs significantly from that in the surrounding normal tissues.

In the process of tumor growth, a gradual increase in tumor/norm ratio of deoxyhemoglobin concentration from 1.08 ± 0.09 to 1.44 ± 0.08 a.u. and a decrease in tumor/norm ratio of oxyhemoglobin concentration from 0.89 ± 0.09 to 0.40 ± 0.01 a.u. from the 5th to the 15th day after strain transplantation respectively was observed while the level of tHb concentration (0.93 ± 0.11 and 0.95 ± 0.23 a.u.) remained unchanged (figure 3). The decrease in tumor/norm ratio of blood oxygen saturation from 0.94 ± 0.05 to 0.65 ± 0.09 a.u. was observed. Changes in oxygen saturation became statistically significant compared to the initial level from the 9th day after inoculation.

4. Discussion

This study demonstrates the possibilities of trans-illumination frequency domain DOS for non-invasive longitudinal monitoring of oxygenation parameters of rodent experimental neoplasms. The method allowed us to identify features of the experimental tumors in comparison with the surrounding normal tissues and track their changes over time. Moreover, by providing data on changes in the concentrations of oxy- and deoxyhemoglobin, which reflects changes in the rate of oxygen supply and consumption, the method makes it possible to evaluate the mechanisms of tumor hypoxia formation.

In the present work, a statistically significant decrease of the tumor oxygenation in the course of tumor growth in parallel with the increase in tumor volume was revealed. These results correspond to Fujii *et al* who described the dynamics of tumor model oxygenation in the course of its growth with the electron paramagnetic resonance method [17]. Diffuse reflectance spectroscopy has been used as a method of experimental tumor oxygenation investigation and has demonstrated a certain decrease in StO_2 level and concentration of

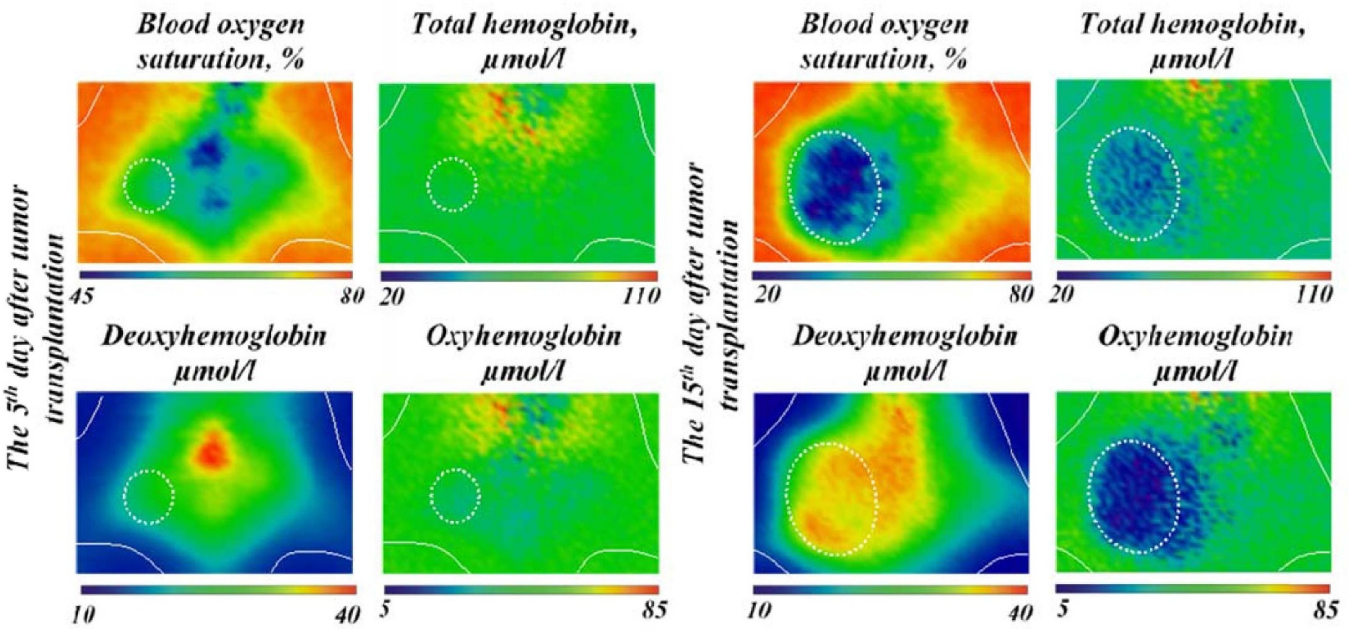

Figure 2. Examples of DOS images of the Pliss lymphosarcoma on 5th (left four images) and 15th (right four images) days after tumor transplantation. Solid lines contour the animal body within the zone of scanning; dotted lines contour the tumor area. Image size 80 × 60 mm.

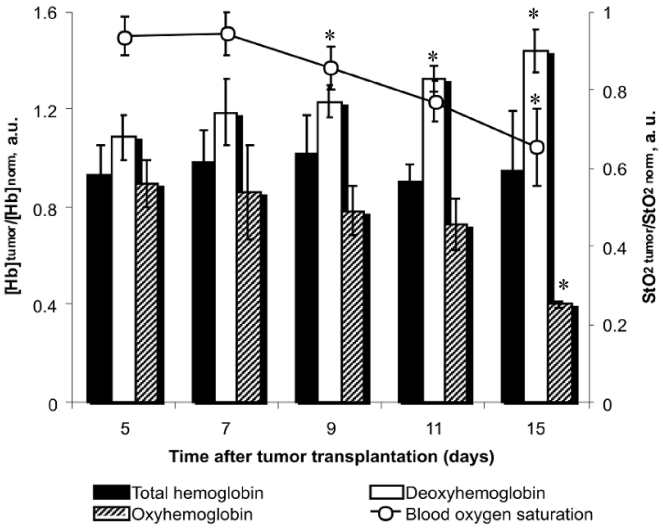

Figure 3. *In vivo* monitoring of oxygen status of Pliss lymphosarcoma in the process of its growth. On the left scale, the ratio of hemoglobin concentration in tumor tissue to hemoglobin concentration in normal tissue is shown, on the right—the ratio of blood oxygen saturation level in tumor tissue to blood oxygen saturation level in normal tissue. Results show mean ± SD (*: statistically significant differences by comparison with the 5th day, $p < 0.05$).

oxyhemoglobin, as well as an increase in deoxyhemoglobin content [18–20].

A temporal rise of blood oxygen saturation level in murine head and neck xenografts was shown in [21]. No changes in the oxygenation level of liver tumor and FSAII tumor models and HCT116 tumor model were demonstrated in [22] and [19] correspondingly. Fujii *et al* showed a gradual decrease in oxygenation of the SCC VII model due to the rise in oxygen consumption rate in the process of tumor growth [17]. A comparison of different tumor models shows the dynamics of oxygenation can be different and does not depend on the growth rate of the neoplasm [19]. Thus, tumor oxygenation changes in the process of its growth may have various character.

The decrease in tumor oxygen saturation arose due to the following effects: the increase of deoxyhemoglobin concentration and the reduction of oxyhemoglobin concentration. Deoxyhemoglobin content reflects oxygen consumption by living tissues. The growth of its concentration in the aggressively growing tumor with a high ratio of parenchyma/stroma [10] may be due to a mismatch between the insufficient level of oxygen supply and the high demands of actively proliferating tumor tissue. According to [23] this inconsistency is one of the reasons for tumor hypoxia. In addition, in the process of tumor growth, the cells moved away more and more from blood vessels, and adequate oxygen uptake became impossible. Another mechanism responsible for the increase of deoxyhemoglobin level may be tumor blood flow disorders caused by abnormalities of the tumor vascular bed [24]. At the same time, a significant reduction in the concentration of oxyhemoglobin in the tumor tissue was observed. It can be associated with a violation of oxygen delivery through an abnormally formed tumor vasculature [25–27]. The absence of statistically significant changes in tHb concentration during the period of observation is an indicator of the stability of its blood filling (figure 3).

Thus, using DOS, the main mechanisms of oxygenation decrease in the process of tumor growth were estimated. Both the increase in oxygen consumption rate (reflected by the increase of deoxyhemoglobin) and the decrease of oxygen supply (reflected by the decrease of oxyhemoglobin)

are responsible for hypoxia enhancement. When monitoring the tumor response to therapy, the DOS method may be useful for revealing the tumor model oxygenation changes demonstrating the mechanism of the effect of therapy on the cellular or vascular component of the neoplasm.

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