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Kinetics of laser-induced photodissociation of oxyhemoglobin and its biomedical applications

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In this study we investigate the kinetics of oxygen tension (pO_2) in skin tissue under the influence of the transcutaneous laser irradiation. The results of *in vivo* experimental measurements of pO_2 by a method of transcutaneous oxygen monitoring (TcOM) are presented. The results show that under laser irradiation the value of tissue oxygenation increases and after approximately 10 minutes of exposure exceeds its initial level up to 1.6 times. The observed increase in pO_2 indicates the process of photodissociation of oxyhemoglobin (HbO_2) in skin blood vessels, which results in local O_2 increase in the tissue. Such laser-induced enrichment of tissue oxygenation can be used in phototherapy of pathologies, where the elimination of local tissue hypoxia is critical.

Keywords: oxyhemoglobin photo-dissociation; tissue oxygenation; oxygen tension.

INTRODUCTION

Tissue oxygenation plays a key role in the cell metabolism, energy supply and life activity of an organism. Many diseases such as diabetes, burns, bedsores and wounds are accompanied with an insufficient supply of oxygen to the tissues [1,2]. Also, the deficit of oxygen in cancer tissue is a major problem limiting the efficiency of photodynamic therapy [3,4]. The wound healing process directly depends on the oxygen pressure in skin tissue ($TcPO_2$). For example, the additional supply of oxygen leads to an increase in the rate of collagen synthesis and an improvement of the protective functions of the skin. It is currently accepted that the adequate tissue oxygen concentration for normal cell metabolism should exceed 40 mmHg. Injuries, infections, and diseases can reduce this vital tissue oxygen level down to almost zero, which indicates tissue hypoxia. Optimal tissue healing occurs when the pO_2 rises to between 50 and 80 mmHg. Consequently, the value of pO_2 is an objective indicator for evaluating the local state of the tissue and the efficiency of cell metabolism. Controlling this mechanism provides the possibility for biological stimulation with a therapeutic effect.

In clinical practice the commonly used method for elimination of tissue hypoxia is ventilation of the lungs by pure O_2 at normal and hyperbaric pressure. Sustained periods at such pO_2 levels can only be realized through the use of hyperbaric oxygen therapy [5], but it leads to the risk of oxygen intoxication. Moreover, this approach has not had a broad clinic application for local treatment due to technical difficulties. Thus the problem of local hypoxia elimination in biological tissues remains significant and the methods of influencing the delivery of oxygen to the tissue are of considerable interest. One of these methods is the light radiation in the optical spectral range.

A new approach for the optically induced increase in the local oxygen concentration due to photodissociation of oxyhemoglobin in cutaneous blood vessels has been proposed in [6]. The absorption of light by blood HbO_2 is connected with the following photochemical and photophysical processes. It is known [7] that absorption of a photon with the activation energy leads to the dissociation of a part of the molecules, resulting in the release of molecular oxygen and the formation of deoxyhemoglobin. Photodissociation is one of the simplest chemical reactions when a compound dissociates under the action of radiation.

$$HbO_2 + h\nu \rightarrow Hb + O_2$$

The quantum efficiency of the photodissociation of oxyhemoglobin is high and reaches 10 % in a wide visible spectral range [7].

The photophysical process is connected with the non-radiative dissipation of the absorbed excitation energy, as the heat generated in this process is transferred to the blood capillaries with a characteristic thermal relaxation time of ~ 0.05-1.2 msec. Estimates show that in the typical case a local increase in temperature of only 0.1 - 0.5 ^{0}C

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may be expected [8]. Such a small rise in the local temperature may produce little improvement in the capillary microcirculation of the blood and can hardly stimulate the cell metabolism [9].

We suppose that in the case of low energy irradiation the most important process is the photodissociation of HbO_2. Experimental proof of the suggested concept has been demonstrated in [10,11]. The observed local decrease in the level of arterial blood saturation during laser irradiation clearly proves the induced photodissociation of HbO_2 in blood capillary vessels. The efficiency of this process depends on the wavelength and the output power of the laser radiation, the blood vessels density in the irradiation area, the optical properties of the skin and the depth of the blood vessels in the tissue.

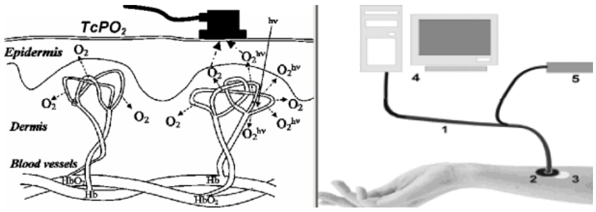
It is interesting but still unclear what fraction of O_2 molecules released by photodissociation can escape from the heme pocket and diffuse through the cell membranes and capillary walls, thereby increasing the tissue oxygen pressure. However, relevant studies mainly refer to *in vitro* experiments or studies of individual cases [12-15]. No investigations have been carried out as yet into whether an immediate improvement in the oxygen saturation occurs *in vivo*, which would be a key to wound healing.

In this paper, we present an experimental study *in vivo* of the pO_2 rate in skin under the influence of local transcutaneous He-Ne laser (628 nm) irradiation.

METHODS

The transcutaneous oximeter method, based on the principle of measuring the oxygen tension pO_2 in arterial blood, is a method for direct registration of gases that dissolve in the blood plasma. The choice of this method is determined by the fact that the TcOM allows the direct determination of the oxygen pressure in the skin tissue, in units of mmHg. Measurements were carried out using a transcutaneous oxygen monitor “Radiometer” TCM-4 with a Clark-type polarographic sensor with a diameter of 2.5 mm ($TcPO_2$ electrode) that consists of a platinum cathode and silver anode, electrolyte and an oxygen permeable membrane; a heating section and an electronic system for measuring and controlling the sensor temperature. (Fig.1 a).

The measurements were carried out on three volunteers, in the conditions of an absence of physical and emotional stress, in a seated position and at room temperature. All the procedures performed in the study were in accordance with the ethical standards. Human studies in the article were approved by the relevant Institutional Review Board. During an individual measurement, all conditions were identical for all the subjects and remained constant throughout the measurement.



a b

Fig. 1. Model of oxygen diffusion in tissue (a) and measurement (b) of tissue oxygen tensions: 1 - Clark sensor, 2 - electrolytic cell, 3 - irradiating zone, 4 - monitor TCM, 5 - He- Ne laser.

The measurement procedure is as follows. The sensor is placed on the skin of the forearm, an area with high capillary pressure then it is heated to 43ºC (Fig. 1 b). Under the influence of temperature, oxygen diffuses from the capillaries into the epidermis and then into the electrolytic cell, where it is measured. The value of oxygen tension, measured transcutaneously ($TcPO_2$), corresponds to the value of pO_2 measured in the arterial blood plasma. The errors in the determination of $TcPO_2$ come from the skin thickness, the subcutaneous blood flow and the physiological factors influencing O_2 delivery to the skin surface (decrease in the blood flow, the arterial blood pressure, the vasoconstriction occurrence). Since oxygenation depends on a number of factors, the laser radiation was only used once and the measurements were carried out immediately, in order to obtain direct observation of the effect of radiation.

RESULTS

For local irradiation of cutaneous blood vessels, a He-Ne (632,8 nm) laser was chosen. As shown in the model calculations of the absorption spectra at the tissue depth [6], this wavelength lies in the effective absorption band of HbO_2 and penetrates deep into the skin issue. The laser output is 1 mW, and the beam diameter – 2,5 mm, providing a laser power density of ~ 20 mW/cm^2. First, after thermostabilization, the initial oxygen pressure in the tissue is measured, then the laser radiation is applied. In Fig.2 the measured values of pO_2 in dependence on the irradiation time are presented. The obtained results are normalized to the initial oxygen pressure value.

As can be seen, the tissue oxygen tension increases for all three patients (although with different rates) and reaches saturation levels after approximately 10 minutes of exposure. The value of $TcPO_2$ in the irradiation zone depends on the time of exposure and the properties of the tissue that differ considerably for all three cases. It is significant to note that this growth of $TcPO_2$ is due to the additional O_2 in the tissue as a result of the laser-induced photodissociation of HbO_2. The oxygen released from HbO_2 first increases the pO_2 in the blood plasma and then diffuses into the surrounding tissue. The value of the $TcPO_2$ measured transcutaneously increases about 1.6 times compared with its initial value. Generally, the diffusion occurs in three directions: toward the skin surface, inward to the muscle tissues and some is carried away by the blood flow. If we assume that the same amount of oxygen is carried away in all these directions, we should expect an increase in pO_2 in the arterial blood plasma by about a factor of 4.8.

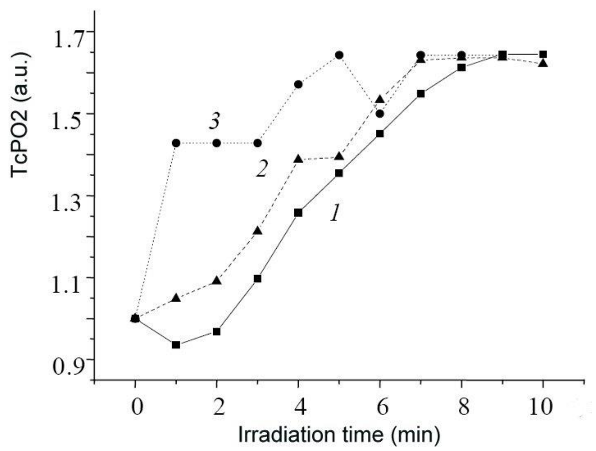


Fig. 2. Kinetics of laser-induced tissue oxygenation.

It is interesting to note that the obtained value of pO_2 realized by laser-induced photodissociation of HbO_2 is comparable with the one typically reachable by the conventional method of hyperbaric oxygen therapy [5].

DISCUSSION

In contrast to other (in vitro) studies, this study was carried out under in vivo conditions, so it is possible to make statements about the feasibility of the measurement effect.

Thus, we demonstrate that after prolonged irradiation (several minutes), a certain fraction of oxygen molecules released due to photodissociation of HbO_2 diffuses in the surrounding tissue and increases the oxygen partial pressure. Since the values of oxygenation depend on a number of factors, the results of this study cannot identify any immediate effect of the radiation on the oxygenation in tissue in vivo. Thus, it would be reasonable if further studies on the immediate effects on oxygen saturation would take into account additional parameters, such as a variation in the wavelength radiation. The results obtained should correlate with the characteristics of light propagation in blood-filled tissues. The visible light in the blue and green spectral range has a small penetration depth into skin tissue, because of their proximity to the absorption bands of some basic skin chromophores such as HbO_2 and melanin. Red and especially near infrared radiation penetrates much deeper into the soft tissues and plays a dominant role in the absorption of laser radiation by oxyhemoglobin in the deeper layers of tissue blood vessels. Such possibility to increase the free oxygen content in tissues can be applied in clinical practice for treatment of a number of diseases related to problems with the microcirculation and oxygen supply and therefore requires further investigations.

It is very important to note that photodissociation of HbO_2 produces only molecular oxygen as a by-product and not singlet oxygen. Lepeshkevich in [16] uses time-resolved luminescence spectroscopy in the near-infrared region and does not measure detectable quantities of singlet oxygen during the photodissociation of O_2 from myoglobin and hemoglobin.

CONCLUSION

The obtained results demonstrate that the laser-induced photodissociation of oxyhemoglobin, whose main biological function is the transport of molecular oxygen, gives the possibility for an additional oxygen supply and allows the development of optical methods for tissue hypoxia elimination. Monitoring the kinetics of tissue oxygenation gives the possibility to control the normal aerobic cell metabolism.

It is shown that the efficiency of laser-induced oxygenation is comparable with the method of hyperbaric oxygenation, at the same time providing advantages by the local action. Advances in technology can lead to further improvement in the management of patients with wounds.

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КИНЕТИКА НА ЛАЗЕРНО-ИНДУЦИРАНАТА ФОТОДИСОЦИАЦИЯ НА ОКСИХЕМОГЛОБИНА В КРЪВТА ЗА БИОМЕДИЦИНСКИ ПРИЛОЖЕНИЯ

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(Резюме)

В тази статия ние изследваме кинетиката на напрежение на кислорода (pO_2) в кожната тъкан под въздействието на транскутанно лазерно облъчване. Експериментални измервания на оксигенацията са направени с помоща на метода на полярографски презкожен мониторинг на кислорода (TcOM). Резултатите показват, че при облъчване с He-Ne лазер (632 нм) степента на тъканната оксигенация се увеличава в зависимост от продължителността на облъчването и след около 10 минути достига стационарно ниво, като максималното измерено увеличение е 1,6 пъти. Трябва да отбележим, че кинетиката на растежа на PO_2 е пряко обусловена от допълнителното освобождаване на O_2 в тъканта като резултат на лазерно-индуцираната фотодисоциация на HbO_2 в кръвоносните съдове на кожата, което води до локално увеличаване на O_2 в тъканта. Това лазерно-индуцирано обогатяване на тъканите с кислород може да се използва в фототерапия на патологии, в зони с нарушена микроциркулация на кръв и тъкани в състояние на хипоксия, например в тумори, изгаряния, рани и язви.