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KINETICS OF OXYGENATION OF SKIN TISSUE EXPOSED TO LOW-INTENSITY LASER RADIATION

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We present the results of modeling the action spectrum and an experimental investigation of the effect of laser-induced photodissociation of oxyhemoglobin in vivo *on the increase in the degree of oxygenation of skin tissue in the exposed area. We have shown that controlling the local concentration of free molecular oxygen in biological tissues together with the possibility of eliminating tissue hypoxia using laser radiation makes it possible to stimulate aerobic cell metabolism and to achieve the needed therapeutic effect.*

Key words: *oxyhemoglobin, tissue oxygenation, transcutaneous oximetry, photodissociation, low-intensity laser radiation.*

Introduction. Oxygen is the key element in cell metabolism, and its concentration in tissues plays an important role in the efficiency of many biochemical reactions. As we know, aerobic metabolism is primary in the mechanism for providing cells with energy. Controlling this mechanism opens up a unique opportunity to use it in phototherapy and laser therapy.

In many medical pathologies such as diabetes, burns, bedsores, and wounds, we observe insufficient supply of oxygen to the tissues. A decrease in the oxygen supply from arterial blood to cells of biological tissues substantially reduces the efficacy of medical treatment, increases the risk of infection and scarring, and ultimately leads to tissue necrosis. This is why considerable attention is focused on the problem of the dependence of wound healing on tissue oxygen pressure [1–4].

Today it has been well established that the wound healing process always includes the following basic stages [3]: fibroblast proliferation, collagen synthesis, angiogenesis, re-epithelization, the rate of which depends on the oxygen pressure in skin tissue, or the transcutaneous O_2 pressure ($TcPO_2$). For example, additional supply of oxygen leads to an increase in the rate of collagen synthesis and improvement of the wound healing process. Increasing $TcPO_2$ in the subcutaneous tissue promotes an increase in the protective function of the body.

The $TcPO_2$ value is a direct indication of O_2 content in the skin and the state of the metabolism. This is the determining parameter for establishing the effectiveness of wound healing, especially after amputation of a limb when chronic ischemia sets in.

It has been experimentally established that treatment of wounds is considerably more complicated under tissue hypoxia conditions, i.e., when the oxygen supply is insufficient. Thus for $TcPO_2$ below 20 torr, wound healing occurs with extreme difficulty. Moderate healing occurs for $TcPO_2$ from 20 torr to 40 torr. For $TcPO_2$ 40 torr, wound healing proceeds successfully. Consequently, the value of $TcPO_2$ is an objective indicator for evaluating the local state of the tissue and the efficiency of cell metabolism.

Note that oxygen deficiency in tissues of cancerous solid tumors is also a major factor limiting the effectiveness of the photodynamic therapy method [5–7].

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In clinical practice, various methods are used to eliminate an oxygen shortage in tissues, in particular the method of forced ventilation of the lungs with pure O_2 at normal atmospheric pressure (normobaric oxygenation). The effectiveness of tissue saturation with oxygen (elimination of tissue hypoxia) increases considerably when using the hyperbaric oxygenation method (HBO). The HBO technology is based on exposure to pure oxygen in a special chamber with O_2 gage pressure above atmospheric [8, 9].

The HBO method is used to treat quite different types of illnesses associated with tissue hypoxia. Moreover, in order to eliminate local tissue hypoxia, the entire body has be exposed to O_2 above atmospheric pressure, i.e., intoxication by pure oxygen must be induced. Attempts have been made to adapt the HBO method for local exposure to oxygen directly in the area where skin tissue is damaged. But this approach has not had broad clinic application due to technical complications.

Thus the problem of eliminating local hypoxia in biological tissues remains an urgent one, and its solution will make it possible to considerably improve the efficacy of treatment for many diseases.

A fundamentally new approach and an original solution to this problem are presented in [10, 11], where for the first time it was suggested to use laser-induced photodissociation (LIP) of blood oxyhemoglobin (HbO_2) *in vivo* to increase the local concentration of free oxygen O_2 in biological tissues. Considering the key role of O_2 in aerobic metabolism, elimination of local tissue hypoxia may be considered as the primary mechanism for the biostimulating and therapeutic effect of low-intensity laser radiation.

The idea essentially is that when blood is exposed to laser radiation *in vivo*, particularly transcutaneously, some of the radiation inevitably is absorbed by HbO_2 in skin blood vessels. In this case, photodissociation of HbO_2 is induced (with quantum yield ~10% [12]) with liberation of oxygen (O_2) and recovery of the hemoglobin (Hb): $[HbO_2] \rightarrow [Hb] + [O_2]$. The additional liberation of oxygen caused by laser-induced photodissociation makes it possible to eliminate tissue hypoxia, stimulate aerobic cell metabolism, and consequently achieve the desired therapeutic effect.

In this paper, we present the results of modeling and experimental studies *in vivo* on elimination of tissue hypoxia using low-intensity laser radiation. We show that in the exposed area, low-intensity laser radiation increases the degree of oxygenation of the biological tissue ($TcPO_2$).

Modeling the Interaction between Laser Radiation and Skin Tissue. Mathematical modeling of the interaction between laser radiation and skin tissue is based on the use of the basic principles presented in [13]. A key factor in considering the effectiveness of interaction between laser radiation and oxyhemoglobin in skin blood vessels is the fact that under *in vivo* conditions, it is impossible to use the absorption spectrum of HbO_2 obtained *in vitro*. This is due to the screening effect of the skin, and consequently the effective absorption spectrum of HbO_2 *in vivo* is considerably different from the original absorption spectrum. So in determining the most effective wavelengths for having an effect on HbO_2 (the action spectrum), we need to consider the optical properties of the skin. It is of practical interest to establish the relationship between the laser parameters, the optical properties, and the depth at which the blood vessels are located in the skin tissue.

Fig. 1 gives the structural model and a diagram showing the interaction between light and skin tissue used in the calculations. The optical properties of the skin in this model are determined by the three basic layers: the horny layer (stratum corneum), the epidermis, and the dermis.

In the calculations, we considered the features of the spectral and optical characteristics of each layer. Thus light incident on the horny layer undergoes partial reflection. The second layer (the epidermis) contains the pigment melanin, one of the major chromophores intensely absorbing light over a broad spectral range. The next layer (the dermis) contains collagens, causing significant scattering of the light.

Skin blood vessels are mainly found at two levels: in the surface layer, close to the epidermis; and in a deep layer, at the boundary between the dermis and the subcutaneous fatty tissue. In order to determine the action spectrum for oxyhemoglobin, we need to take into account all the factors listed above.

The action spectra were calculated using the Kubelka–Munk optical model [14] for light-colored skin, constructed assuming a homogeneous distribution of the major chromophores over the corresponding layers. Such an approach makes it possible to obtain qualitative data on the interaction between light and the components of skin tissue. In the calculations, the thickness of the horny layer was 10 μm, the thickness of the epidermis was 100 μm, and the thickness of the dermis was 3 mm. Furthermore, the extinction coefficients of aqueous solutions of HbO_2 and melanin at the maximum of the absorption band in the visible region were 10^5 and $1.3 \cdot 10^5$ $L \cdot m^{-1} \cdot cm^{-1}$ respectively.

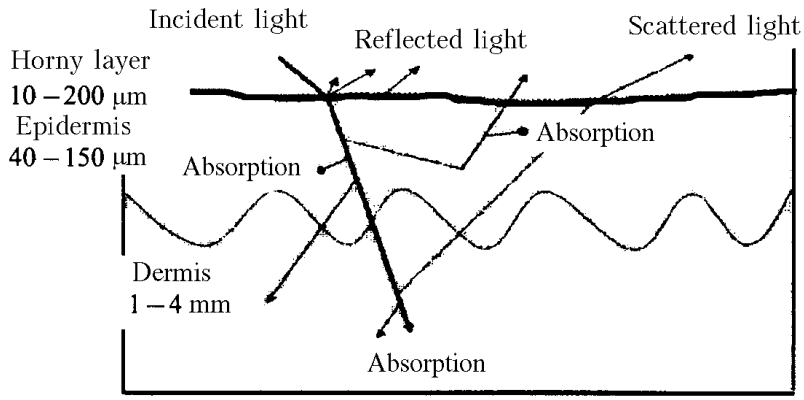

Fig. 1 Structural model and diagram showing interaction between light and skin tissue.

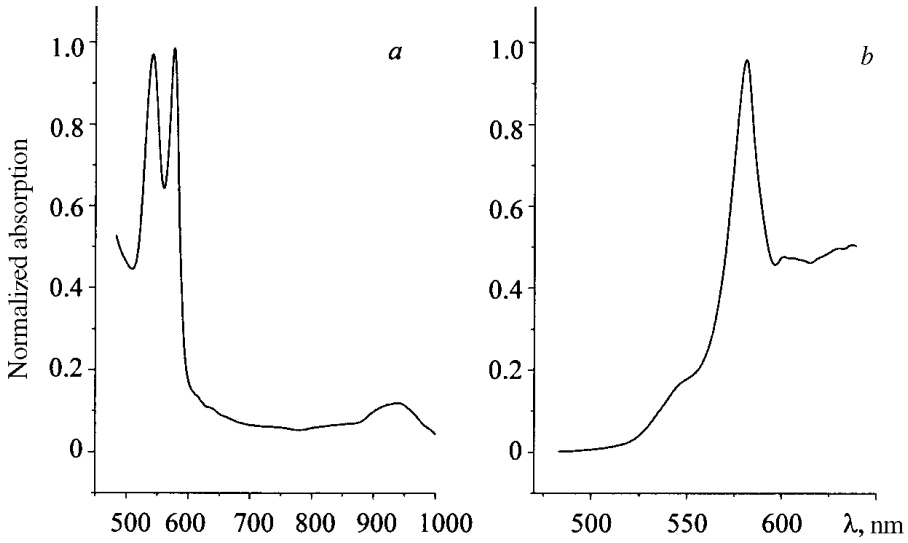

Fig. 2 Typical absorption spectrum of the *Q* band for HbO_2 *in vitro* (a) and the calculated action spectrum for HbO_2 in blood vessels located at a depth of 2 mm from the skin surface (b).

As we know, the HbO_2 absorption spectrum *in vitro* has pronounced bands over a broad spectral range (Fig. 2a). Under *in vivo* conditions, the effectiveness of action of the light on the blood components in skin blood vessels is mainly determined by absorption of the pigment melanin in the epidermis. Taking into account light scattered from collagens, the effective absorption by HbO_2 also depends on the depth at which the blood vessels are located in the skin tissue. These factors are key in determining the wavelengths of the laser radiation which will ensure effective irradiation of the blood components, in particular HbO_2.

The modeling results in fact demonstrate a substantial difference between the absorption spectra of blood HbO_2 *in vitro* and *in vivo*. Fig. 2b shows the calculated effective absorption spectrum for HbO_2 at a depth of 2 mm in the skin tissue. As we see, under *in vivo* conditions, the HbO_2 absorption band is shifted toward longer wavelengths (λ_{max} = 585 nm). Furthermore, *in vivo* the absorption band ($\Delta\lambda \approx$ 25–30 nm) is narrowed compared with the original band *in vitro*. Consequently, the need increases for selectivity of the source for effective irradiation of HbO_2 *in vivo* through the skin. Thus the calculations show that at a depth of 2 mm, HbO_2 in skin blood vessels is most effectively irradiated by radiation in the visible spectral range with λ= 585 nm.

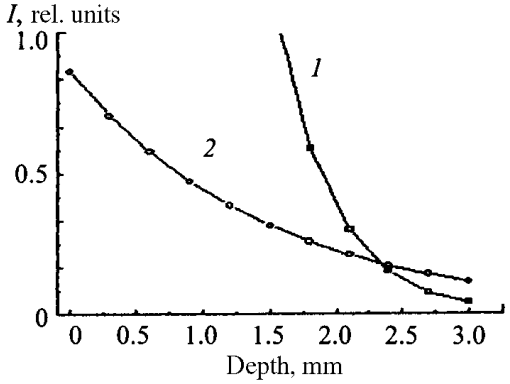

Fig. 3 Effectiveness of absorption of laser radiation by oxyhemoglobin at the maxima of the absorption bands at 585 nm (1) and 960 nm (2) at different depths from the skin surface.

For irradiation of blood HbO_2 in deeper skin blood vessels, it is more effective to use IR radiation in the spectral range 600–1200 nm. This band has a flat shape with maximum at $\lambda \approx 960$ nm and plays a dominant role in absorption of laser radiation by HbO_2 in blood vessels located at a significant depth. Note that the quantum yield for photodissociation of HbO_2 at this wavelength is the same as in the visible region of the spectrum [12]. This makes it possible to induce photodissociation of HbO_2 in blood vessels rather far away from the skin surface.

Fig. 3 shows the effectiveness of absorption of laser radiation by oxyhemoglobin for $\lambda \approx 585$ nm and 960 nm vs. the depth of the skin tissue. As we see from Fig. 3, the effectiveness of HbO_2 absorption at $\lambda = 585$ nm is higher than for $\lambda = 960$ nm all the way down to a depth of 2 mm. At a depth of ≈2.5 mm, oxyhemoglobin absorbs equally well at both wavelengths. For irradiation of deeper blood vessels, it is advisable to use laser radiation in the IR range.

The modeling results obtained allow us to not only determine the qualitative characteristics of the process of interaction between laser radiation and skin tissue, but also to properly select the effective wavelength for stimulation of HbO_2 photodissociation *in vivo*.

Experimental Procedure. The experimental measurements of the increase in the degree of oxygenation of skin tissue when exposed to laser radiation were made by the polarographic method, using a Radiometer (Denmark) TCM-2 oxygen monitor. This method was chosen because transcutaneous oxygen monitoring (TCOM) makes it possible to directly determine $TcPO_2$ in torr.

We know that a direct method for estimating oxygen pressure in arterial blood is analysis of blood samples. A Clark sensor is used for this purpose, which is an electrolytic cell separated from the analyte blood by an oxygen-permeable membrane. The oxygen electrode contains a platinum cathode and a silver anode, connected to a voltage source. Oxygen penetrating the membrane as a result of the electrochemical reaction ($O_2 + 2H_2O + 4e^- R 4OH^-$) forms hydroxyl ions at the platinum electrode. The current in the circuit depends on the number of added electrons, which in turn is determined by the amount of oxygen diffusing into the electrolytic cell. In this case, the current detected in the electrode circuit is proportional to the value of PO_2 in the analyte blood plasma sample.

For the transcutaneous method for PO_2 determination, we use membrane sensors containing a Clark electrode and a heating element. The membrane of the electrode is brought into contact with the skin, which is warmed up to a temperature of ~44°C. At the warmer temperature, the oxygen from the capillary vessels diffuses into the epidermis and then into the electrolytic cell, where the measurement is made. The $TcPO_2$ values correspond to the PO_2 values determined in arterial blood plasma samples.

Errors in the $TcPO_2$ determination depend on the thickness of the skin, the subcutaneous blood flow, and physiological factors affecting the supply of O_2 to the skin surface (decrease in cardiac output and arterial blood pressure, appearance of central vasoconstriction/narrowing of blood vessel lumina).

In order to reduce errors in the $TcPO_2$ determination, the sensor of the instrument was placed on the skin surface at sites with high capillary pressure and minimal breakdown of blood vessels. The setup for measuring oxygen pressure in biological tissue during laser irradiation is shown in Fig. 4.

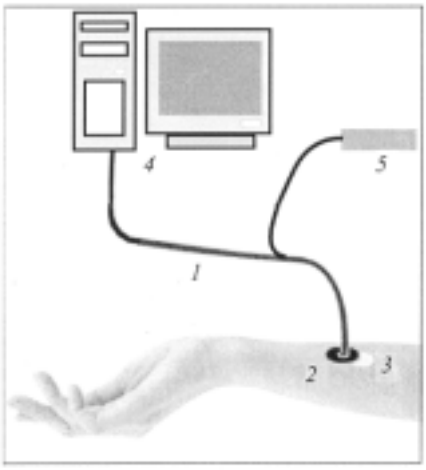

Fig. 4 Schematic drawing of $TcPO_2$ measurements during irradiation by an He–Ne laser: 1) Clark oxygen sensor 2) electrolytic cell, 3) irradiated area, 4) TCOM monitor, 5) He–Ne laser.

For local irradiation of the blood in skin blood vessels, we selected the emission from an He–Ne laser (Fig. 4) with λ = 632.8 nm, which as shown in the model calculations, falls within the effective absorption band for HbO_2 and penetrates rather deeply into skin tissue. The output power of the laser emission was 1.1 mW; the emission had a gaussian profile and, for a beam diameter of 2.5 mm, provided an irradiation power of $\approx$225 W/m^2.

The effect of laser radiation on the degree of oxygenation was studied on forearm skin tissue of volunteers under conditions eliminating prior physical and emotional stress, in a seated position at room temperature (22.5^{o}C). These precautions were needed to eliminate the influence of the indicated factors on the measurement results.

Results and Discussion. The results for measurements of the $TcPO_2$ dependence on laser radiation exposure time taken for the skin blood vessels of forearm tissues for three patients are given in Fig. 5. The initial value of $TcPO_2$ (in the absence of irradiation) was individual for each of the three patients, and characterized the initial O_2 pressure in the tissues. The dependence of $TcPO_2$ on exposure time to the emission from an He–Ne laser is shown normalized to the initial $TcPO_2$ value.

As we see from Fig. 5, the degree of oxygenation of the tissue increases as the exposure time to laser radiation increases, and after 10 min goes to a steady-state level. The data obtained clearly demonstrate that in the three studied cases, exposure to laser radiation leads to an increase in $TcPO_2$. We should note that, despite some scatter in the $TcPO_2$ measurements made at the same time, we observe a common pattern of an increase in $TcPO_2$ and going to a steady-state level. The scatter in the $TcPO_2$ values is due to individual characteristics of the state of the skin tissue, which also introduces some error into the $TcPO_2$ measurement by the TCOM method.

It is significant that the growth kinetics for $TcPO_2$ are due directly to the additional liberation of O_2 in the tissue as a result of laser-induced photodissociation of HbO_2 [15]. The oxygen liberated from HbO_2 initially increases PO_2 significantly in the blood plasma, and then diffuses into the tissue. In this case, diffusion occurs in three directions: toward the skin surface, inward toward 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, then when $TcPO_2$ increases on the skin surface by a factor of 1.6, we should expect an increase in PO_2 in the arterial blood plasma by about a factor of 4.8. It is interesting to note that such an increase in PO_2 is comparable with that achieved in the HBO method. Further studies in this direction to determine the optimal conditions for laser-induced oxygenation of tissues will make it possible to develop new laser optical methods for eliminating local hypoxia.

Thus if the blood microcirculation ensures oxygen transport into the tissue and maintains its pressure at a level of 30 torr, then by exposure to laser radiation from a He–Ne laser of only 1.1 mW power, we can increase the local $TcPO_2$ up to 50 torr. This means that using laser-induced photodissociation of oxyhemoglobin *in vivo* opens up a fundamentally new opportunity for controlling the local concentration of free molecular oxygen in tissues.

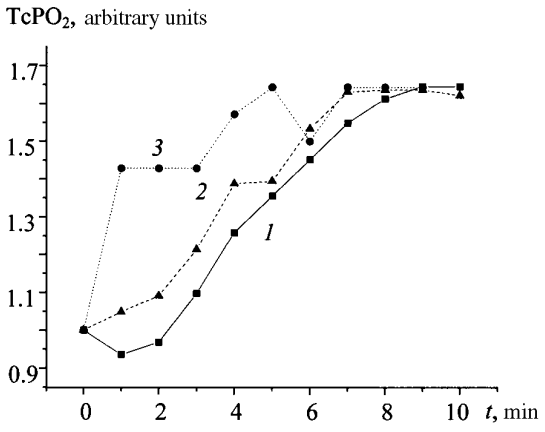

Fig. 5 Degree of oxygenation of the skin vs. exposure time to radiation from a He–Ne laser for three patients.

In [16, 17], it is shown that *in vivo* photodissociation of oxyhemoglobin is apparent in the change in the degree of oxygen saturation in arterial blood (the saturation SaO_2) when exposed to laser radiation. Thus using the pulse oximetry method, it has been established that exposure to the emission from a He–Ne laser leads to a decrease in the maximum SaO_2. And the observed effect disappears at the same time as when the laser irradiation is stopped, which leads to recovery of the maximum SaO_2 level. The appearance of HbO_2 photodissociation *in vivo* with exposure to laser radiation has been demonstrated in [16] from the response (SaO_2) by the pulse oximetry method.

The decrease in SaO_2 when exposed to laser radiation convincingly demonstrates the process of additional liberation of free molecular oxygen into the tissue as a result of photodissociation of oxyhemoglobin. Furthermore, it has been shown [17] that the effectiveness of exposure to laser radiation significantly increases as the body temperature rises (above 40^oC) compared with normal temperature.

It is interesting to note that photodissociation of oxyhemoglobin and additional liberation of free molecular oxygen are also achieved with intravenous laser irradiation of blood. Thus in [18], an increase in the partial pressure PO_2 in capillary blood plasma was experimentally detected for intravenous laser irradiation of blood. We should point out that venous blood contains ~60% HbO_2, and when exposed to the emission of a He–Ne laser, the process of photodissociation is initiated: $[HbO_2] + h\nu \rightarrow [Hb] + [O_2]$. Thus intravenous laser irradiation of blood can also be effectively used as an additional way to eliminate tissue hypoxia and to control anaerobic infections.

The results obtained show that using TCOM in monitoring $TcPO_2$ combined with other data (pulse, temperature, state of the skin) makes it possible to predict wound behavior. In this case, elimination of tissue hypoxia using laser radiation opens up an opportunity to increase $TcPO_2$ to the level needed for normal collagen synthesis and conglomeration of collagen, which plays a key role in wound healing. The results convincingly demonstrate that laser-induced photodissociation of HbO_2 *in vivo* makes it possible to increase the local concentration of free molecular oxygen in tissues, and can be used to eliminate hypoxia and to stimulate aerobic cell metabolism with the aim of achieving the needed therapeutic effect.

Conclusion. We have used transcutaneous oximetry to experimentally detect *in vivo* an increase in the degree of oxygenation of skin tissue in the area exposed to low-intensity laser radiation, due to photodissociation of oxyhemoglobin in arterial blood.

We have shown that exposure of oxyhemoglobin to low-intensity laser radiation leads to an increase in the local concentration of free molecular oxygen in tissues. This makes it possible to eliminate hypoxia, to stimulate aerobic cell metabolism, and to achieve the needed therapeutic effect.

Using laser-induced photodissociation of oxyhemoglobin in skin blood vessels opens up a fundamentally new opportunity for controlling the local concentration of free molecular oxygen in tissues.

The prospects seem good for further studies of laser-induced oxygenation of tissues, combined with transcutaneous oxygen pressure monitoring in tissue, in the direction of development of new diagnostic and therapeutic methods for determination and elimination of tissue hypoxia.

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