Journal of Engineering Physics and Thermophysics, Vol. 80, No. 1, 2007

MODEL OF PHOTODYNAMIC THERAPY OF SKIN TUMORS

S. A. Gubarev, A. A. Makhanek, and Z. P. Shul'man UDC 536.24 + 532.135]:577.344.3

We have constructed a physico-mathematical model of photodynamic therapy of skin tumors taking into account the photochemical reactions and the features of the radiation propagation, the heat and mass transfer, and the mass transfer of oxygen in the irradiation zone. The numerical solution has shown that depending on the light intensity and the degree of injury of exchange vessels the concentration of oxygen in a tumor can decrease to below the hypoxic limit, which limits the photodynamic effect. We have analyzed different methods of conducting this therapy, thus increasing its success — changing the radiation intensity and hyperoxygenation.

Introduction. Photodynamic therapy (PDT) is a promising method for treating malignant tumors combining the action of a special dye — photosensitizer (PS), oxygen, and radiation on biological tissues. Treatment consists of several sequential stages: injection of the PS (intravenous or local), leaving it for up to 24 h, and irradiation for 15–20 min. In general, any coherent or incoherent light source with a proper spectrum can be used for therapy. The most widely used devices are continuous nonionizing lasers with a power density of up to 250 W/cm^2. The wavelength is, as a rule, within the limits of the "therapeutic window" of 600–1200 nm. In this case, the light penetrates deeper into the tissue compared to the remaining part of the visible spectrum.

PDT is based on the photodynamic action (PA) affecting living structures, which was discovered at the end of the 19th to the beginning of the 20th centuries. In the experiment, death of infusoria cells stained with certain fluorescent substances exposed to radiation of sufficient intensity was shown [1]. Later the role of oxygen was established, and by PA one began to imply sensitized photooxidation of biological structures. In so doing, macromolecules and cell organelles, which do not absorb light directly, decompose. The mediators in the photoreaction — PS molecules — under the action of photons go to energetically excited states and initiate chains of biochemical reactions injuring cell elements. The dye makes the biochemical reaction sensitive to light of a certain wavelength.

PDT is characterized by high efficiency and selective action on cancer cells; locality, it is relatively cheap and easy to use. Its limitation is an increased sensitivity of patients to light for some time after intravenous injection of the PS. Injury of healthy portions of the skin and the retina of the eyes when intensive solar light falls on them is possible. Therefore, patients are recommended to wear sunglasses and stay indoors. The duration of such a period depends on the type of the dye and lasts from several days to several months.

At the molecular level destruction of cancer cells by PDT occurs through the formation of singlet oxygen (reaction of the second kind) — a very active oxidizer [1–3]:

$$\Phi C + h\nu \rightarrow \Phi C^* + O_2 \rightarrow \Phi C + \text{singlet oxygen} +$$

$$+ \text{substrate (organelles)} \rightarrow \text{oxidation of cell structures}\ , \qquad (1)$$

where $h\nu$ is the photon energy; PS* is the energetically excited PS molecule.

At the first stage, the PS molecule absorbs a light quantum and goes to an energetically excited state. Then it transfers the energy to the oxygen, changing it to the singlet form. The lifetime of singlet oxygen in biological liquids does not exceed 3 μsec, and the distance at which oxidizer molecules have time to diffuse is up to 0.1 μm.

A. V. Luikov Heat and Mass Transfer Institute, National Academy of Sciences of Belarus, 15 P. Brovka Str., Minsk, 220072, Belarus. Translated from Inzhenerno-Fizicheskii Zhurnal, Vol. 80, No. 1, pp. 76–82, January–February, 2007. Original article submitted October 10, 2006.

1062-0125/07/8001-0081

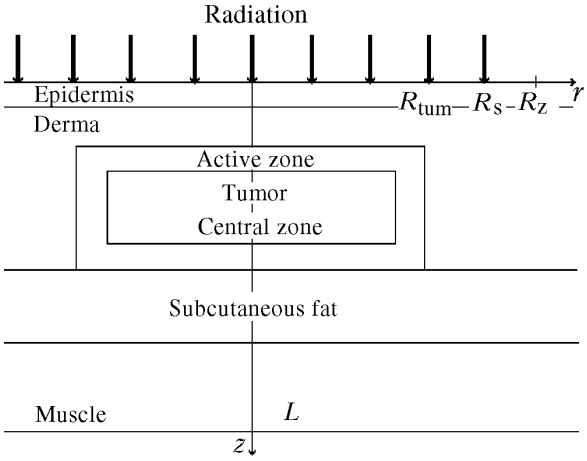

Fig. 1. Scheme of the PA zone.

Therefore, the sites of primary injury of cells and tissue are determined by the PS localization. The PDT selectivity is provided by the ability of tumors to accumulate the dye in larger quantities compared to healthy skin. A certain influence on the therapeutic effect is produced by disorders in the microcalculation system, apoptosis, inflammatory processes, and immune reactions caused by the PA [4].

As a PS, compounds of the porphyrin series: hematoporphyrin, its and derivatives, and Photofrin 2 were first used [5]. Then chlorophyll-based sensitizers — chlorins and purpurins — gained wide recognition [4]. As a PS, molecular oxygen can act (activation occurs at wavelengths near 760 and 1270 nm). At present, active work is being carried out to modify the old dyes and develop new ones — both natural and synthetic. The PS dose for intravenous injection is 1–10 mg/kg of body mass [6–8]. The method of PS injection is an important parameter of the therapy influencing the dye distribution in the body. In [9], it was shown that injection of the sensitizer directly into the tumor can lead to a considerable increase in the ratio between the dye concentrations in the tumor and adjoining healthy biological tissues. The basic requirements for the PS are: a high selectivity to malignant cells; nontoxicity and fast removal from the body; maximum adsorption in the therapeutic window; and effective generation of singlet oxygen.

Photodynamic therapy was initially used to treat skin tumors, which was due to the frequency of cases, as well as to the possibility to observe the course of therapy and its results. With the aid of waveguides and endoscopes PDT can be used to treat neoplasms localized on inner surfaces (mucous membranes, esophagus, lungs, etc.).

PDT is a complex multifactor process. It includes radiation transfer in the biological tissue, photochemical transformations of active components, heat exchange, and mass transfer of oxygen. The basic controlled parameters of therapy are: the kind and dose of the PS, the time interval between the introduction of the dye and the beginning of illumination, the wavelength and power of the radiation source, and the radiation dose. At present they are chosen mainly heuristically. Deeper understanding of the cytotoxic mechanisms of therapy, as well as substantiated choice of its conditions on the basis of the quantitative estimation of the photodynamic effect, call for the development of a physico-mathematical model of PDT taking into account the accompanying heat and mass transfer processes and the rheological properties of the blood flow.

Formulation of the Problem. The skin was modeled by plane-parallel layers, and the tumor — by a region with a central and a peripheral zone (Fig. 1). The problem was solved in a cylindrical coordinate system with the origin on the skin surface at the center of the light spot. The photochemical, thermophysical, and optical properties of the biological tissues were assumed to be constant within the limits of each layer and temperature-independent. The blood flow intensity was calculated by the temperature-time analogy (TTA) model [10]. The absence of considerable hemorheological shifts in the course of PDT [11] permitted using the TTA model parameters obtained for healthy tissues.

The description of the destruction kinetics of oncocells according to scheme (1) is based on information on the population of the ground and excited levels of PS and O_2 molecules. Figure 2 shows the Yablonskii diagrams for PS and O_2 molecules [12]. Open arrows point to radiative transitions (with absorption or emission of photons) and solid ones — to nonradiative transitions.

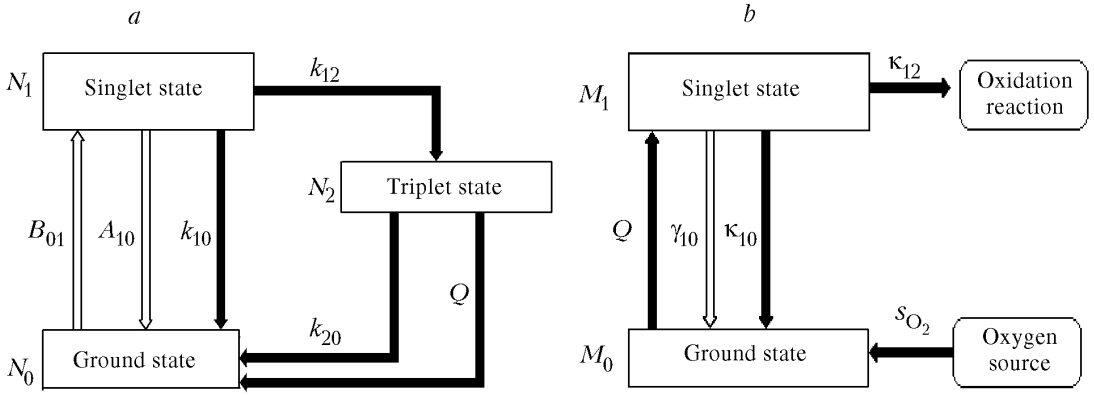

Fig. 2. Energy levels and possible transitions of PS (a) an oxygen (b) molecules.

The estimates show [1, 12] that the lifetime of the PS in the excited singlet form is 4–5 orders of magnitude shorter than in the triplet one. Therefore, N_1 is neglected in theoretical models. Assuming the dye concentration to be constant in the course of therapy, we describe the PS and O_2 balances by the system of equations

$$dN_0/dt = -N_0 \Phi B_{01} I + N_2 (k_{20} + QM_0) , \tag{2}$$

$$N_2 = N_{00} - N_0 , \tag{3}$$

$$dM_0/dt = -M_0 Q N_2 + M_1 (1/\tau_\Delta - \kappa_{12}) + s_{O_2} , \tag{4}$$

$$dM_1/dt = M_0 Q N_2 - M_1/\tau_\Delta . \tag{5}$$

The initial conditions are

$$N_0 (t=0) = N_{00} , \quad N_2 (t=0) = 0 , \quad M_0 (t=0) = M_{00} , \quad M_1 (t=0) = 0 . \tag{6}$$

The photodynamic effect is estimated by the parameter Ω — the number of malignant cells in a unit volume destroyed as a result of the PDT:

$$d\Omega (t)/dt = \varepsilon M_1 (t) \kappa_{12} . \tag{7}$$

Note that Ω includes only cells killed by the PA and does not take into account the possible death of cells caused by prolonged hypoxia or destruction of microvessels. The values of the photochemical parameters of Eqs. (2)–(7) are as follows [12–16]: Φ = 0.7, $B_{01} = 2.1 \cdot 10^{-3}$ m^2·J^{-1}, $Q = 1.4 \cdot 10^6$ m^3·mole^{-1}·sec^{-1}, $\tau_\Delta = 3 \cdot 10^{-6}$ sec, $\kappa_{12} = 10^{-6}$ sec^{-1}, $N_{00} = 7 \cdot 10^{-4}$ mole·m^{-3}, $M_{00} = 0.03$ mole·m^{-3}, $\varepsilon = 10^5$ mole^{-1}.

The index ε depends on the PS and the type of biological tissue. Its estimate was obtained in [14] by the original method of circulating a perfusate with a given content of oxygen through a colony of cells with a dye additive and simultaneous irradiation. The decrease in the oxygen concentration at the output is determined by the joint action of the metabolic and photochemical reactions; the decrease in the metabolic activity upon irradiation permits estimating the number of cells killed by the PA. The value of N_{00} has been obtained for Photofrin (molecular mass 3000) on the assumption of a uniform distribution of the PS throughout the body of an average man (0.072 m^3). It is comparable to the values of $8.7 \cdot 10^{-4}$ mole/m^3 (*in vivo* experiments on rats [15]) and $8 \cdot 10^{-4}$ mole/m^3 (*in vivo* experiments on carcinoma patients [17]). Variations in the PS distributions within one and the same tumor, as well as between different patients, are frequently observed [9, 16]. Real neoplasms can be highly heterogeneous, which should be taken into account when conducting PDT in a hospital. Quantitative estimation of the PS concentration *in vivo* before

PDT (e.g., by the fluorescence [18]) will make the prognosis more reliable and increase the effect. The value of M_{00} has been obtained on the assumption of a uniform distribution of O_2 in the biotissue before a session and corresponds to the partial pressure of O_2 — 20 Torr. In tumors, hypoxic zones (especially in the region of the necrotized nucleus) are frequently observed, and the O_2 distribution can be highly nonuniform. In the numerical experiment, we varied the N_{00} and M_{00} values. In prognosing the course of the PDT in particular patients, more exact values of N_{00}, M_{00}, and ε are needed.

For a theoretical description of PDT, the greatest problem is presented by the oxygen source s_{O_2}, which is determined by the intensification of the blood flow upon heating (and, consequently, by the increase in oxygenation) and the decrease in the metabolic utilization of oxygen O_2 because of the death of cells and the vascular stasis. Account of s_{O_2} requires a simultaneous solution of system (2)–(5) and the equations of heat transfer and mass transfer of O_2 in the course of the PA:

$$\rho c \frac{\partial T}{\partial t} = \lambda \nabla^2 T - \rho_{bl} c_{bl} W (T - T_{art}) + \rho Q_0 \cdot 1.07^{\frac{T-T_0}{b}} + \mu_a I , \tag{8}$$

$$W = W_0 \left[1 + \exp (\beta_1 \Delta T)\, \xi \exp (-\xi) \right], \quad \xi = \frac{1}{t_0^*} \int_0^t \exp (\beta_2 \Delta T)\, d\tau , \tag{9}$$

$$s_{O_2} = f (W) , \tag{10}$$

where Q_0 is the thermal flow in the biotissue due to the metabolism at temperature T_0, $W \cdot kg^{-1}$; W_0 is the blood flow intensity at temperature T_0; ΔT is the difference between the current temperature and T_0; $b = 0.5^oC$.

The radiation intensity I was calculated by the modified Bouguer law with allowance for the scattering anisotropy

$$I (z) = I_0 (1 - r_{sp}) \exp \left\{ - [\mu_a + (1 - g)\, \mu_s]\, z \right\} , \tag{11}$$

where I_0 is the light intensity on the skin surface, W/m^2.

The account of the oxygen source (10) presents the greatest analytical problem. In the numerical experiment, we considered two limiting cases — $s_{O_2} = 0$ and

$$s_{O_2} = \frac{\partial \langle M_0 \rangle_t}{\partial t} . \tag{12}$$

The method for calculating the mean concentration of O_2 in the tissue $\langle M_0 \rangle_t$ was described in [19]. In the first case, there is no additional inflow of O_2 (the oxygen is completely utilized by the metabolism in the cells or a considerable part of microvessels has been destroyed). In the second case, the effect of the PA is insignificant. In practice partial photodestruction of the vascular network is not infrequently observed, and its account requires extensive experimental-theoretical studies and is beyond the scope of the present paper.

At the initial instant of time we assume that the PS and O_2 molecules are in the ground state and are uniformly distributed, the ratio between the PS concentrations in the tumor and the adjoining healthy skin is known, the blood flow intensity is constant, and the temperature is given by the stationary heat conduction equation (8) with constant perfusion W.

We presume that by the beginning of PDT the PS molecules have been localized on the cell organelles. The characteristic time of elution of the dye from the tumor and normal tissues (the removal half-time is 16–57 h [20]) considerably exceeds the duration of the PDT session, and, therefore, we neglect the PS transport in the source of irradiation. To determine the concentrations of N_0, N_2, M_0, and M_1, we solve the Cauchy problem.

The boundary condition on the skin surface was given by means of the effective heat conductivity coefficient which took into account both the thermal radiation and the free convection of air. Under normal conditions at an am-

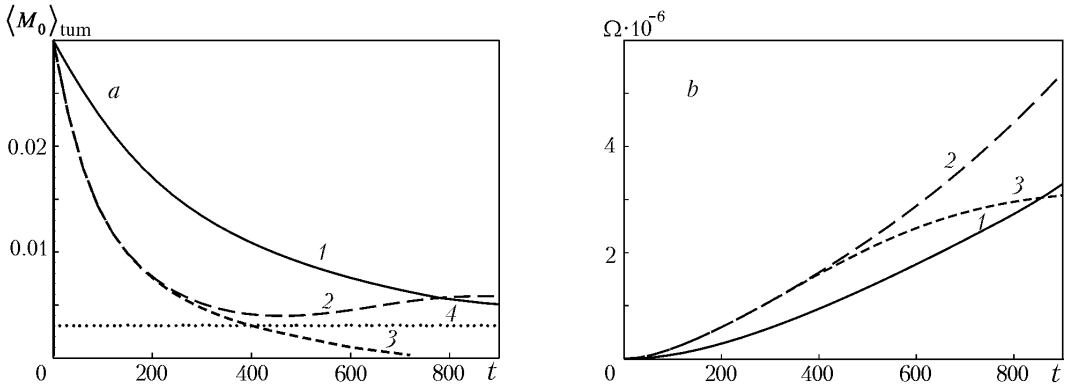

Fig. 3. Change in the average concentration of O_2 in the tumor (a) and in the cytotoxic effect (b) in the course of PDT: 1) I_0 = 50; 2) 150 mW/cm^2 and s_{O_2} calculated by (12); 3) 150 mW/cm^2 and s_{O_2} = 0; 4) hypoxic limit. $\langle M_0\rangle_{tum}$, mole·m^3; Ω, m^{-3}; t, sec.

bient temperature T_{env} = 20–23°C α_{ef} = 10 W·K^{-1}·m^{-2}. On the axis with r = 0 the flow is zero because of the temperature field symmetry. The thermal action in PDT is local; therefore, at the right and lower boundaries (Fig. 1) the flows were also taken as zero (the thermal perturbation caused by the laser energy absorption was within the outer boundaries):

$$\lambda \left.\frac{\partial T}{\partial z}\right|_{z=0} = \alpha_{ef}\left(T\big|_{z=0} - T_{env}\right), \quad \left.\frac{\partial T}{\partial z}\right|_{z=L} = 0, \quad \left.\frac{\partial T}{\partial r}\right|_{r=0} = 0, \quad \left.\frac{\partial T}{\partial r}\right|_{r=R_z} = 0. \tag{13}$$

Calculations on a new temporal layer were started from the system of photochemical equations (2)–(5) which was solved numerically by the Runge–Kutta method with automatic choice of the time step (Merson method). The criterion of changing the time step was as follows: at any point of the region the new concentration of singlet oxygen $M_1 > 0$. If this condition was met, then the step was doubled. Otherwise it was divided by 2 and the system was solved again. Then the temperature field was determined by (8) and the perfusion was calculated by formula (9). In so doing, we used an additional two-dimensional array to store the intermediate values of the parameter ξ, which permitted avoiding recalculation of the integral in (9). In the case of (12), the additional inflow of oxygen from the blood was calculated by the known values of W on the current and previous temporal layers.

Results and Discussion. Figure 3 presents the results of the calculations by model (2)–(5), (8)–(10) for various values of I_0 and s_{O_2}. At I_0 = 50 mW/cm^2 the maximum heating of the tissue did not exceed 3°C and did not lead to any considerable intensification of the blood flow. Therefore, the conditions of tumor oxygenation at s_{O_2} = 0 and by (12) were similar and, as a result, destruction of cancer cells in both cases occurred in much the same manner (Fig. 3, curve 1). In the case where I_0 = 150 mW/cm^2 and s_{O_2} = 0, the average concentration of oxygen in the tumor $\langle M_0\rangle_{tum}$ decreased to below the hypoxic limit 6.5 min after the beginning of irradiation, and by the end of the session it approached zero. If condition (12) was fulfilled, then the intensification of the blood flow caused an additional flow of oxygen which compensated for the O_2 utilized in the photochemical reactions, and the concentration of $\langle M_0\rangle_{tum}$ stabilized by the end of a PDT session at a level higher than the hypoxic one. These differences directly influenced the cytotoxic effect of the therapy (Fig. 3b). For I_0 = 150 mW/cm^2 and s_{O_2} = 0, the rate of growth of Ω decreased following the decrease in $\langle M_0\rangle_{tum}$ to below the hypotoxic limit, and at $\langle M_0\rangle_{tum} \to 0$ the cytotoxic effect reached a steady state. In case 2, this did not happen, and the number of dead cells by the end of a PDT session was 1.8 times higher than in case 3. Consequently, if destruction of cells under the PA occurs through singlet oxygen according to scheme (1), then it is more efficient to choose a PS with a weaker vascular action.

In the literature, the possibility of increasing the PA effect by decreasing the radiation intensity but preserving the aggregate irradiation dose is being analyzed. It should be noted that the rate of utilization of oxygen in the photochemical reactions will decrease and will be compensated by its inflow from the blood. This hypothesis has been verified by us on the PDT model. In case 3 (Fig. 3), molecular O_2 in the tumor burns up practically completely 10

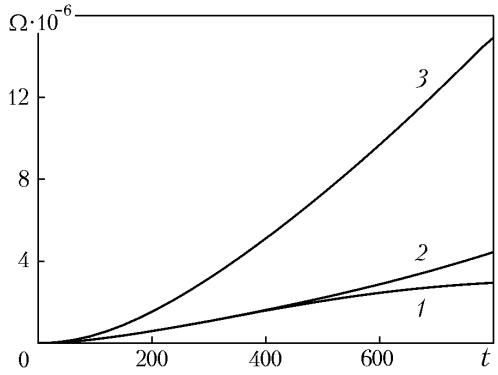

Fig. 4. Influence of hyperoxygenation on the PDT effect.

min after the beginning of irradiation. This leads to stabilization of the number of damaged cells, so that further illumination is ineffective. The decrease in the source intensity to I_0 = 50 mW/cm^2 caused a decrease in the rate of destruction of cells at the beginning of the procedure. However, as Ω stabilized, at I_0 = 150 mW/cm^2 the difference between curves 1 and 3 decreased, and 14 min after the beginning of irradiation the number of destroyed cells in both cases became equal. Continuation of irradiation heightened the cytotoxic effect of the PDT in case 1 compared to case 3. Thus, at a strong destruction of exchange vessels in the course of the PA a decrease in I_0 does potentiate the effect of therapy.

If the photodynamic action on microvessels in weak and (12) is fulfilled, then complete burnup of O_2 in the tumor does not occur and the cytotoxic effect for I_0 = 150 mW/cm^2 grows throughout the irradiation time. As a result, a decrease in I_0 will only lead to a decrease in the rate of destruction of oncocells, which will require a longer irradiation time to reach an analogous effect. Therefore, at a weak destruction of exchange vessels in the course of PDT a decrease in the laser intensity does not lead to an increase in the therapeutic efficiency.

A promising method for increasing the PDT efficiency is hyperoxygenation of tissues. It is realized by inhaling oxygen-enriched gaseous mixtures at atmospheric or higher pressure. In this case, it is possible to considerably reduce the share of zones with a lower concentration of oxygen in the tumor, as well as to compensate, partially or completely, for the O_2 utilized in the photochemical reactions. The results of the numerical experiment with inhalation of 98% oxygen at a pressure of 1 atm are shown in Fig. 4. The PDT effect by the end of a session for M_{00} = 156 mmole/m^3 is about five times higher than in the case of M_{00} = 31.2 mmole/m^3. Upon inhalation of 98% oxygen after 15-min irradiation the average concentration of oxygen in the tumor turns out to be much higher than the hypoxic limit, which permits increasing the duration of actions. Another way of increasing the cytotoxic effect of PDT is hyperbaric breathing, as well as inhalation of an oxygen-enriched gaseous mixture throughout the procedure and only before its beginning.

A further improvement of the PDT model requires determination of the photochemical constants for a wide range of dyes and biotissues, account of the photoburnup of the PS and the partial destruction of the vascular network in the course of therapy, and detailed account of the mass transfer of oxygen.

Conclusions. We have constructed a physico-mathematical model of PDT of skin tumors taking into account the photochemical reactions, the features of the radiation propagation, the heat transfer, the rheological factor of the blood flow, and the mass transfer of oxygen in the region of the photodynamic action. In the numerical experiment, it has been shown that in the course of therapy a decrease in the oxygen concentration to below the hypoxic limit with a strong destruction of exchange vessels is possible. This limits the cytotoxic effect of PDT. It has been shown that a decrease in the therapeutic laser intensity in the case of a strong injury of microvessels in the course of the PA increases the result of therapy (a decrease in I_0 from 150 to 50 mW/cm^2 has led to an 80% increase in the number of dead cells upon 15-min irradiation). If the destruction of the vascular system is insignificant, then a decrease in the laser intensity is ineffective. Hyperoxygenation considerably intensifies the cytodestruction in the course of PDT: for I_0 = 150 mW/cm^2 the number of cancer cells killed in the course of therapy upon inhalation of 98% oxygen at atmospheric pressure is five times larger than in the case of usual air, and the oxygen concentration in the tumor does

not fall below the hypoxic limit, which permits prolonging the irradiation. Hyperbaric breathing, as well as inhalation of oxygen-enriched gaseous mixtures throughout the session, permits additional amplification of the effect.

NOTATION

A_{10}, fluorescence rate of the PS, $\sec^{-1}$; $B_{01} = 2.1 \cdot 10^{-3}$, activation parameter of the PS, $m^2 \cdot J^{-1}$; c, specific heat capacity, $J \cdot kg \cdot K^{-1}$; g, mean cosine of the radiation scattering phase function in the biotissue; $h = 6.626 \cdot 10^{-34}$, Planck constant, J·sec; I, radiation intensity, $W \cdot m^{-2}$; k_{10}, k_{12}, $k_{20} = 4 \cdot 10^4$, rates of nonradiative spontaneous transitions of the PS, $\sec^{-1}$; L, skin thickness, m; M_0, oxygen concentration in the ground state, $mole \cdot m^{-3}$; M_{00}, initial concentration of oxygen, $mole \cdot m^{-3}$; M_1, concentration of singlet oxygen, $mole \cdot m^{-3}$; N_0, PS concentration in the ground state, $mole \cdot m^{-3}$; N_1, PS concentration in the excited singlet state, $mole \cdot m^{-3}$; N_{00}, initial concentration of the PS, $mole \cdot m^{-3}$; N_2, PS concentration in the excited triplet state, $mole \cdot m^{-3}$; $Q = 1.4 \cdot 10^6$, characteristic of photosensitizer quenching, $m^3 \cdot mole^{-1} \cdot sec^{-1}$; R, radius, m; r, variable, m; r_{sp}, coefficient of specular reflection of light on the skin surface; s_{O_2}, source/sink of oxygen in biotissue, $mole \cdot m^{-3} \cdot sec^{-1}$; T, temperature, ^oC; t, time, sec; $t_0^* = 4.7 \cdot 10^5$, parameter of the TTA model, $\sec^{-1}$; W, perfusion, $\sec^{-1}$; z, variable, m; α_{ef}, effective heat conductivity coefficient, $W \cdot m^{-2} \cdot K^{-1}$; β_1, β_2, parameters of the TTA model, $^oC^{-1}$; γ_{10}, phosphorescence rate of oxygen, $\sec^{-1}$; ε, sensitivity of biotissue to the action of singlet oxygen (number of cancer cells killed in a unit volume per 1 mole of oxidizer), $mole^{-1}$; κ_{10}, $\kappa_{12} = 10^{-6}$, rates of nonradiative transitions of oxygen, $\sec^{-1}$; λ, heat conductivity coefficient, $W \cdot m^{-1} \cdot K^{-1}$; μ_a, radiation absorption coefficient, m^{-1}; μ_s, radiation scattering coefficient, m^{-1}; ν, therapeutic laser radiation frequency, Hz; ξ, parameter of the TTA model; ρ, density, $kg \cdot m^{-3}$; $\tau_\Delta = (\gamma_{10} + \kappa_{10} + \kappa_{12})^{-1}$, lifetime of singlet oxygen, sec; $\Phi = A_{10}/(A_{10} + k_{10} + k_{12})$, quantum outlet of PS in excited triplet state; Ω, photodynamic destruction rate, m^{-3}. Subscripts: O_2, oxygen; a, absorption; s, scattering; sp, specular; art, arterial; bl, blood; z, irradiation zone; tum, tumor; s, light spot; env, environment; t, tissue; ef, effective.

REFERENCES

1. A. A. Krasnovskii, Photodynamic action and singlet oxygen, *Biofizika*, **49**, Issue 2, 305–321 (2004).
2. A. Sienkiewicz and A. Graczyk, Photodynamic therapy — photochemical and photophysical principles, *Biocybern. Biomed. Eng.*, **11**, Nos. 1–2, 23–36 (1991).
3. K. R. Weishaupt, C. J. Gomer, and T. J. Dougherty, Identification of singlet oxygen as the cytotoxic agent in photo-inactivation of a murine tumor, *Cancer Res.*, **36**, 2326–2329 (1976).
4. I. Wang, *Photodynamic Therapy and Laser-Based Diagnostic Studies of Malignant Tumours*, Doctoral Thesis, Department of Oncology, Lund University Hospital (1999).
5. T. J. Dougherty, Hematoporphyrin as a photosensitizer of tumors, *Photochem. Photobiol.*, **38**, No. 3, 377–379 (1983).
6. C. J. Gomer, N. Rucker, N. J. Razum, et al., In vitro and in vivo light dose rate effects related to hematoporphyrin derivative photodynamic therapy, *Cancer Res.*, **45**, 1973–1977 (1985).
7. B. W. Henderson and V. H. Fingar, Relationship of tumor hypoxia and response to photodynamic treatment in an experimental mouse tumor, *Cancer Res.*, **37**, 3110–3114 (1987).
8. C. H. Sibata, V. C. Colussi, and N. L. Oleinick, Photodynamic therapy: a new concept in medical treatment, *Braz. J. Med. Biol. Res.*, **33**, 869–880 (2000).
9. C. C. Lee, Spatial heterogeneity and temporal kinetics of photosensitizer (A1PcS$_2$) concentration in murine tumors RIF-1 and MTG-B, *Photochem. Photobiol. Sci.*, **2**, 145–150 (2003).
10. A. S. Gubarev, Thermal effect in photodynamic therapy of tumors, in: *Proc. of 5th Minsk Int. Forum on Heat and Mass Transfer "Heat and Mass Transfer–MIF-2004"* [in Russian], May 24–28 2004, Minsk (2004), pp. 126–127.
11. A. A. Makhanek, Yu. P. Istomin, Z. P. Shu'man, et al., Influence of the photodynamic action on the rheological properties of the blood of donors and experimental animals, *Vestsi Nats. Akad. Navuk Belarusi*, *Ser. Biyal. Navuk*, No. 3, 89–95 (2004).

12. H. J. C. M. Sterenborg and M. J. C. van Gemert, Photodynamic therapy with pulsed light sources: a theoretical analysis, *Phys. Med. Biol.*, **46**, 835–849 (1996).
13. R. Bonnet, C. Lambert, E. J. Land, et al., The triplet and radical species of hematoporphyrin and some of its derivatives, *Photochem. Photobiol.*, **38**, No. 1, 1–8 (1983).
14. T. Schunck and P. Poulet, Oxygen consumption through metabolism and photodynamic reactions in cells cultured on microbeads, *Phys. Med. Biol.*, **45**, 103–119 (2000).
15. B. W. Pogue, R. D. Braun, J. L. Lanzen, et al., Analysis of the heterogeneity of pO_2 dynamics during photodynamic therapy with verteporfin, *Photochem. Photobiol.*, **74**, No. 5, 700–706 (2001).
16. X. Zhou, B. W. Pogue, B. Chen, et al., Pretreatment photosensitizer dosimetry reduces variation in tumor response, *Int. J. Rad. Oncol. Biol. Phys.*, **64**, No. 4, 1211–1220 (2006).
17. B. W. Henderson, T. M. Busch, and L. A. Vaughan, Photofrin photodynamic therapy can significantly deplete or preserve oxygenation in human basal cell carcinomas during treatment, depending on fluence rate, *Cancer Res.*, **60**, 525–529 (2000).
18. B. Pogue and T. Hasan, Fluorophore quantitation in tissue-simulating media with confocal detection, *IEEE J. Sel. Top. Quant. Electron.*, **2**, No. 4, 959–964 (1996).
19. S. A. Gubarev, A. A. Makhanek, and Z. P. Shul'man, Model of human skin oxygenation under thermal action, *Inzh.-Fiz. Zh.*, **80**, No. 1, 70–75 (2007).
20. A. A. Makhanek, É. A. Zhavrid, Yu. P. Istomin, et al., *Physical and Mathematical Description of the Processes of Heat and Mass Transfer in Human Skin in Photodynamic Therapy of Tumours* [in Russian], Preprint No. 4 of the A. V. Luikov Heat and Mass Transfer Institute, National Academy of Sciences of Belarus, Minsk (2002).
21. Q. Chen, Z. Huang, H. Chen, et al., Improvement of tumor response by manipulation of tumor oxygenation during photodynamic therapy, *Photochem. Photobiol.*, **76**, No. 2, 197–203 (2002).