Optimization of Light Action Spectra for Porphyrins *in Vivo*

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***Abstract* — Light action spectra for main skin chromophores are simulated. Porphyrins are selected as target chromophores. They can produce singlet oxygen (SO) reactions under tissue irradiation, which acts as a natural photosensitizer. The SO is toxic for, e.g. cancer cells. This process is known to be widely used in photodynamic therapy (PDT). It is shown by way of examples that skin irradiation by red light, where the porphyrins have local maxima of light absorption, can improve the generation of SO as compared with the irradiation at a wavelength corresponding to the absolute maximum of light absorption. Taking etio- and rhodoporphyrin as target chromophores, we obtained that the said improvement of SO generation can be about 5 to 100 times at specific depths inside tissue and 10 to 20 times integrally for the whole dermis thickness. The presented results can provide new opportunities for the selection of the irradiation wavelengths under application of traditional PDT methods.**

***Index Terms* — multi-layered biological tissues, skin, singlet oxygen, porphyrins, radiative transfer equation, light action spectra.**

I. INTRODUCTION

Natural and artificial porphyrins are sometimes used as photosensitizers (PS) for the effective selective destruction of malignant tumors. Tetrapirrol compounds localized selectively in tumor tissues were shown to be able to provide effective death cancerous and bacterial cells via the photosensitization process. Now it is generally accepted that singlet oxygen (SO) generated during energy transfer from the triplet state of a PS to molecular oxygen is the main active cytotoxic agent for photodynamic therapy of cancerous tissues *in vivo*. Porphyrins can be introduced into a human organism from outside. They are exogenic ones in this case. Porphyrins are also formed by a human organism itself (endogenic porphyrins) and can have an increased concentration under some pathologies, for example, Acne vulgaris. Porphyrins are transferred by blood over the whole organism to be natural PS for photodynamic therapy.

Generation of SO by porphyrins is a multistage process. Its first step is light absorption. Then, via several photochemical reactions, there can be formed SO molecules, which are highly toxic for cancer cells. One can roughly assume that the more light power is absorbed by porphyrins, the more SO will be formed. The objective of this work is to find, what irradiation wavelengths provide maximal SO generation or, in other words, provide maximal absorbed light power. The idea of the optimization is rather simple. Really, optical properties of soft biotissues, especially their absorption coefficients, are spectrally selective, so that the tissue acts as a spectral filter with complex transmittance. The light power absorbed by a target chromophore at specific depth z is proportional to the product of the fluence rate at this depth by the absorption coefficient of the chromophore. By varying the irradiation wavelength, one can change the filter transmittance and, hence, the fluence rate to maximize the said product. It is obvious that the optimal wavelength does not necessarily coincide with the maximum of the absorption coefficient. The below results are obtained by using the optical tissue model [1] and the analytic techniques [2 – 4] for describing radiative transfer though multilayered human skin.

II. SIMULATION METHOD

Introduce a concept of differential effective absorption coefficient (DEA) or differential action spectrum, which is meant as the number of singlet oxygen molecules $\alpha(z,\lambda)$, being formed per unit time per unit volume at depth z under the irradiation of skin surface by unit power E_0 of monochromatic light at wavelength λ:

$$\alpha(z,\lambda) \approx \mu_a(\lambda)E(z,\lambda)/\ \mu_a(\lambda_{max})E(z,\lambda_{max}) \qquad (1)$$

Here $\mu_a(\lambda)$ is the absorption coefficient of a chromophore, $E(z,\lambda)$ is the fluence rate, and λ_{max} is the wavelength corresponding to the maximal absorption of a specific chromophore. By definition, $E(z,\lambda)$ is $E(z,\lambda) = \int_{4\pi} I(\lambda, z, \vartheta, \phi)d\Omega$, where $I(\lambda,z,\Omega)$ is the light intensity (or radiance) as a function of angular coordinates ϑ and ϕ, $d\Omega = \sin(\vartheta)d\vartheta d\phi$ is the solid angle element. The $E(z,\lambda)$ quantity has dimension W/cm^2. Absolute (non-normalized) values of DEA have dimension cm^{-3}·s^{-1}. The quantity of Eq. (1) characterizes how effective the irradiation of the tissue surface at wavelength λ is as compared with that at wavelength λ_{max}. The more α values are, the more SO molecules are formed under the same light power incident on skin surface.

Figure 1 shows the spectra of optical density [5] of some typical synthetic porphyrins. Note several absorption peaks in the visible, for example at $\lambda \approx$ 490 – 510, 530 – 540, 565

– 575, and 620 – 635 nm. There are also the intense absorption maxima in the violet – blue range (the Sauret band), but they will be omitted below, because the light penetration depth at $\lambda \approx 400$ nm is small to be on the order of fractions of millimeter [3]. By this reason, the violet – blue light acts on the near-surface tissue layer only and has essentially no effect on deeper dermis layers. The spectra shown in Fig. 1 are typical for other porphyrins, including endogenic ones.

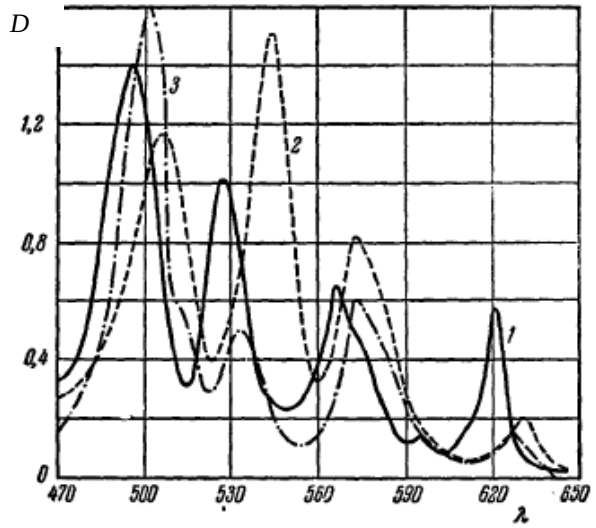

Fig. 1. Dependence of optical density D on λ, nm for etioporphyrin (1), rhodoporphyrin (2), and phylloporphyrin (3) according to data of [5]

Integration of Eq. (1) by dermis thickness gives the integral effective absorption coefficient (IEA), which characterizes the total number of SO molecules formed in dermis layer of thickness $z_2 - z_1$ per unit time per unit area due to light absorption by a chromophore:

$$\beta(\lambda) = \mu_a(\lambda) \int_{z_1}^{z_2} E(z,\lambda)dz \,/\, \mu_a(\lambda_{\max}) \int_{z_1}^{z_2} E(z,\lambda_{\max})dz \,. \qquad (2)$$

Therefore, to simulate the mechanism of the SO formation, one needs to determine fluence rate E at different depths and to further calculate DEA and IEA by the simple formulas of Eqs. (1) and (2). The light fields in biotissue are obviously to depend on its biophysical, structural and optical characteristics. We will use below the analytical procedure of Refs. [2 – 4].

III. STRUCTURE AND OPTICAL CHARACTERISTICS OF SKIN

The base for making the investigations is the model of structural and spectral properties of near-surface tissue layers over the wavelength range of 300 to 100 nm. The model has been constructed by the way of critical evaluation and generalization of various published experimental and theoretical data. Skin is assumed to be a three-layered medium composing of stratum corneum, epidermis, and dermis. For the two latter layers, the volume fractions of the main absorbing chromophores, melanin f_m in epidermis and blood C_v in dermis, can vary. The model enables one to set optical dimensions and spectral characteristics of tissue by its known structural (layer thicknesses) and biophysical parameters. The spectral characteristics are meant as the dependences of extinction and absorption coefficients and phase functions in each skin layer on the wavelength, and the biophysical ones are meant as the concentrations f_m and C_v, capillary hematocrit H (blood volume fraction in capillaries occupied by erythrocytes), fraction C_h of erythrocyte volume occupied by hemoglobin, and blood oxygenation degree S (the ratio of HbO_2 volume to the total hemoglobin). We will assume further that the following parameters are fixed: $S = 0.75$, $H = 0.4$, $C_h = 0.25$. Besides, set the thicknesses of stratum corneum and epidermis constant for concreteness. Dermis is assumed to be a semi-infinite layer.

IV. DEPTH DEPENDENCES OF FLUENCE RATE AND LIGHT ACTION SPECTRA FOR BLOOD AND TISSUE

Light absorption is known to be one of possible mechanisms of light action on biological tissues. In any case, it always accompanies phototherapeutic irradiation of a living organism in practice. As one can see from Eqs. (1) and (2), light action spectra depend on depth distributions of fluence rate. Consider first the dependences of E on z. Two cases are represented below, namely, uniform and inhomogeneous dermis. Table [6] gives the structural and biophysical properties of dermis in the latter case.

Table. Structural and biophysical parameters of multi-layered dermis and subcutaneous fat [6]

	Upper bound z, mm	Thickness, mm	C_V
Dermis with capillary loops	0.08	0.25	0.04
Dermis with superficial vascular network	0.33	0.08	0.08
Dermis	0.41	1.5	0.05
Dermis with deep vascular network	1.91	0.18	0.14
Subcutaneous fat	2.09	6	0.06

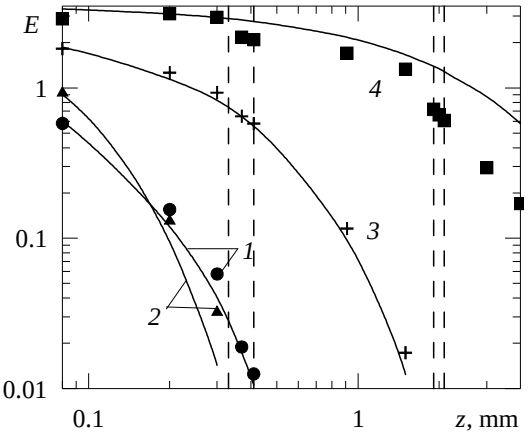

Fig. 2. Depth profiles of normalized fluence rate in uniform (curves, $C_V = 0.0595$) and multi-layered dermis (see Table) at λ = 350 (*1*), 418 (*2*), 550 (*3*), and 800 nm (*4*), $f_m = 0.08$

Figure 2 [4] illustrates profiles $E(z)$ in tissue (normalized by incident flux E_0), which are created by light at different wavelengths in uniform (curves) and inhomogeneous dermis (symbols). The bounds of dermis sublayers are

shown by vertical dashed lines in Fig. 2. The mean volume concentration of blood capillaries for the both cases is set the same, namely C_V = 0.0595. One can see from Fig. 2 that $E(z) > 1$ at λ = 550 and 800 nm (curves *3* and *4*) up to depth $z \cong 0.3$ and 2 mm, respectively. This is due to the multiple light re-reflections between tissue layers.

As follows from Fig. 2, fluence rate is essentially independent of the dermis structure in the upper skin layers at $z < 2$ mm. With z increasing, there can appear differences in depth profiles $E(z)$ for the two skin models. This is especially evident at λ = 800 nm (symbols *4*). There are kinks of $E(z)$ at the sublayers boundaries for multi-layered dermis, where optical properties of the medium change. For uniform dermis in this case (curve *4*), values of $E(z)$ change smoothly with z increasing. At all the considered wavelengths of the 300 to 1000 nm range, absolute values of $E(z)$ change too at $z > 2$ mm. This can be seen more obviously at λ = 800 nm, where the fluence rate for inhomogeneous dermis is substantially smaller than for the uniform one. Here is observed the shadowing of lower sublayers by upper ones due to generally higher capillary concentration of as compared with the uniform dermis. Below, the dermis will be considered as an uniform layer.

The main absorbing chromophores of skin dermis are blood and bloodless tissue. Fig. 3 shows absorbed fluence rate E as a function of depth z beneath the skin surface. One can see that at λ = 575 nm, where blood absorbs light strongly, E values are mainly determined by blood only. At λ = 630 and 800 nm, E values are due to the combined absorption by blood and tissue collagen fibers. One can isolate three regions in the shown dependences. The first and third ones are at, respectively, small and large depths, where absorbed power P slowly or rapidly decreases with z to be approximately described by an exponential function with low and big exponents, correspondingly. The second region is intermediate. Here the $E(z)$ dependence cannot be represented by an exponential function.

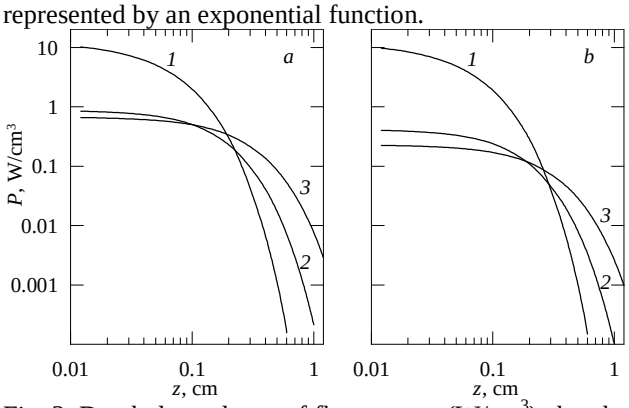

Fig. 3. Depth dependence of fluence rate (W/cm^3) absorbed by unit volume of skin tissue (a) and blood (b) at wavelengths λ = 575 (curves 1), 630 (2) or 800 nm (3), f_b = 0.04, f_m = 0.08, S = 0.75, E_0 = 1 W/cm^2

V. OPTIMIZATION OF SINGLET OXYGEN GENERATION UNDER LIGHT ACTION FOR PORPHYRINS

Figure 4 shows the DEA and IEA spectra for etioporphyrin normalized by the corresponding values at λ_{max} = 495 nm (see Fig. 1). One can see from Fig. 2*a* that, in the upper dermis layers at $z \le 0.15$ mm (curve *2*), light at wavelength λ_{max} corresponding to the maximal absorption provides the most effective SO generation. However in the deeper dermis layers (кривые *3* to *5*), there is the definite increase in the absorbed light power under the irradiation of the surface by light with λ = 620 – 630 nm near the local extreme of optical density $D(\lambda)$ of etioporphyrin. The said increase can be 50- and more-fold as compared with the irradiation at λ_{max} = 495 nm.

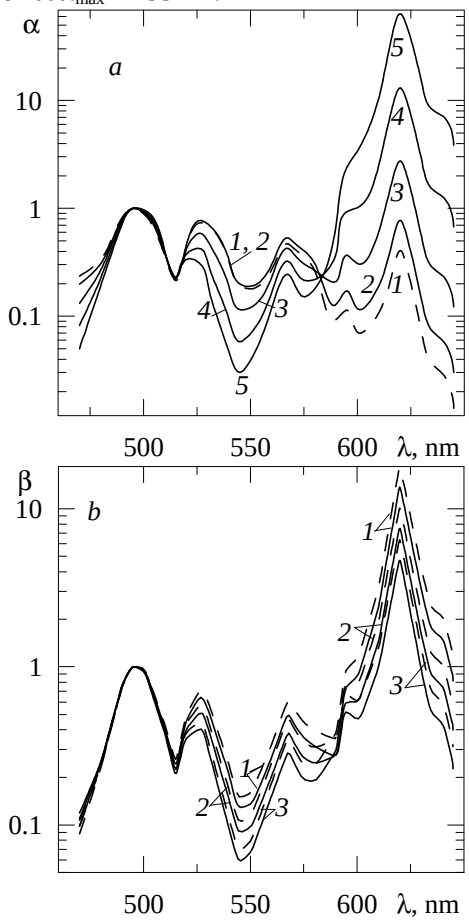

Fig. 4. Normalized DEA (*a*) and IEA spectra of etioporphyrin (*b*) at (*a*) – z = 0.15 (curve *2*), 1 (*3*), 2 (*4*), and 3 mm (5), f_m =0.08, C_V = 0.04; (*b*) – f_m =0.08 (solid) и 0.16 (dashed curves), C_V = 0.02 (1), 0.04 (2), and 0.08 (3), z = 1 mm. Dashed curve *1* in Fig. 4*a* shows the original normalized absorption spectrum of etioporphyrin

The IEA spectra (Fig. 4*b*) demonstrate also the considerable increase in the absorbed light power under the irradiation by red light, λ = 620 – 630 nm. The increase here can be 10- and more-fold. The β values depend on structural and biophysical tissue parameters and, in particular, on volume fraction f_m and C_V of melanin and blood capillaries. However, the variations of these concentrations do not lead to substantial shift of the maximal IEA.

Figure 5 gives the DEA and IEA spectra of rhodoporphyrin, normalized by the corresponding values at при λ_{max} = 545 nm (see Fig. 1). Here (Fig. 5*a*), as in the case of etioporphyrin, the irradiation at wavelength λ_{max} near the absolute absorption maximum provides the most effective SO generation in the upper dermis regions. However, as opposed to the case of etioporphyrin, there are two maxima of normalized DEA with z increasing (curves *3 – 5*). They occur at $\lambda \approx$ 510 and 630 nm near the local

extremes of optical density $D(\lambda)$ of rhodoporphyrin. The increase of α in the short-wave spectral region as compared with λ_{max} = 545 nm is due to the features in the spectral light penetration depth into tissue [3] and, in particular, due to the spectral absorption of hemoglobin derivates [1]. Really, blood absorption near $\lambda \approx 510$ nm the blood absorption is lower and, hence, the light penetration depth is larger than at λ_{max} = 545 nm. Therefore, green light attenuates more, and product $\mu_a(\lambda)E(z,\lambda)$ assumes smaller values in this spectral region than near $\lambda \approx 510$ nm. Note also that the increase in DEA under irradiation of skin surface by red light can be up to 100 times and higher as compared with λ_{max} = 545 nm. The essential increase in the absorbed power (Fig. 5*b*) is also inherent to the IEA spectra under the irradiation at wavelengths $\lambda \approx 510$ and 630 nm.

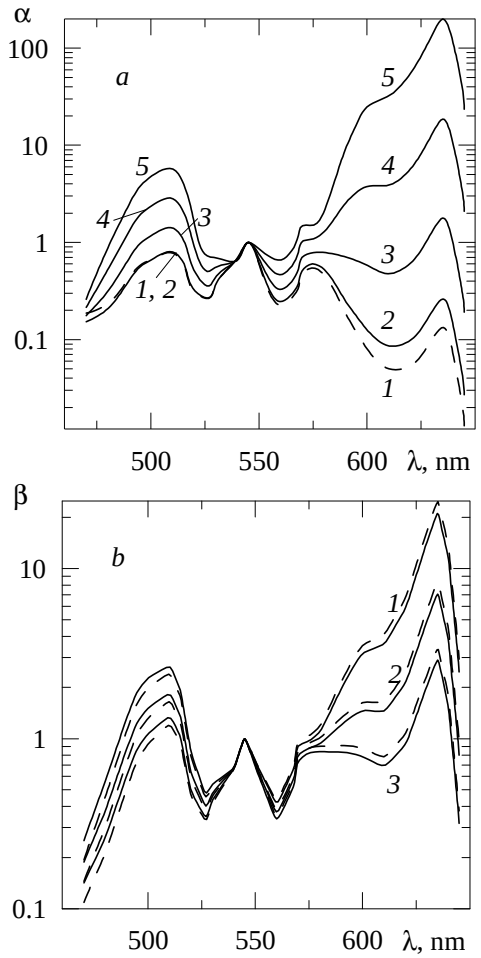

Fig. 5. The same as in Fig. 4, but for rhodoporphyrin. Dashed curve *1* in Fig. 5*a* shows the original normalized absorption spectrum of rhodoporphyrin

VI. CONCLUSION

Analytical methods for solving the radiative transfer equation were successfully used to simulate light absorption by the main tissue chromophores and the SO production by porphyrins generated under skin irradiation at different wavelengths. It was somewhat surprisingly that the big shift of the irradiation wavelength to the red spectral region located far from the absolute absorption maxima (near about 495 nm for etioporphyrin and 545 nm for rhodoporphyrin) can provide essential increase in the absorbed light power and, hence, in the SO generation. The irradiation at approximately 620 nm can deliver the maximal SO production by etioporphyrin at both a local tissue depth and in the whole dermis thickness. The increase in SO concentration can be up to 50 times at $z \geq 1$ mm and up to 20 times in the whole dermis as compared with the irradiation at λ_{max} = 495 nm. On the other hand, two irradiation wavelengths, about 510 and 630 nm, provide the maximal SO production by rhodoporphyrin as compared with the irradiation at λ_{max} = 545 nm. For 510 nm the increase in SO generation can be several times, whereas for 630 nm it can achieve 200 and more times. The observed features in the light action spectra for porphyrins are due to the spectral selectivity of optical characteristics of skin tissue and, in particular, due its absorption properties. As noted, the tissue acts as a spectral filter with complex transmittance. For example, the maximal light power absorbed by rhodoporphyrin at 510 nm is provided by complex combination of blood and porphyrin absorption as well as by multiple light scattering in the medium. The global extremes of DEA and IEA in the red have the same origins. The obtained results can be a basis for designing new methods for improving the SO generation by porphyrins as applied to practical photodynamic therapy procedures. The essential increase in SO concentration as compared with the traditional irradiation at absolute maximal absorption of porphyrins can provide such an improvement.

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