

PROMISING NEW-GENERATION PHOTSENSITIZERS FOR PHOTODYNAMIC THERAPY

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The photodynamic method for therapy of malignant tumors is extensively used all over the world. The photodynamic therapy (FDT) method is based on the capability of photosensitizers to be accumulated in tumor tissues and to acquire the marked cytotoxic properties due to optical activation. The photosensitizers are represented mainly by the porphyrin-series compounds – derivatives of hematoporphyrin and chlorine e6. Positive results have been obtained for PDT of carcinoma cutaneum, malignant tumors of head and neck, bladder, bronchi, female genital organs, metastatic cutis in the case of breast cancer, esophagus and stomach.

Further success of using the PDT method is associated with phototherapeutic compounds of new generation which are characterized by a strong absorption of light in the region of so-called phototherapeutic window from 700 to 900 nm, where light scattering by the biological tissues is low and there is practically no light absorption by such endogenic biomolecules as hemoglobin, melanin, and water. Because of this, light penetrates deep into the tissues making it possible to use PDT in treatment of both the surface and deep-lying tumors. The new-generation photosensitizers must be accumulated predominantly within tumor tissues, be minimally toxic and rapidly removable from the normal tissues to preclude the development of side effects due to the prolonged phototoxicity. One of the groups of compounds representing the new-generation phototherapeutic preparations is a group of the polymethine dyes showing particular advantages over the porphyrin series compounds. The tricarbocyanine dyes are characterized by the presence of the light absorption band with a high molar absorption factor ($>10^5 \text{ M}^{-1}\text{cm}^{-1}$) in the longer wavelength region (700–900 nm) as compared to the porphyrin derivatives.

The development of the new-generation sensitizers for photochemotherapy was based on studies of their most important parameter – ability of activation by optical radiation with the wavelength corresponding to the transparency window of biological tissues. For the majority of photosensitizers, damage of tumor cells on PDT is dependent on the medium oxygenation. To attain the identical oxygen consumption rates in the process of phototherapy, it is required to provide the same numbers of the absorbed photons in unit time at every of the irradiation wavelengths

used. This may be achieved by adequate selection of the power density for light incident on a sample. As demonstrated by the obtained results, when numbers of the absorbed photons in unit time is the same, efficiency of the photoactivated damage of cancerous HeLa cells is identical at all the three wavelengths. At the same time, the data obtained for HeLa cells and *in vivo* reveal particular differences. Damage of the tumor cells *in vivo* is growing thrice when the phototreatment wavelength is varying over the range from 668 to 780 nm. Increase in depth of necrosis for the light source with the wavelength 740 nm, compared to irradiation at 668 nm, is in line with the increased light transmission by the tissues on going to the near IR spectral region. But situation is different when compared with the wavelengths 740 and 780 nm. Proceeding from the spectrum given in the literature for the photodissociation effect of oxyhemoglobin, at the wavelength 780 nm the process efficiency is nearly twice as that at 740 nm. Therefore, a difference in the tumor damage depth for sources with different wavelengths is determined both by the difference in transmission of tissues depending on the phototreatment wavelength and by differences in the photodissociation efficiency of hemoglobin complexes in blood vessels. In this way the photochemotherapeutic damage of tumor tissues is growing thrice when the irradiation wavelength is varying from 668 to 780 nm. In the process of phototreatment *in vivo* one can observe a growth of the half-width and a blue shift in fluorescence spectra for tricarboyanines in tumor tissues due to different transmission of the tissues and growing of the local oxygen concentration.

To establish mechanisms of damage for the tumor cells, the spectral, kinetic, and photochemical properties of tricarboyanine dyes have been studied on condition that the ionic equilibrium is shifted to the increased portion of close contact pairs. The studies have been conducted considering the fact that introduction of an additional salt in low-polar solutions of polymethine dyes leads to the ionic equilibrium shifted to the increased portion of contact ion pairs including the close ones as well. In low-polar dichlorobenzene the introduction of tetrabutyl ammonium chloride results in lowering of the fluorescence quantum efficiency and fluorescence lifetime; besides, quantum efficiencies of photodestruction and of the singlet oxygen formation are growing. The latter is indicative of a considerable increase of the molecules passing to the triplet state upon addition of the salt. Thus, the introduction of salt leads to significant changes of the photophysical and photochemical characteristics of the dye due to the increased portion of close contact ion pairs. The influence exerted by the increased portion of close contact ion pairs in a solution on the free radical formation process has been investigated by examination of nonstationary absorption spectra for tricarboyanine in low-polar dichlorobenzene and in dioxane. As demonstrated by the induced absorption spectrum for tricarboyanine dye upon addition of tetrabutyl ammonium chloride, the contribution made by the additional band with $\lambda_{\max} = 735$ nm into the total spectrum of nonstationary absorption is increased indicating a growth in the number of radicals. In the process the salt addition is responsible for lowering of the dye's fluorescence quantum efficiency and lifetime in proportion correlating with a change in the intensity of a new band.

The formation of radicals may influence the patterns of the dye photodestruction in deoxygenated solutions. As photodestruction of the dyes because of self-sensitized oxidation in deoxygenated solutions is excluded, with growing numbers of radicals in such conditions, the probability of finding the results of interaction between the dye molecules and these intermediates or products of reactions with the solvent is greater.

Photodestruction of the dyes with different counter-ions in oxygenated alcoholic solutions proceeds with identical quantum efficiencies independent of the presence of the salt. In deoxygenated alcoholic solutions the dye photodestruction quantum efficiency is decreased by more than an order of magnitude and is identical (within the scope of definition) for tricarbocyanine dyes with different counter-ions, being invariable upon the salt addition.

Also, deoxygenation in low-polar dichlorobenzene leads to lowering of the dye photodestruction quantum efficiency by an order and more and, as distinct from oxygenated solutions, the efficiency is invariable for the dyes with different counter-ions. Note that for a tricarbocyanine dye with the counter-ion I⁻ the quantum efficiency of the singlet oxygen generation in low-polar solutions is by an order of magnitude higher than that for a tricarbocyanine dye with the counter-ions ClO₄⁻ and Br⁻. Therefore, identical values of the photodestruction quantum efficiencies in deoxygenated solutions for dyes with different counter-ions point to the fact that under these conditions a mechanism of the polymethine dye photodestruction is not associated with transition of molecules to the triplet state.

Addition of salt results in a considerable (by a factor of 3.5) increase in the quantum efficiency of the photoreaction of a tricarbocyanine dye with the counter-ion Br in deoxygenated solutions of dichlorobenzene. As this takes place, addition of the salt leads to the growing formation of radicals. Because of this, we can conclude that the radical formation is a decisive factor for photodestruction of tricarbocyanine dyes in deoxygenated solutions.

A change in the photophysical and photochemical characteristics of tricarbocyanine dyes in low-polar solvents upon addition of tetrabutyl ammonium bromide is caused by a greater portion of close contact ion pairs and by the formation of free radicals due to the ultrafast (<150 fs) electron transfer between cation and anion in the molecules.

So, the studies conducted *in vitro* and *in vivo* for the developed compounds have revealed their potentialities and advantages over other classes including their applicability as photosensitizers for diagnostics and phototherapy of deep lying tumors and also in hypoxic conditions. The mechanisms responsible for the photoactivity in hypoxic conditions have been interpreted; the methods to establish the optimum doses of phototreatment have been proposed on the basis of recording the changes in the spectral characteristics of photosensitizers; the diagnostic methods for the neoplasm localization regions have been optimized.