Hydroxyaluminium Tetra-3-Phenylthiophthalocyanine is a New Effective Photosensitizer for Photodynamic Therapy and Fluorescent Diagnosis

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We studied the possibility of using liposomal forms of hydroxyaluminium tetra-3-phenylthiophthalocyanine as a near infrared band photosensitizer. Experiments on mice with solid Ehrlich tumor and subcutaneously transplanted P-388 leukemia revealed high selectivity of accumulation of the photosensitizer in tumors in comparison with normal tissues and high photodynamic activity of the preparation. This photosensitizer can be used as the basis for creating an effective preparation for photodynamic therapy and fluorescent diagnosis.

Key Words: *photodynamic therapy; fluorescent diagnosis; photosensitizer; phthalocyanine; liposomal form*

Photodynamic therapy (PDT) and fluorescent diagnosis (FD) of tumors are important problems in experimental and clinical oncology [1]. Clinical studies are paralleled by the search and selection of new photosensitizers (PS) with improved characteristics. PS with absorption and fluorescence in the near infrared band (700-850 nm), where natural absorption of biological tissue is minimum [3], are the most perspective for FD and PDT. Optical radiation of this band penetrates into biological tissues most deeply, which is essential for PDT of large tumors.

The possibility of creating PS of the near infrared band on the basis of bacteriochlorophyll derivatives, naphthalocyanines, and phthalocyanines is discussed in papers reviewing the data on the mechanisms of photodynamic effect and choice of potential agents for PDT with consideration for the molecular structure of compounds of different classes [4,7].

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Photodynamic characteristics of bacteriochlorophyll-based PS were studied in detail [6]. The efficiency of PDT with bacteriophyllide serine [3,15] and palladium bacteriopheophorbide [9] was studied. Spectral peaks of absorption for these PS are about 770-780 and 750-760 nm, respectively. The possibility of using these agents for PDT of melanoma [15], prostatic cancer [9], and some other tumors is intensely investigated. However, modern technology of preparing these PS is very difficult.

Liposome-entrapped naphthalocyanine derivatives were studied in detail as the near infrared band PS [13]. The efficiency of these PS was not high.

Lutetium texaphyrin PS with the absorption maximum at 720-740 nm was studied [8]. Its photodynamic efficiency was lower than that of photofrine II and chlorines.

Cheap, technological, and effective synthetic PS phthalocyanine derivatives are extensively tested in experimental and clinical studies (excitation in the red band). The possibility of creating infrared PS based on octabutoxy phthalocyanines with maximum absorption about 750 nm was demonstrated [5]. Phosphinylme-

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thyl-substituted phthalocyanine PS with absorption at 690-740 nm are known [11]. We investigated the possibility of using a new class of substances, phenylthio-substituted phthalocyanines, as the near infrared band PS. The presence of phenylthiogroups in the benzene rings of the phthalocyanine macrocycle appreciably shifts the long-wave absorption band of these phthalocyanines towards longer waves in comparison with their non-substituted and tert-butyl-substituted analogs, and hence these compounds can be used as PS sensitive in the near infrared band.

We studied the characteristics of a representative of this class hydroxyaluminium tetra-3-phenylthiophthalocyanine.

MATERIALS AND METHODS

Hydroxyaluminium tetra-3-phenylthiophthalocyanine [3-$(PhS)_4$-PcAlOH] was synthesized from 3-phenylthiophthalodinitrile in the reaction with aluminum chloride [2].

Since [3-$(PhS)_4$-PcAlOH] is a hydrophobic compound, a liposomal composition based on lecithin, cholesterol, and cardiolipin with a molar ratio of 10:4:0.2 was created for *in vivo* studies (the total lipid/[3-$(PhS)_4$-PcAlOH] ratio was 235:1). The liposomes were prepared as described previously [12]: homogeneous film containing lipids and photosensitizer after evaporation of chloroform solution was washed with water and sonicated for reducing the size of vesicles.

The efficiency of PDT was evaluated on mice with Ehrlich tumor and P-388 leukemia. Ehrlich tumor (0.1 ml ascitic fluid containing 500,000 tumor cells) was transplanted to first-generation F_1 (C57Bl/6×DBA/2) hybrid mice intramuscularly (hind leg), P-388 leukemia was transplanted to first-generation BDF_1 (C57Bl/6 ×CBA) hybrid mice subcutaneously into the same area in the same volume. Photosensitizer was injected in a single dose of 4 mg/kg intravenously on day 5 after transplantation, when the size of tumor nodes reached 1500-1800 mm^3 (Ehrlich tumor) and 700-800 mm^3 (P-388 leukemia). Irradiation was carried out on day 6 after transplantation.

The spectrum of PS absorption in biological tissues was evaluated by diffuse reflection spectroscopy [14]. The level and selectivity of PS accumulation in the tumor in comparison with normal tissue (skin) was evaluated by the spectral fluorescent method [10]. Spectral studies were carried out using LESA-01-Biospek spectroanalyzer (Biospek).

During PDT the tumors were irradiated with an LS5-PDT light source (Biospek) with rearrangeable interference filter (730 nm spectral maximum wavelength, 35 nm band half-width, 250-500 mW/cm^2 power density, and 250-600 J/cm^2 dose power). The efficiency of PS for PDT was evaluated by inhibition of tumor growth at different terms of observation. Inhibition of tumor growth (ITG) was estimated by the formula:

$$ITG(\%)=\frac{V_c-V_e}{V_c}\times 100,$$

where V_c and V_e are the mean volume of tumor in the control and experimental groups, respectively (mm^3).

Experimental groups consisted of 5-6 mice, control group consisted of 8-10 mice.

The results were processed statistically using Fisher—Student test at $p<0.05$.

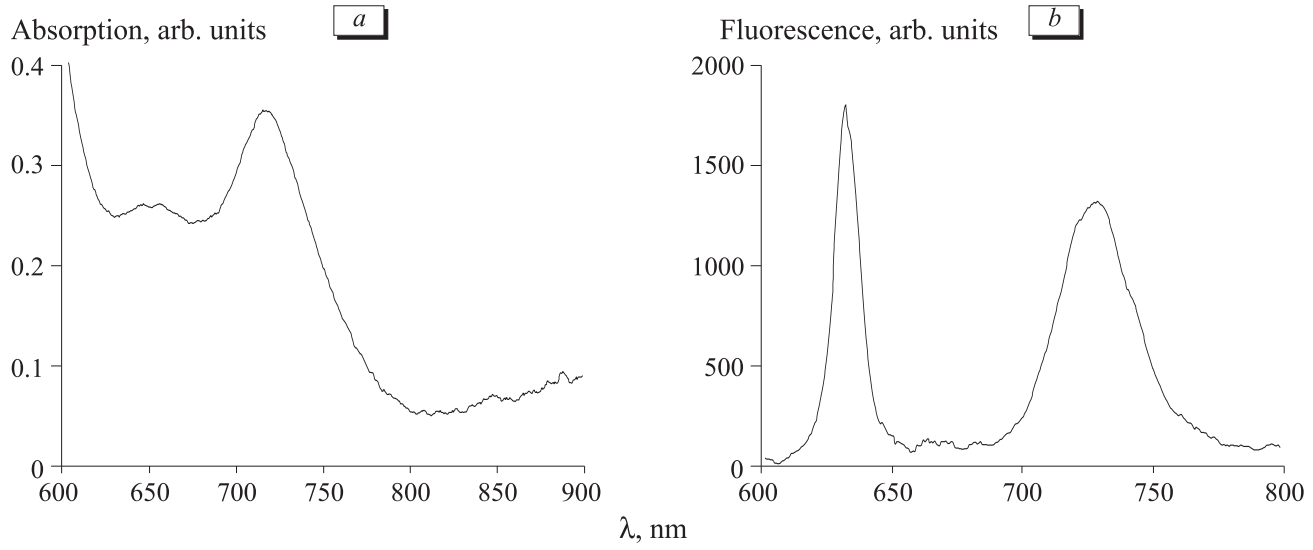

Fig. 1. Absorption (*a*) and fluorescence spectra (*b*) of sensitized tumor tissue in mice 24 h after injection 4 mg/kg hydroxyaluminium tetra-3-phenylthiophthalocyanine.

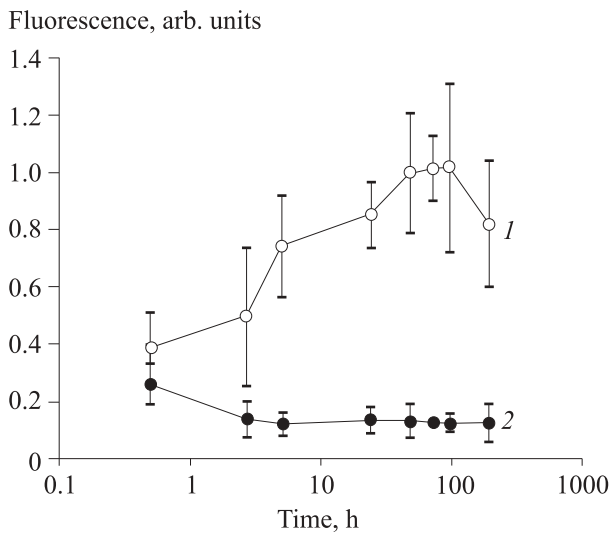

Fig. 2. Relationship between the intensity of hydroxyaluminium tetra-3-phenylthiophthalocyanine fluorescence and time of its injection in a dose of 4 mg/kg to F_1 mice with Ehrlich tumor. *1*) tumor; *2*) normal value.

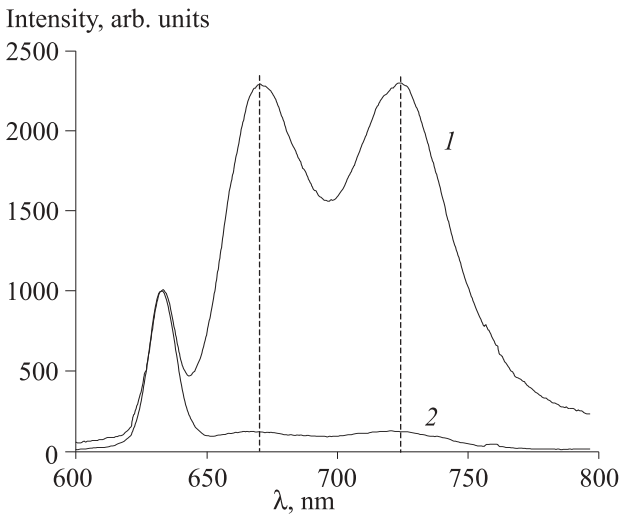

Fig. 3. Fluorescence spectra of P-388 tumor. *1*) in the center of a tumor with necrosis; *2*) at the periphery of tumor under intact skin. Dotted lines show position of fluorescence spectral maxima.

RESULTS

The absorption spectrum of [3-$(PhS)_4$-PcAlOH] in the tumor *in vivo* is a band of about 30-nm half-width and spectral maximum at 720 nm (Fig. 1, *a*). A band with half-width of about 40 nm and spectral maximum at 730 nm dominates in the fluorescence spectrum of this photosensitizer *in vivo* under conditions of excitation with He-Ne-laser (Fig. 1, *b*).

Changes in the [3-$(PhS)_4$-PcAlOH] fluorescence intensity in Ehrlich solid tumor indicate that the level of PS accumulation in the sensitized tumor tissue increases over 20-30 h, remains stable during the next 2-3 days, and then gradually decreases (Fig. 2). The level of PS accumulation in the skin 1 h after its injection in a dose of 4 mg/kg was 1.5-2 times lower than in the tumor and rapidly decreased to a level comparable with tissue autofluorescence.

The index of selectivity for liposome-incorporated 3-$(PhS)_4$-PcAlOH 30 min after injection is about 1.5, rapidly increases to 6 over the next 5 h, and increases slower. Twenty-four hours postinjection the index of selectivity is 6.3. The maximum value (8.2) is observed 48 h after injection.

After PDT a necrotic crust formed in the central zone of irradiation over 24 h. PDT appreciably inhibited tumor growth (Tables 1, 2): by 80% and 84% for Ehrlich tumor and P-388 leukemia, respectively.

Due to high selectivity of PS accumulation in the tumor in comparison with the adjacent normal tissues, the skin of normal tissues within the 16-mm zone of intensive exposure was damaged. It is noteworthy that PDT damaged the tumor even under intact skin, which was shown by spectral studies of the zone of exposed tumor. We analyzed a characteristic fluorescence spectrum (Fig. 3, *1*) of the surface of necrotic crust in the center of the exposed zone (where the skin was damaged) and the fluorescence spectrum (Fig. 3, *2*) of intact skin situated above the tumor edge beyond the irradiated zone. This latter spectrum corresponded by

TABLE 1. ITG (%) after PDT with Liposome-Incorporated [3-$(PhS)_4$-PcAlOH]

Exposure	Day after PDT						
	3	7	10	14	17	21	24
PS (4 mg/kg)+irradiation	41	49	62*	80*	77*	80*	78*
PS (4 mg/kg) without irradiation	12	+10	+21	+12	+14	0	4
Irradiation without PS	21	29	17	21	18	22	23

Note. Irradiation parameters: λ=730 nm, power density 300 mW/cm², duration 20 min (dose power 360 J/cm²). "+": stimulation of tumor growth. *p<0.05 compared to the control.

TABLE 2. Inhibition of P-388 Solid Leukemia Growth (%) after PDT with Liposome-Incorporated [3-$(PhS)_4$-PcAlOH]

Exposure	Day after PDT			
	1	5	8	12
PS (4 mg/kg)+ irradiation	21	74*	84*	84*

Note. Irradiation parameters: λ=730 nm, power density 400 mW/cm², duration 20 min (dose power 480 J/cm²). *$p<0.05$ compared to the control.

its shape to the fluorescence spectrum of necrotic tissue, but was characterized by lesser intensity. This suggests the presence of necrotic tumor tissue under intact skin in this area.

Hence, liposome-incorporated hydroxyaluminium tetra-3-phenylthiophthalocyanine was characterized by high absorption in the 710-740 nm spectral band in the sensitized biological tissue far surpassing natural absorption of the corresponding tissue; high index of selectivity of accumulation in the tumor, reaching 8.2; high photodynamic efficiency (inhibition of Ehrlich tumor growth by 80% and P-388 leukemia growth by 84%).

PDT with [3-$(PhS)_4$-PcAlOH] is most effective 20-30 h after PS injection, when the concentration of the photosensitizer in the tumor and selectivity of accumulation in comparison with normal tissues are close to the maximum values.

High selectivity of [3-$(PhS)_4$-PcAlOH] accumulation in the tumor suggests that this PS can be used for fluorescent diagnosis.

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