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Near-Infrared Photosensitizers based on Nanostructured Forms of Phthalocyanine Derivatives

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Abstract—The research is devoted to development and study of photosensitizers on a base of injectable nanoparticular formulations of phthalocyanine derivatives absorbing at long wavelength (near-infrared) range of 710–740 nm: Thiosens [liposomal form of aluminum hydroxide 1,8,15,22-tetrakis(phenylsulfanyl)phthalocyanine] and Octasens [polymeric micellar form of zinc 1,4,8,11,15,18,22,25-octachloro-2,3,9,10,16,17,23,24-octakis-(decylsulfanyl)phthalocyanine based on domestic poloxamer Emuxol-268]. Experiments have shown that the photosensitizers under study show high selectivity of accumulation and prolonged retaining at high concentration in tumor, relatively fast clearance from normal tissue and high therapeutic efficiency of photodynamic treatment on model tumors.

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INTRODUCTION

Photodynamic the rapyis a modality of combined therapy of tumors is based on the of photosensitizers. The photosensitizer is administered to a patient, and then the pathological site is subjected to optical irradiation, absorbed by the photosensitizer. Light performs the addressing function as a source of energy for photobiochemical reaction leading to the formation of cytotoxic agents (reactive oxygen species) in tumor tissue and/or tumor vessels.

Diagnostic methods based on the use of fluorescent markers selectively accumulated in abnormal tissues are widely used in oncology. This allows on detecting site with increased fluorescence intensity, to suggest development of pathological processes. Undoubted advantages of the method of fluorescent diagnostics, non-invasive, high-speed performance, the possibility of combining treatments with therapeutic laser action for the photodynamic therapy, make this promising method for the detection of nodes and evaluation of a number of diseases, mostly of superficial and intra-cavitary localizations.

Creating new photosensitizers with high photo-dynamic activity and selectivity of accumulation in tumors is a current challenging task.

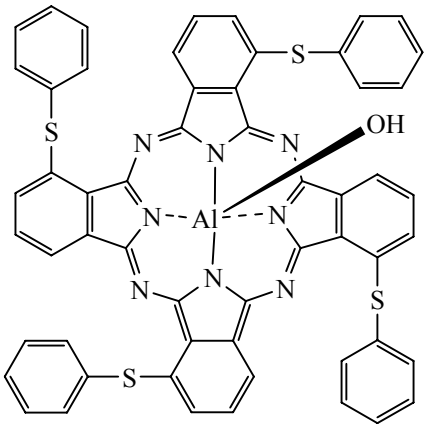
No less important and actual task is to optimize the pharmacokinetics of photosensitizers. Distribution and dynamics of changes in the concentration of photosensitizer in tissues and organs should provide:

– achievement of high selectivity of accumulation in tumor relative to normal tissue and skin;

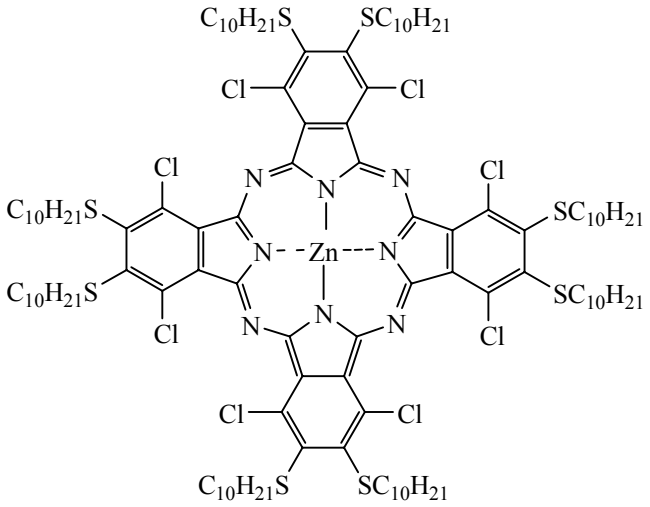
– maintenance of a high concentration in the tumor for a time necessary for the therapeutic and/or diagnostic procedures;

– sufficiently rapid decrease in concentration in normal tissue, especially in the skin after the therapeutic procedures through their excretion from the body and/or metabolism to a residual level excluding or minimizing possible side effects.

Scheme 1.



Thiosens



Octasens

In search of improving the efficiency of photodynamic therapy and fluorescent diagnostics photosensitizers absorbing light in the spectral range of 700–800 nm, in which the self-absorption of biological tissues is minimal are of great interest. The use of such photosensitizers allows to minimize losses to its own tissue uptake, increase the depth of penetration of the excitation light and with the optimal choice of their concentration in tissues to provide a preferential effect on the pathological site. Studies [1, 2] show that the efficacy of photodynamic therapy with irradiation of deep tumors increases with the photosensitizer dose only up to a certain limit, and then it is reduced because of the shielding of deep layers by sensitized subsurface layers.

According to our estimates, the high efficiency of the therapeutic irradiation is achieved if the absorbance of the photosensitizer exceeds the intrinsic absorption of biological tissue about three times [1]. At a high intrinsic absorption of biological tissue energy efficiency of the therapeutic radiation is significantly reduced. Therefore, for effective therapy of tumors of considerable size the most promising photosensitizers are those with absorption in the main band corresponding to the spectral region of maximum transparency ("window of transparency") of biological tissues, 700–850 nm. It should be noted, that in this spectral region lies deoxyhemoglobin absorption band (750–760 nm), in sensitized tumor it may be observed due to hypoxia, therefore minimal absorption will be in the ranges 710–740 nm and 780–830 nm.

In this paper we present the results of a study of two photosensitizers based on phthalocyanine derivatives: Thiosens [3] and Octasens [4], which absorb in the infrared region of the spectrum (Scheme 1).

Photosensitizers: Photodynamic Active Substances and Injectable Forms

Thiosens—Liposomal Photosensitizer

Thiosens [aluminum hydroxide 1,8,15,22-tetrakis(phenylsulfanyl)phthalocyanine] was prepared by reaction of 3-(phenylsulfanyl)phthalonitrile with aluminum chloride in the presence of dry urea and a catalytic amount of ammonium molybdate. Product analysis was performed by chromatography on Al_2O_3 as described in [3].

Thiosens is hydrophobic semisolid soluble in common organic solvents, such as chloroform. Injectable form for systemic (intravenous) administration was pre-pared as a liposomal dispersion. Experimentally was found optimal composition of the liposomes with a molar ratio of lecithin: cholesterol: PEG-2000–DSPE, equal to 1 : 0.22 : 0.002 [5]. The molar ratio of phthalocyanine : lecithin of 1 : 270, provides an acceptable medium size liposomes (~160 nm) and high degree of inclusion of photosensitizer (98.0%).

For liposomal form solutions of the lipid components and Thiosens substance in chloroform were prepared. Both solutions were combined and the chloroform was evaporated on a rotary evaporator at a water bath temperature 38±2°C until a homogeneous

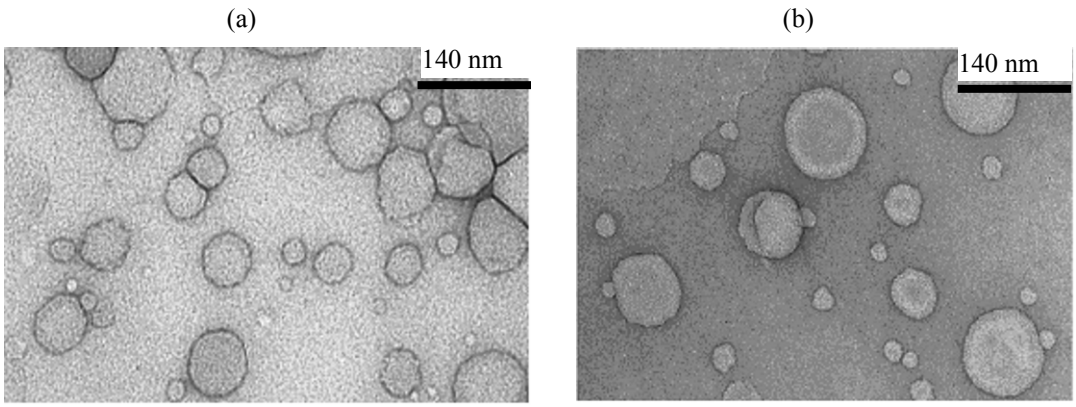


Fig. 1. Electron micrographs of Thiosens liposomes (a) before and (b) after freeze drying.

light green film formed. The film was rinsed with water for injections and then the dispersion was passed through a filter (pore size 1.2 and 0.45 microns) and was extruded at a high pressure homogenizer Microfluidizer M-110S to produce vesicles of optimal size. The dispersion was filtered sequentially through a membrane with a pore size of 0.45 and 0.22 microns. For long-term storage liposome form was subjected to gradient freezing and freeze-drying, using sucrose as cryoprotectant in a weight ratio to lecithin 2 : 1. Lyophilisate was stored at –18°C. Immediately prior to the administration lyophilisate was resuspended at room temperature in injection solution.

Form of liposomes and their distribution in the dispersion size are important quality parameters of liposome preparations. It was shown [6] that the size of the liposomes to a large extent determines the pharmacokinetics, selectivity of accumulation, elimination from normal tissues and photodynamic efficiency of Thiosens. As follows from the results obtained in mice used as Ehrlich carcinoma (ELD) model, these characteristics are achieved with an average diameter of Thiosens liposomes in the range of 80–160 nm.

The size distribution of the liposomes in the dispersion was determined by dynamic light scattering on a Nicomp 380 Submicron Particle Sizer device. Furthermore, for one Thiosens batch the shape and size of the liposomes before and after freeze drying were measured by electron microscopy with the negative contrast agent (Fig. 1) [5]. For the preparation of a sample for microscopy on a collodion-coal base liposomal dispersion was applied, dried, and contrasted with 1% uranyl acetate solution. Electron micrographs were obtained on an electron microscope JEM-100CX (JEOL) at a magnification of 20000 with a scanning resolution of 1.200 dpi.

Octasens as a Micellar Photosensitizer

Octasens [zinc 1,4,8,11,15,18,22,25-octachloro-2,3,9,10,16,17,23,24-octakis(decylsulfanyl)phthalocyanine] was synthesized by reaction of 3,6-dichloro-4,5-bis(decylsulfanyl)phthalonitrile with zinc acetate as described in [4]. Octasens is hydrophobic semisolid soluble in nonpolar and chlorinated organic solvents such as chloroform. Pluronic analog Emuxol-268 (manufactured by FSUE SSC "NIOPIK") was used for the preparation of injectable formulation as a dispersion of polymeric micelles.

Micellar form was obtained as follows. Chloroform solution of Octasens was dropwise added to the 4% aqueous Emuxol at a temperature of 65–70°C, then chloroform was removed from the solution, heating and bubbling were seised, the dispersed system was cooled to room temperature and filtered using a filter system Millipore SWINNEX-25 equipped with filters Pall ("Pall Eurasia," Russia) with a pore diameter of 0.22 microns.

To determine the particle size distribution in an aqueous emuxol micellar dispersion was diluted with water for injection at a ratio of 1 : 10. Analysis using laser correlation spectrometer Submicron Particle Sizer NICOMP-CW380 ("Particle Sizing Systems") showed that the composition of the dispersion is represented mainly by the two factions, micelle diameter ~186 nm (~92% of the total) and 52 nm micelles (~8%). It should be noted that using the method of laser correlation spectroscopy one measure hydrodynamic radius of the micelles that is much greater than the size of their "cores," in which concentrated photosensitizer molecules. Apparently, both these two dimensions determine the interaction of the micelles with the reticuloendothelial system and endothelial layer

defects in tumor vasculars and the probability of extravasation of micelles.

Experimental Investigation of Photosensitizers *in vivo*

Study of the efficacy of photodynamic therapy using Thiosens liposomal form were performed on transplantable mouse tumors: Ehrlich carcinoma (ELD), lymphocytic leukemia P388, Lewis lung carcinoma (LLC).

Ehrlich carcinoma was transplanted to the first generation mice hybrids F1 (C57 Bl/6×CBA) intramuscularly in the right shin with 0.1 mL of ascitic liquid containing 10^6 tumor cells.

The photosensitizers were injected intravenously via the tail vein.

Absorption and fluorescence spectra of photosensitizers and sensitized biological tissues were recorded using fiber-optic spectrum analyzer LESA-01-Biospec ("Biospec," Russia). Fluorescence spectra were recorded using excitation by helium-neon laser (emitting at 632.8 nm). Additionally, absorption spectra were recorded *in vivo* by diffuse reflectance spectroscopy [7, 8].

Fluorescence and absorption spectra of biological tissue were collected at various time points after administration of photosensitizer in several (5–10) points of tumor and normal tissue contralateral to tumor. To achieve sufficiently high accuracy of study dynamics of accumulation and selectivity, each test group of mice consisted of at least three animals and measurements were made at 5–10 points of tumor surface. The obtained data were averaged using software UnoMomento ("Biospec," Russia). Photosensitizer content in the tumor and normal tissues was evaluated (in relative units) from the integral values of the fluorescence intensity normalized to the intensity of the scattered laser radiation, or by the values of the integrated absorption in the selected spectral range of the given photosensitizer. From the ratio of the content of the drug in the tumor and normal tissue selectivity accumulation index was calculated.

Semiconductor laser LPhD-01/730-Biospec ("Biospec," Russia) emitting at 732 nm was used for the irradiation of tumors during photodynamic therapy. The effectiveness of photodynamic therapy was evaluated by the index of TGI (tumor growth inhibition), which was calculated using the following equation:

$$\mathrm{TGI} = \frac{V_c - V_0}{V_c} \times 100\%,$$

where V_c is an average tumor volume in the control group (cm^3) calculated as the multiplication of the results of three orthogonal tumor measurement ($a \times b \times c$); V_0 is an average tumor volume in the experimental group (cm^3).

Experimental groups for the photodynamic therapy were formed from 5–6 mice, control from 7–10 mice. The data obtained were subjected to statistical processing using Fischer-Student method. Only difference values with $p \leq 0.05$ were considered to be significant.

Absorption and Fluorescence of Photosensitizers in Tissues of Experimental Animals

The fluorescence spectrum of Thiosens *in vivo* is a narrow band with a maximum at 730 nm and a half-width ~40 nm, similar to the fluorescence spectrum of the liposomal form of Thiosens. The fluorescence intensity of sensitized tumor is significantly higher than such of sensitized normal tissues (more than 4-fold at 24 h after administration).

The absorption spectrum of Thiosens in liposomal form has a major band with a maximum at 720 nm and a half-width ~40 nm and adjacent band with a maximum in the region of 650–670 nm, the intensity of which increases with decrease of ration of "total lipid: phthalocyanine." Presumably, the main contribution to this absorption band are made by aggregates of phthalocyanine molecules.

The absorption spectrum of tumor sensitized with Thiosens has an intense major band at 710–740 nm, much greater than both the intrinsic absorption of unsensitized tissue and normal sensitized tissue. This ensures high efficiency of photoexcitation of a photosensitizer by irradiation of the tumor with light of a wavelength corresponding to the maximum of its absorption as a photosensitizer molecules absorb the main part (about 80%) of light energy in the specified range.

Dynamic of change of the shape of Thiosens absorption spectrum in tumor over time is shown on Fig. 2. Immediately after administration (within the first three hours) the spectral position of the main peak (720 nm) and the intensity ratio of the main and the short-wave absorption bands are approximately the same as in the spectrum of the Thiosens liposomal dispersion. Subsequently (after approximately 24 h

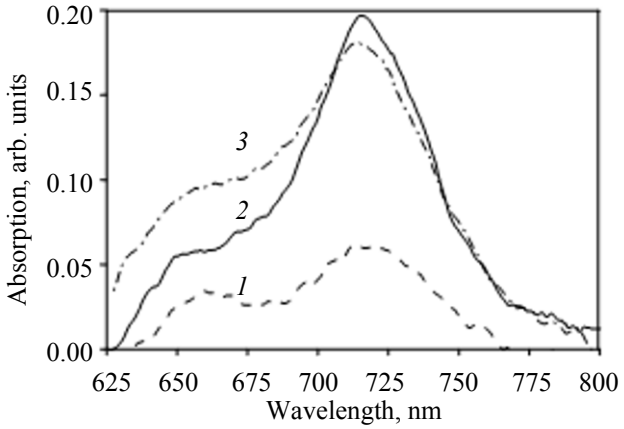


Fig. 2. Thiosens spectra in tumor at different times after administration: (*1*) after 3 h; (*2*) after 24 h; (*3*) after 72 h.

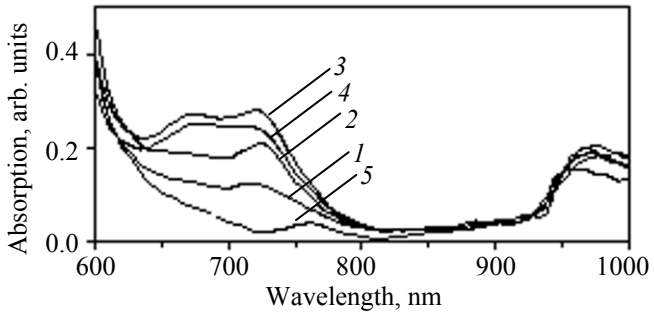


Fig. 3. Absorption of tumor, sensitized with Octasens afterintravenous administration to mice with Ehrlich tumor (3 mg/kg dose): (*1*) after 1 h; (*2*) after 4 h; (*3*) after 24 h; (*4*) after 48 h; and (*5*) control (before administration).

after administration), the absorption intensity increases considerably, mainband spectral absorption maximum is shifted to shorter wavelengths by about 5 nm (to 714–716 nm) and the intensity ratio of the short-wave and main bands decreases to about 0.25. Over the next 70 h the maxi-mum position of mainband remains approximately the same as that after 24 h, the intensity of the main absorption band remains approximately constant or decreases slightly while absorption in the short-wave band significantly increases.

We can assume that the observed spectral changes are associated with changes in the interaction of molecules of Thiosens with the environment. At the initial stage after administration (Fig. 2, curve *1*), most of its molecules are within a lipid bilayer of liposomes, long circulating in blood. Part of the molecules is presumed to be in an aggregated state, which is manifested in the form of short-wavelength wing (650–670 nm) absorption band. Thanks to the extravasation from newly formed blood vessels liposomes enter the tumor. Through the merger of the liposomes with the membrane of tumor cells or endocytosis of liposomes [9] photosensitizer molecules are released from the liposomal lipid environment and interact with cell organelles. Changing in environment of Thiosens molecules leads to a shift of the absorption band. At the initial stage of the process the photosensitizer accumulates in the cells mainly in monomeric form, which leads to an increase in the intensity of the main absorption band observed at 24 h after administration of the liposomal formulation. We can assume that it is at this time interval that the tumor would be most sensitive to photodynamic treatment. Subsequent accumulation of Thiosens molecules in cells also leads to their interaction with each other and aggregation thereby increasing the absorption in the 650–670 nm range.

Apparently, similar phenomenon can also be observed for other nanostructured (in particular, liposomal) photosensitizers.

Octasens administered in the form of a micellar dispersion based on emuxol-268 at a dose of 1.5 to 6 mg/kg body weight of the animals does not exhibit appreciable fluorescence. In all subsequent experiments we investigated the absorption of Octasens in biological tissues.

Shortly after administration Octasens is represented in absorption spectrum of sensitized tumor by 710–745 nm band corresponding with main absorption band of a substance (Fig. 3). Its intensity continued to grow up to about 24 hours after injection of dispersion. In addition it is possible to observe secondary wide absorption band in 630–690 nm supposedly associated with aggregation of phthalocyanine molecules. Aggregation of photosensitizer molecules during first 1–3 hours (when most of micelles are still circulated in blood stream) after administration is relatively high and relative intensity of the band is close to that observed for micellar dispersion in vitro. By 4–5 hours post injection absorption in main band grows while aggregation band decreases as photosensitizer gets delivered to tumor cells and disaggregates in cytoplasm. After 24 post administration main band remains at about the same level while absorption at 630–690 nm significantly regrows representing secondary aggregation of photosensitizer as it accumulated at the cellular level.

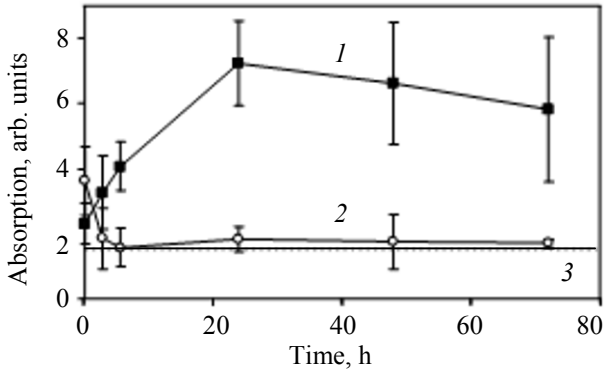


Fig. 4. Thiosens absorption after intravenous administration to mice F_1 bearing Ehrlich tumor: (*1*) tumor; (*2*) normal tissue; and (*3*) absorption of non-sensitized tissue at 710–740 nm.

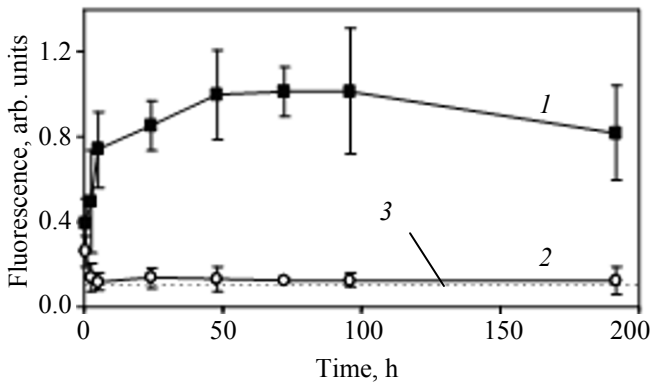


Fig. 5. Fluorescence of Thiosens, administered to F_1 mice with Ehrlich tumor: (*1*) tumor; (*2*) normal tissue, and (*3*) non-sensitized tissue.

Dynamics and Selectivity of Photosensitizers Accumulation

Studying the dynamics of Thiosens accumulation was performed on F_1 mice with solid Ehrlich tumors and BDF_1 mice with subcutaneously transplanted lymphocytic leukemia P388 on the fifth day after inoculation. The photosensitizer was administered to animals intravenously at a dose of 4 mg/kg. The level of accumulation in the tumor and normal tissues was evaluated for its absorption and fluorescence of sensitized tissues.

The intensity of the main Thiosens absorption band in tumor gradually increased over 24 h, and then remained virtually unchanged for several days, followed by a slow decline (Fig. 4).

In normal tissue the absorption in this band within 5 h after administration of the photosensitizer decreased about 2.5 times, and then remained almost unchanged. The latter can be explained by the fact that the measured value of the absorption consists of the absorption of the photosensitizer and the intrinsic absorption of biological tissue. Within 5 h photosensitizer was largely excreted from normal tissue, and the total absorption was determined by the intrinsic absorption of the tissue. Thiosens fluorescence intensity risen for the first day 2-fold, reaching the maximum values (approximately 2.5–3 times higher than initial level) 48 h after the administration, and after 96 h began to slowly decline (Fig. 5). The content of the photosensitizer in the normal tissue is appreciably smaller than in the tumor and it monotonously decreased during the observation time ranging from 0.5 h (the first observation point) up to 7 days after administration. After 1 h after administration Thiosens content decreased by approximately 1.5-fold, and 24 h later, 2–2.5 times and quite quickly decreased to a level roughly corresponding to the intensity of the fluorescence of unsensitized tissue (before the administration of Thiosens). Studies in mice with Ehrlich tumor showed that the selectivity index of Thiosens accumulation in tumors (compared to normal tissue) through 1 h after its administration was 3.5–6 over 6 h, and after 2–4 days (with a maximum accumulation in tumors) reaches the value of 8 (see Fig. 5). Even higher values of selectivity index of accumulation of the photosensitizer reached in mice inoculated subcutaneously with P388 lymphocytic leukemia (value 8 was achieved already 1 h after administration).

Study of absorption after administration of Octasens micellar dispersion at different doses showed that the time interval between injection and Octasens

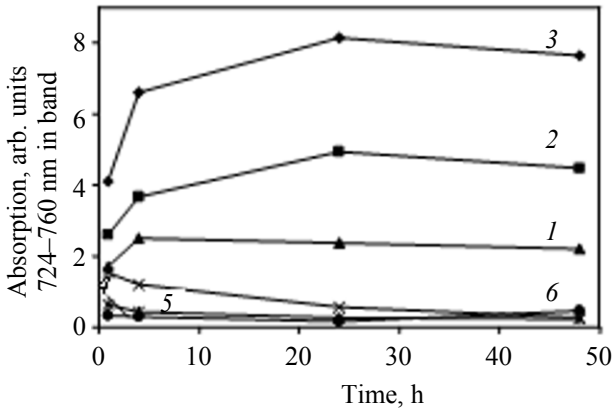


Fig. 6. Absorption of tissues, sensitized with Octasens in 724–760 nm range, time after administration: (*1*, *4*) 1.5 mg/kg; (*2*, *5*) 3 mg/kg; (*3*, *6*) 6 mg/kg. Curves (*1*, *2*, *3)* for tumor and (*4*, *5*, *6*) for normal tissue.

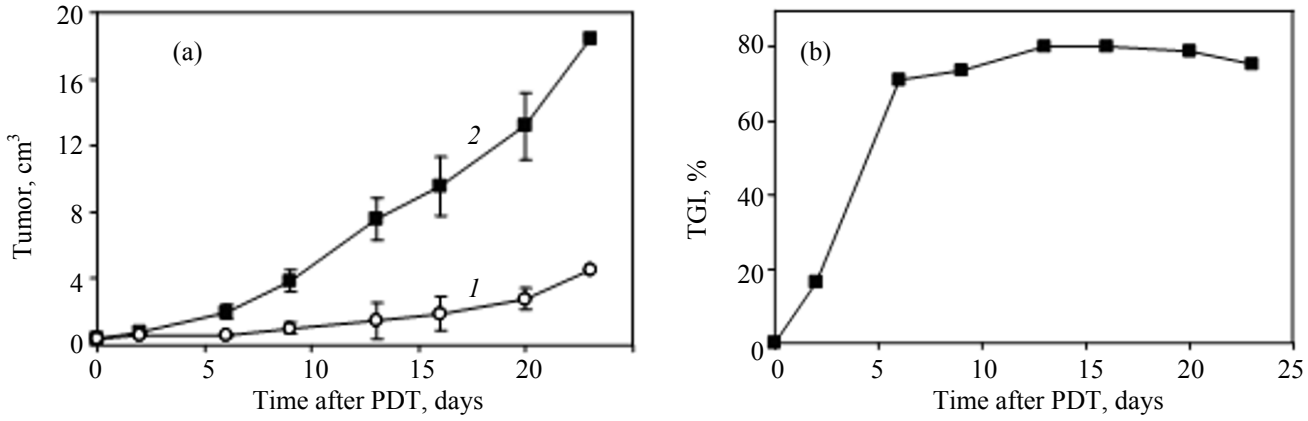


Fig. 7. (a) Growth and (b) growth inhibition of Ehrlich tumor after photodynamic therapy (PDT) with Thiosens in liposomal form (dose 4 mg/kg): (*1*) control; (*2*) group after PDT. For 6–26 days after PDT, $p < 0.05$.

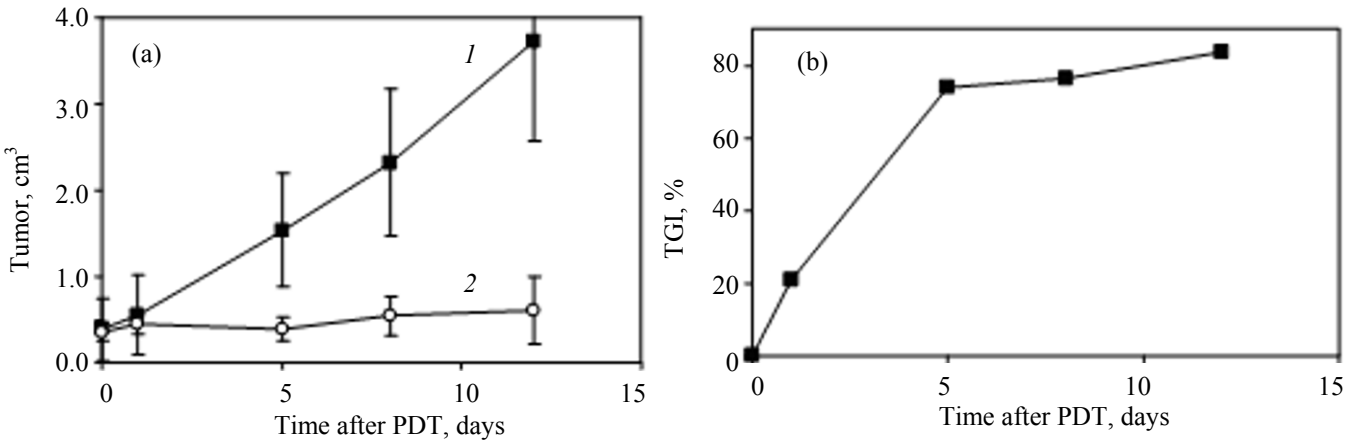


Fig. 8. (a) Growth and (b) TGI of P388 tumor after PDT with Thiosens in liposomal form (4 mg/kg dose): (*1*) control; (*2*) test group after therapy on 5–12 days after therapy $p < 0.05$.

mainband absorption increases with the dose administered from about 4 h for low doses (1.5–2 mg/kg body weight of mice) to 24 h for higher doses (more than 3 mg/kg) (Fig. 6). The dependance of intensity of absorption in the mainband on the dose administered is sublinear (at least for doses exceeding 3 mg/kg).

The content of the photosensitizer in the normal tissue was appreciably lower than in the tumor and decreased monotonically during the observation period since the first hour (the first observation point) up to seven days after administration. 4 h after administration photosensitizer content decreased by approximately 1.5-fold, and 24 h later, in 2 times and fast enough (within 5 days) decreased to the level of unsensitized tissue.

In studies in mice with Ehrlich's tumor Octasens accumulation selectivity index in tumors (compared to normal tissue) within 4–5 h after injection exceeded the value 4–5, and after 1 day (at a time of maximum accumulation in tumors) reached values of 6–8.

In vivo Studies of Photodynamic Effectiveness of Photosensitizers

Photodynamic therapy with thiosens in liposomal form was performed 24 h after administration of the photosensitizer in three tumor models: Ehrlich tumor (ELD), solid lymphocytic leukemia P-388, mouse Lewis lung carcinoma (LLC). The photosensitizer was injected into the tail vein at a dose of 4 mg/kg. The animals were irradiated with liser light at 730 nm, power density of radiation was 250–300 mW/cm^2 for 20 min. The data presented in Fig. 7 shows that treatment with Thiosens liposomal form exerts on Ehrlich tumor significant and prolonged (20 days) antitumor effect (TGI = 80%). It was noted that

thiosens in liposome form, unlike other photosensitizers does not cause swelling of the irradiated zone. Because of this, a statistically significant antitumor effect (TGI = 56%) was detected already at day 7 after laser irradiation and lasted up to 27 days (TGI = 70%).

Photodynamic therapy of another tumor model, solid variant of lymphocytic leukemia P388, 24 h after administration of thiosens in liposomal form 4 mg/kg (power density 250–300 mW/cm^2, irradiation time 20 min) resulted in remarkable inhibition of tumor growth up to 84%. The therapeutic effect manifested on the third day after laser irradiation, TGI index was 70%, and on the seventh day the antitumor effect increased to 76% (Fig. 8). Photodynamic therapy with thiosens liposomal form also gave lengthy moderate antitumor effect in mice with Lewis lung carcinoma (Fig. 9). On the seventh day after laser irradiation index TGI was 59%, and on the 23 day, 53%. Maximal therapeutic effect was observed on the 14th day after laser irradiation, TGI index was 70%.

Photodynamic efficiency of Octasens in micellar form was investigated on Ehrlich tumor (ELD) upon irradiation at 4 h after administration of the photosensitizer. Tests were carried out on mice hybrids F1 (females) with Ehrlich tumor, intramuscularly inoculated in a shank of the right hind paw 4 days prior to Octasens administration. The photosensitizer was administered as emuxol-268 based micellar dispersion at 2 mg/kg dose. Test animals were irradiated 24 h after administration of the photosensitizer with the laser power density of 200 mW/cm^2. Results of the study are shown in Fig. 10. Within 4 days after exposure irradiated animal paw edema was observed. Biologically significant inhibition of tumor growth

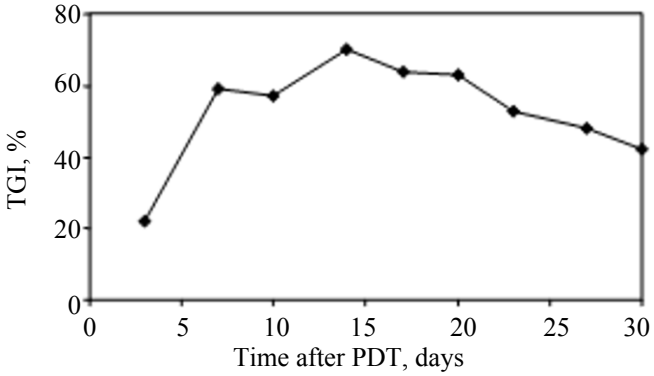


Fig. 9. TGI of mouse Lewis lung carcinoma (LLC) after PDT with Thiosens in liposomal form ($p < 0.05$).

was achieved on the 8th day after irradiation and persisted for 3 weeks, with the maximal index TGI reached 83%.

CONCLUSIONS

The studies have allowed to develop prototypes of two effective nanostructured infrared photosensitizers based on hydrophobic substances: aluminum hydroxide 1,8,15,22-tetrakis(phenylsulfanyl)phthalocyanine (Thiosens) and zinc 1,4,8,11,15,18,22,25-octachloro-2,3,9,10,16,17,23,24-octakis(decylsulfanyl)-phthalocyanine (Octasens). These photosensitizers are characterized by high efficacy in photodynamic therapy of larger model tumors, selectivity, rapid clearance from normal tissues. For injectable form of Thiosens sterically stabilized liposomal form was prepared, incorporating the photosensitizer substance into lipid bilayer. Laboratory protocols and the draft preliminary pharmacopoeia monograph on “Thiosens

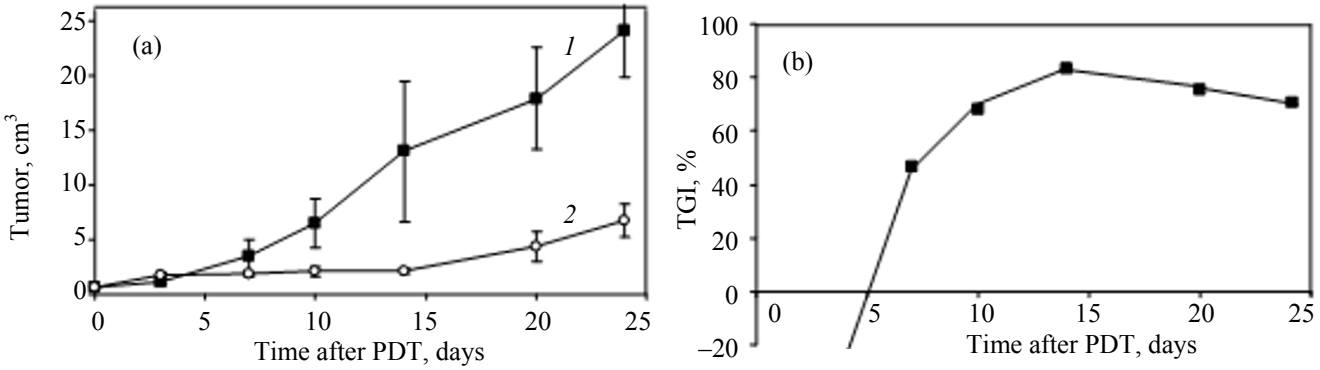


Fig. 10. (a) Growth and (b) TGI of Ehrlich tumor after PDT with Octasens (2 mg/kg dose): (*1*) control; (*2*) test group.

liposomal, lyophilisate for injections 1.5 mg." have been developed and implemented. Standardization of obtained batches of dosage form according to the selected parameters have been performed. Studies conducted for one and a half year revealed no significant change of quality indicators of Thiosens in liposomal form specified in the preliminary pharmacopoeia monograph. The results of biological experiments allow to conclude that high photodynamic efficacy of the Thiosens formulation maintains at least for 1 year. In-depth preclinical study are now in progress.

Micellar Octasens formulation was developed using domestic (Russian) proxanol Emuxol-268. Studies suggest technological convenience of preparation of the dosage form.

Both photosensitizers show promise for development of drugs for the treatment of tumors of large size.

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REFERENCES

1. Meerovich, I.G., Meerovich, G.A., Oborotova, N.A., and Baryshnikov, A.Yu., Russ. Biotherap. Zh., 2006, vol. 5, no. 3, pp. 93–97.
2. Meerovich, I.G., Stratonnikov, A.A., Meerovich, G.A., et al., *Proc. SPIE*, 2005, vol. 5973, p. 59730H1-H10.
3. Russian Patent no. 2 257 898, issued 10.08.2005.
4. Russian Patent no. 2 340 615, issued 10.12.2008 .
5. Sanarova, E.V., *Cand. Sci. (Pharm.) Dissertation*, Moscow, 2012.
6. Meerovich, I.G., Oborotova, N.A., Smirnova, Z.S., Baryshnikov, A.Yu., Meerovich, G.A., Loshchenov, V.B., Derkacheva, V.M., Luk'yanets, E.A., and Vorozhtsov, G.N., Abstracts of Papers, *Rusnanotech-08, Int. Nanotechnology Forum*, St. Peperspurg, 3–5.12.2008, vol. 2, pp. 416–418.
7. Loschenov, V.B., Konov, V.I., and Prokhorov, A.M., *Laser Physics*, 2000, vol. 10, no. 6, pp. 1188–1207.
8. Stratonnikov, A.A., Edinats, N.E., and Klimov, D.V., et al., *Proc. SPIE*, 1996, vol. 2924, pp. 49–60.
9. Margolis, L.B. and Bergelson, L.D., *Liposomy i ikh vzaimodeistvie s kletkami* (Liposomes and Their Interaction with Cells), Moscow: Nauka, 1986.