PHOTODYNAMIC THERAPY OF THE EXPERIMENTAL TUMORS OF DIFFERENT MORPHOLOGICAL TYPES WITH LIPOSOMAL BORONATED CHLORIN E6

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Abstract

The article summarizes the results of studies of the effectiveness of photodynamic therapy using a new domestic photosensitizer liposomal borated chlorin e6 (LBC) after its parenteral administration (intraperitoneal and intravenous). Antitumor efficacy was evaluated in rats with M-1 sarcoma and PC-1 alveolar liver cancer and mice with B16 melanoma and Ehrlich's carcinoma, which were transplanted subcutaneously into the thigh area of the animals. The aim of the study was to determine the optimal regimes of photodynamic therapy that would allow achieving the maximum antitumor effect up to 21 days after the photodynamic therapy. The therapy was carried out under the control of the accumulation of the photosensitizer in the tumor and surrounding tissues of the thigh by selecting the doses of the drug and the parameters of laser radiation (energy density and power density). The effectiveness of therapy was assessed by the inhibition of tumor growth, by the percentage of animals with complete tumor regression, by the absolute growth rate in animals with continued tumor growth compared to controls. The results of our studies have shown that the domestic photosensitizer liposomal borated chlorin e6 has high antitumor activity *in vivo*. In an experimental study of the photosensitizer under certain PDT modes, the maximum antitumor effect (complete tumor regression in 100% of animals) was obtained up to 21 days after PDT in all tumor models used.

Keywords: photodynamic therapy, pharmacokinetics, laser, liposomal borated chlorin e6, rat sarcoma M-1, rat alveolar cancer liver PC-1, mouse melanoma B16, mouse Ehrlich's carcinoma.

For citations: Abramova O.B., Drozhzhina V.V., Churikova T.P., Kozlovtceva E.A., Arkhipova L.M., Kaplan M.A., Ivanov S.A., Kaprin A.D. Photodynamic therapy of the experimental tumors of different morphological types with liposomal boronated chlorin e6, *Biomedical Photonics*, 2021, T. 10, No. 3, pp. 12–22. doi: 10.24931/2413–9432–2021–10–3–12–22

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ФОТОДИНАМИЧЕСКАЯ ТЕРАПИЯ ЭКСПЕРИМЕНТАЛЬНЫХ ОПУХОЛЕЙ РАЗЛИЧНЫХ МОРФОЛОГИЧЕСКИХ ТИПОВ С ЛИПОСОМАЛЬНЫМ БОРИРОВАННЫМ ХЛОРИНОМ Е6

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Резюме

Обобщены результаты исследований эффективности фотодинамической терапии (ФДТ) с использованием нового отечественного фотосенсибилизатора липосомального борированного хлорина е6 после его парентерального введения (внутрибрюшинное и внутривенное). Противоопухолевую эффективность препарата оценивали на моделях перевивных опухолей: саркома М–1 и альвеолярный рак печени РС–1 у крыс, меланома В16 и карцинома Эрлиха у мышей. Опухоли перевивали подкожно в область бедра животных. Цель исследования состояла в определении оптимальных режимов ФДТ, позволяющих добиться максимального противоопухолевого эффекта до 21 сут после проведения ФДТ. Терапию проводили под контролем накопления фотосенсибилизатора в опухолевой и окружающих тканях бедра, осуществляя подбор доз препарата и параметров лазерного излучения (плотность энергии и плотность мощности). Эффективность терапии оценивали по следующим параметрам: торможение роста опухоли, процент животных с полной регрессией опухоли, коэффициент абсолютного прироста опухоли у животных с продолженным ростом. Результаты исследований показали, что отечественный фотосенсибилизатор липосомальный борированный хлорин е6 обладает высокой противоопухолевой активностью *in vivo*. При экспериментальном исследовании фотосенсибилизатора при определенных режимах ФДТ получен максимальный противоопухолевый эффект (полная регрессия опухоли у 100% животных) до 21 сут после проведения ФДТ на всех использованных опухолевых моделях.

Ключевые слова: фотодинамическая терапия, фармакокинетика, лазер, липосомальный борированный хлорин е6, саркома М–1 крыс, альвеолярный рак печени РС–1 крыс, меланома В16 мышей, карцинома Эрлиха мышей.
Для цитирования: Абрамова О.Б., Дрожжина В.В., Чурикова Т.П., Козловцева Е.А., Архипова Л.М., Каплан М.А., Иванов С.А., Каприн А.Д. Фотодинамическая терапия экспериментальных опухолей различных морфологических типов с липосомальным борированным хлорином е6 // Biomedical Photonics. – 2021. – Т. 10, № 3. – С. 12–22. doi: 10.24931/2413–9432–2021–10-3–12–22

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Introduction

The problem of combating malignant neoplasms remains a priority for the modern society. The possibilities of clinical oncology have significantly increased with the introduction of the method of photodynamic therapy (PDT), and now it is successfully taking its place in the treatment of cancer patients [1, 2]. PDT is an effective method of treatment carried out using modern semiconductor lasers and photosensitisers (PS). PS are selectively accumulated in tumor tissue and, upon local exposure to laser irradiation, generate the formation of singlet oxygen and other active radicals that have a cytotoxic effect on the tumor. The realization of PDT effects directly depends on the structures of the tumor node in which PS has accumulated during the therapy session. The targets of photochemical effects are many cellular structures: cell membranes, mitochondria, and microtubules. As membrane damage progresses, other electrolyte disturbances and cytokine reactions can be observed due to the stimulation of tumor necrosis factor production, activation of macrophages, leukocytes and lymphocytes. Sublethal damage to cells through the involvement of many signaling systems can induce apoptosis. In addition to direct cytotoxic effects on tumor cells, indirect effects such as ischemic necrosis due to damage to the endothelium of blood vessels and thrombosis can play an important role in the destruction of neoplasms [3, 4].

Further improvement of the PDT method requires the search for new PSs with higher photoactivity, tumor affinity, and the excitation ability in the near infrared range of the spectrum. It is necessary to create safe medicines that provide a sufficient therapeutic effect with minimal damage to surrounding tissues and the absence of a general toxic effect. Chlorophyll derivatives are of great interest as PSs [5–8]. Active work is underway to create new forms of chlorin PS, for example, by conjugating existing PS forms with liposomes, which expands the range of their capabilities. They exhibit an order of magnitude greater light toxicity in the absence of dark toxicity, increase the selectivity of accumulation in the tumor, which increases the efficiency of PDT when using lower doses of PS [9–16].

Boronated chlorin e6 is an original domestic drug with both photoactive properties and can be used for radiation therapy, in particular for neutron capture therapy (NCT). Boron, which possesses neutron-capture properties, was introduced into the PS. In addition, the modification of PS by the addition of boron clusters to the tetrapyrrole macrocycle significantly optimized the properties of the antitumor drug. The boronated derivatives of chlorin e6 action mechanism includes deep penetration into the lipid bilayer of the cell membrane due to the properties of the boron polyhedron, which provides irreversible damage to tumor cells due to the induction of primary necrosis. Thus synthesized boronated chlorin e6 allows PDT to be carried out and, if necessary, supplemented with NCT. Experimental studies carried out with boronated chlorin e6 indicated its good photoactive properties and low toxicity [17–21].

The aim of the study was to summarize the results of experimental research on the study of the antitumor efficacy of a new pharmaceutical formulation based on boronated chlorin e6 - liposomal boronated chlorin e6 (PS LBC) on various tumor models: sarcoma M – 1 and alveolar liver cancer RS – 1 of rats, melanoma B16 and carcinoma Ehrlich of mice.

Materials and methods

Liposomal boronated chlorin e6 was used as a PS.

At the Nesmeyanov Institute of Organoelement Compounds of Russian Academy of Sciences was synthesized (RF patent No. 2406726) drug - boronated chlorin e6 (Fig. 1).

In State Educational Institution of Higher Professional Education I.M. Sechenov First Moscow State Medical University at the Department of Pharmaceutical Engineering and Pharmacology on the basis of boronated chlorin e6, a new dosage form of membrane-active PS for PDT and NCT "Borchlorin liposomal lyophilisate" was developed and synthesized, used to prepare a solution for injection.

The composition of the liposomal formulation: borchlorin/lecithin 1:200 and lecithin/cholesterol 3:1, ensures the incorporation of borchlorin at a level of 99%, PEG-DSPE, an acceptable liposome size of 185 ± 10 nm and a pH value of 6.9 [12].

The drug was administered to animals intraperitoneally and intravenously. Sarcoma M–1 and alveolar liver cancer RS–1 of rats, melanoma B16 and Ehrlich carcinoma of mice were used as experimental models of tumors. The work was carried out in compliance with international recommendations for research using laboratory animals.

(13 (1)-N-{2- [N- (клозо-монокарбадодекаборат-1этил) метил] аминоэтил} амид-15 (2), 17 (3) – диметилового эфира хлорина е6

(The patent of the Russian Federation № 2406726)
(13 (1)-N – {2 [N – (closo-monocarbadodecaborate-1ethyl) methyl] aminoethyl} amide-15 (2), 17 (3) – dimethyl ether chlorin e6

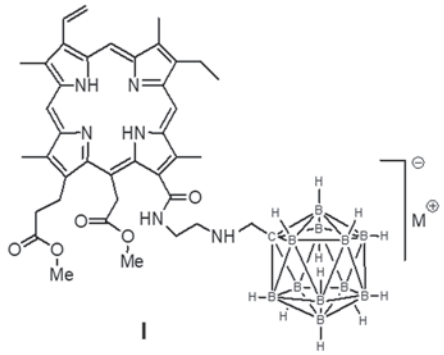

Рис. 1. Структурная формула борированного хлорина е6.
Fig. 1. The structural formula of boronated chlorin e6.

Studies of the effectiveness of PDT for sarcoma M–1 (62 rats) and alveolar liver cancer PC–1 (42 rats) were carried out on outbred rats weighing 150–180 g. Tumors were transfered under the skin in the thigh area in the form of donor tumor pieces. Animals with sarcoma M–1 were included in the experiment on days 7–9, when the palpable tumor reached 0.7–1.0 cm in diameter, animals with PC–1 - on days 11–13, when the tumor reached a diameter of 1.2 up to 1.6 cm.

The study of the effectiveness of PDT of melanoma B16 was carried out on 183 mice-hybrids of the first generation F1 (CBA x C_{57}BL/6j) weighing 20 g. Melanoma was transfered into mice in the form of a cell suspension with a volume of 0.10–0.15 ml. The mice were included in the experiment on days 4–5, when the tumor diameter reached 0.4–0.6 cm. The response of PDT for Ehrlich's carcinoma was studied in outbred mice (n = 87). To reproduce a solid tumor, 0.05 ml of ascitic fluid from donor mice was injected under the skin in the thigh area. Mice were included in the experimental group on the 4th day after the transfer, when the tumor diameter reached 0.8–1.0 cm.

Control animals were tumor-bearing animals that were not exposed to PDT. The study design is presented in Table 1.

The drug-light interval, that is, the time from the moment of PS administration to laser irradiation, was determined on the basis of pharmacokinetic studies carried out by the method of laser fluorescence diagnostics using the LESA-01– "Biospec" device. Irradiation was performed at a high level of PS accumulation in the tumor and the maximum tumor/surrounding tissue contrast index.

The source of laser radiation was an Atkus-2 semiconductor laser device manufactured by Poluprovodnikovye pribory ZAO (St. Petersburg, Russia) with a radiation wavelength of 662 ± 1 nm. The light spot diameter was 1.0–2.0 cm.

Animals during irradiation were under general thiopental anesthesia. Thiopental sodium was administered intraperitoneally: to mice, 0.03 ml of 1.25% solution per 10 g of body weight; rats, 0.2 ml of 2.5% solution per 100 g of animal weight.

Tumor volume was measured: before PDT (V_0) and on days 3, 7, 10, 14 and 21 (V_t) after therapy.

The effectiveness of PDT was assessed in accordance with the recommendations of the Pharmacological Committee for preclinical approbation of drugs [22] according to the following parameters:

1. Coefficient of absolute tumor growth (K).

For this, the tumor volumes were first calculated using the formula:

$$V = \frac{1}{6}\pi * d_1 * d_2 * d_3 \quad (1), \text{ where:}$$

d_1, d_2, d_3 - three mutually perpendicular tumor diameters;
V is the volume of the tumor in cm^3.

K was calculated by the formula:

$$K = \frac{V_t - V_0}{V_0} \quad (2), \text{ where:}$$

V_0 is the volume of the tumor before exposure;
V_t is the volume of the tumor at a certain period of observation.

2. Tumor growth inhibition (TGI, %) was calculated by the formula:

$$TGI\ \% = \frac{V_k - V_o}{V_k} * 100\ \% \quad (3), \text{ where:}$$

V_k is the average tumor volume in the control group;
V_o is the average tumor volume in the experimental group.

Таблица 1
Схема экспериментов
Table 1
Experimental design

Опухоль / Tumor	Тест–система/ Test system	Доза ФС мг/кг / PS dose mg/kg	Метод введения / Method of administration	Параметры облучения / Irradiation parameters	
				E Дж/см² / E J/cm²	Ps Вт/см² / Ps W/cm²
Саркома М–1 / Sarcoma M–1	крысы / rats	0,75 1,25 2,5 5,0	Интраперитонеально / Intraperitoneal	150	0,25
Альвеолярный рак печени РС–1/ Alveolar liver cancer RS–1	крысы / rats	2,5 5,0	Интраперитонеально / Intraperitoneal	150	0,25
Меланома В16 / Melanoma B16	мыши / mice	5,0 5,0 5,0 7,5 7,5 10,0 10,0 10,0	Интраперитонеально / Intraperitoneal	150 150 300 150 30 150 150 300	0,25 0,51 0,44 0,51 0,44 0,25 0,51 0,44
Карцинома Эрлиха / Ehrlich Carcinoma	мыши / mice	0,7 1,25 2,5 2,5	Интраперитонеально / Intraperitoneal	100 100 100 100	0,51 0,51 0,28 0,51
Карцинома Эрлиха / Ehrlich Carcinoma	мыши / mice	1,25 2,5	Внутривенно / Intravenous	150 100	0,51 0,51

3. The percentage of animals in the group with complete tumor regression (CR,%) (K = -1.00). The absence of a visible and palpable tumor was taken as TR of the tumor.

4. In some studies, the percentage of animals cured was determined: no tumor recurrence within 90 days after PDT.

Statistical processing of research results was carried out in the computer program "Statistica" by nonparametric methods for independent groups (descriptive statistics, the significance of differences in features). The statistical significance of the differences between the compared traits in the groups was assessed using the Mann – Whitney U test. The differences were considered statistically significant at the level $p < 0.05$.

Research results and discussion

Sarcoma M–1

The level of high and maximum accumulation of the drug in the tumor and the highest contrast index were observed after 3–3.5 h, this interval is optimal for laser irradiation. When PDT was performed at the optimal time with a laser energy density (E) of 150 J/cm^2 and a power density (Ps) of 0.25 W/cm^2, PS doses were selected, with the introduction of which the maximum antitumor effect was observed: 21 days after PDT (see Fig. 2, Table 2).

The maximum antitumor effect was obtained with the intraperitoneal injection of PS LBC in a low dose of 1.25 mg/kg of body weight (Table 2).

When the animals were observed up to 90 days after PDT with an applied dose of LBC of 1.25 mg/kg of body weight, a relapse was observed in 10% of rats. Recovery in 100% of animals was obtained with a PS dose of 2.5 mg/kg of body weight with the same parameters of laser exposure (E=150 J/cm^2 and Ps=0.25 W/cm^2) (Table 2) [23].

PC-1

Based on the pharmacokinetic studies carried out, the highest contrast index was observed after 2.5 hours, that is, this time of laser irradiation was optimal. Despite the significant initial tumor size and aggressiveness, intraperitoneal administration of PS LBC after PDT achieved the maximum antitumor effect: tumor CR in 100% of animals under observation up to 21 days after exposure (E = 150 J/cm^2 and Ps = 0.25 W/cm^2) (Fig. 3, Table 3).

A significant inhibitory effect, consisting in the CR of the tumor in 78.4% of animals, was obtained with the introduction of PS at a dose of 2.5 mg/kg of body

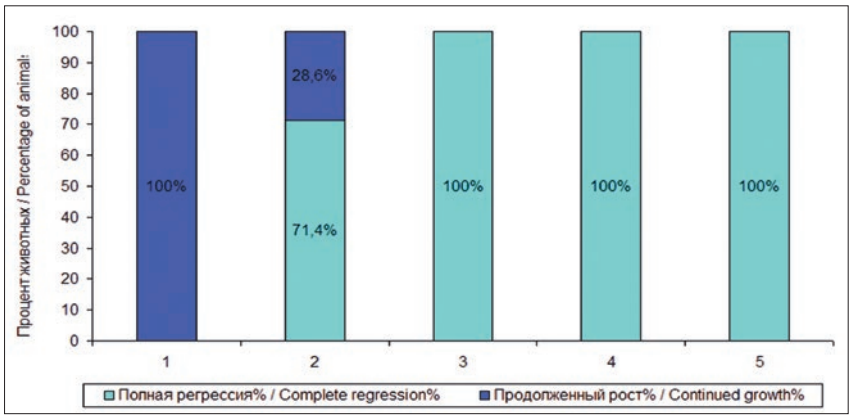

Рис. 2. Эффективность ФДТ на 21 сутки в группе животных с саркомой М–1 при использовании разных доз ФС ЛБХ (мг/кг массы тела): 1 – контроль; 2–0,75; 3–1,25; 4–2,5; 5–5,0.
Fig. 2. Efficiency of PDT on the 21st day in the group of animals with sarcoma M–1 when using different doses of PS LBC (mg/kg of body weight): 1– control; 2–0.75; 3–1.25; 4–2.5; 5–5.0.

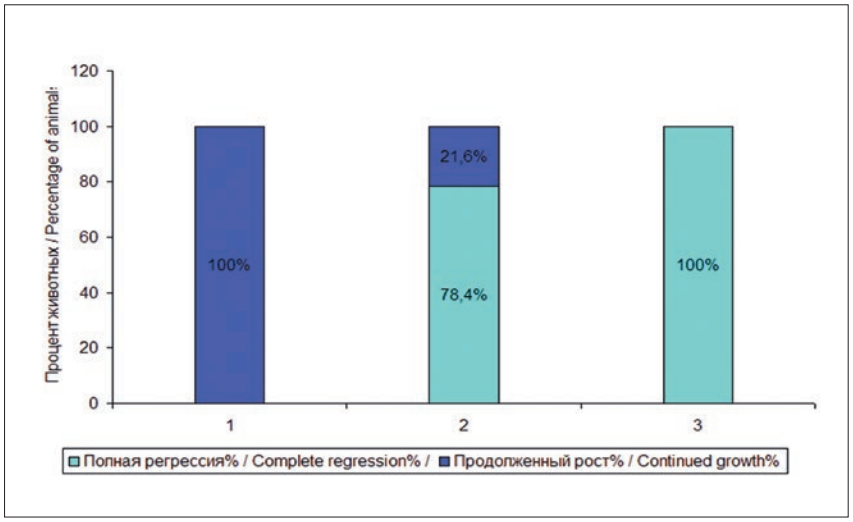

Рис. 3. Эффективность ФДТ на 21 сутки в группе животных с РС–1 при использовании разных доз ФС ЛБХ (мг/кг массы тела): 1 – контроль; 2–2,5; 3–5,0.
Fig. 3. The effectiveness of PDT on day 21 in the group of animals with PC–1 when using different doses of PS LBC (mg/kg of body weight): 1 – control; 2–2,5; 3–5,0.

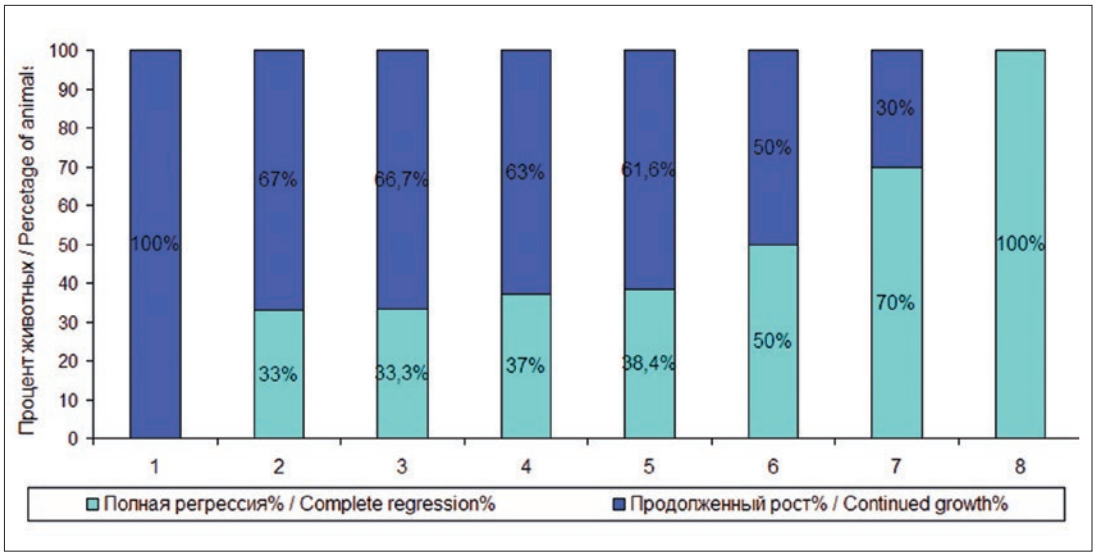

Рис. 4. Эффективность ФДТ на 21 сутки в группе животных с меланомой В16 при использовании разных доз ФС ЛБХ и с разными параметрами облучения: 1 – контроль; 2–2,5 мг/кг; 150 Дж/см^2; 0,51 Вт/см^2; 3–5,0 мг/кг; 100 Дж/см^2; 0,51 Вт/см^2; 4–5,0 мг/кг; 150 Дж/см^2; 0,44 Вт/см^2; 5–5,0 мг/кг; 300 Дж/см^2; 0,44 Вт/см^2; 6–5,0 мг/кг; 300 Дж/см^2; 0,25 Вт/см^2; 7–10 мг/кг; 150 Дж/см^2; 0,25 Вт/см^2; 8–10 мг/кг; 300 Дж/см^2; 0,44 Вт/см^2.
Fig. 4. Efficiency of PDT with different doses of PS LBC and different irradiation parameters on the 21st day in the group of animals with melanoma B16: 1 – control; 2–2.5 mg/kg; 150 J/cm^2; 0,51 W/m^2; 3–5,0 mg/kg; 100 J/cm^2; 0,51 W/cm^2; 4–5,0 mg/kg; 150 J/cm^2; 0,44 W/cm^2; 5–5,0 mg/kg; 300 J/cm^2; 0,44 W/cm^2; 6–5,0 mg/kg; 300 J/cm^2; 0,25 W/cm^2; 7–10 mg/kg; 150 J/cm^2; 0,25 W/cm^2; 8–10 mg/kg; 300 J/cm^2; 0,44 W/cm^2.

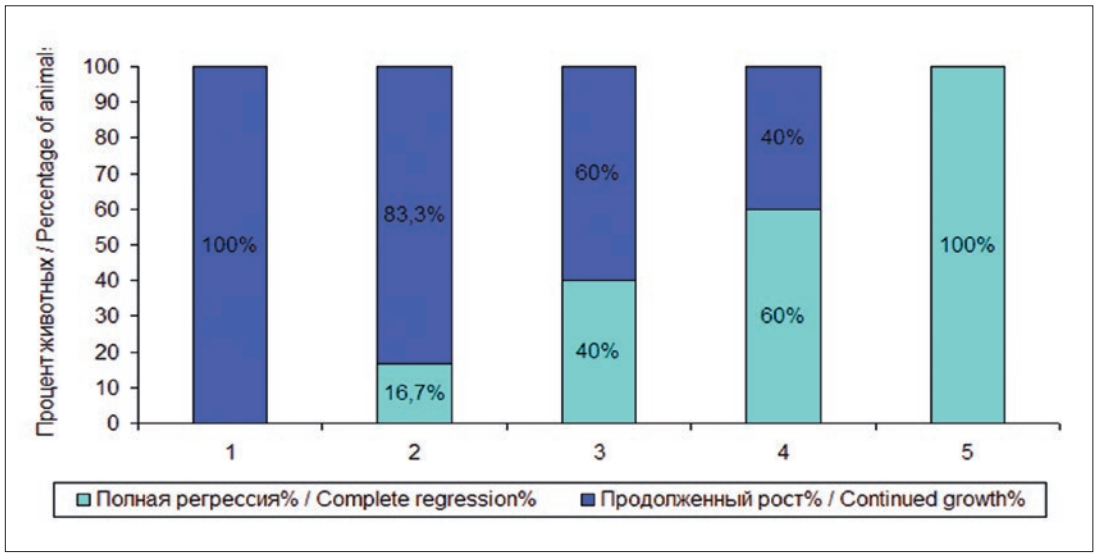

Рис. 5. Эффективность ФДТ при интраперитонеальном введении разных доз ФС ЛБХ и с разными параметрами облучения на 21 сутки в группе животных с карциномой Эрлиха: 1 – контроль; 2 – 0,70 мг/кг; 100 Дж/см^2; 0,51 Вт/см^2; 3 – 1,25 мг/кг; 100 Дж/см^2; 0,51 Вт/см^2; 4–2,5 мг/кг; 100 Дж/см^2; 0,28 Вт/см^2; 5–2,5 мг/кг; 100 Дж/см^2; 0,51 Вт/см^2.
Fig. 5. Efficiency of PDT with different doses of PS LBC (intraperitoneal administration) and different irradiation parameters on day 21 in the group of animals with Ehrlich's carcinoma: 1 – control; 2 – 0,70 mg/kg; 100 J/cm^2; 0,51 W/cm^2; 3 – 1,25 mg/kg; 100 J/cm^2; 0,51 W/cm^2; 4–2,5 mg/kg; 100 J/cm^2; 0,28 W/cm^2; 5–2,5 mg/kg; 100 J/cm^2; 0,51 W/cm^2.

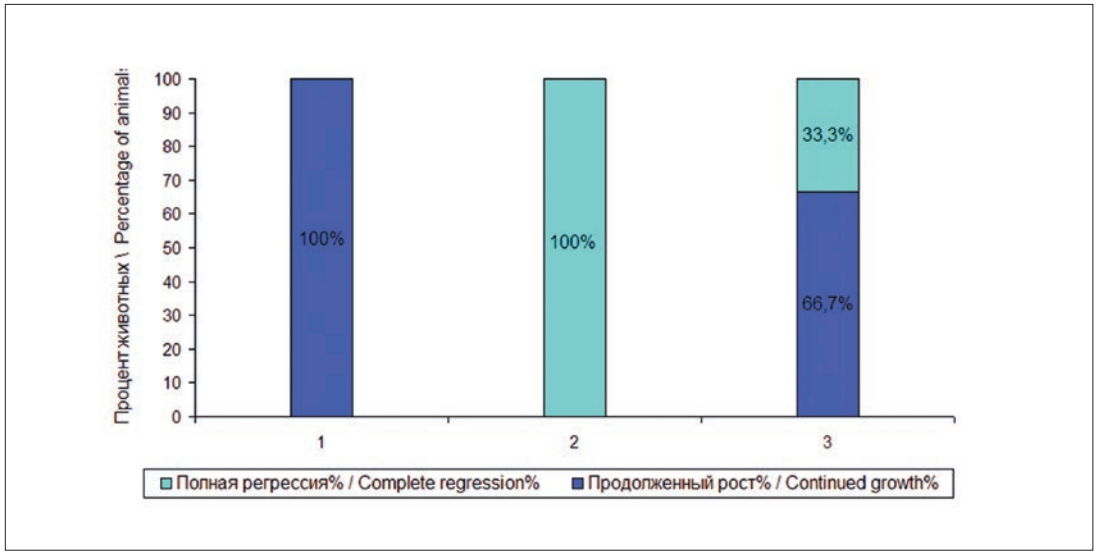

Рис. 6. Эффективность ФДТ при внутривенном введении разных доз ФС ЛБХ и с разными параметрами облучения на 21 сутки в группе животных с карциномой Эрлиха: 1 – контроль; 2 – 1,25 мг/кг; 150 Дж/см^2; 3 – 2,5 мг/кг; 100 Дж/см^2.
Fig. 6. Efficiency of PDT with different doses of PS LBC (intravenous administration) and different irradiation parameters on the 21st day in a group of animals with Ehrlich's carcinoma: 1 – control; 2 – 1,25 mg/kg; 150 J/cm^2; 3 – 2,5 mg/kg; 100 J/cm^2.

weight. The maximum effective dose of PS, leading to the CR of the MS-1 tumor in 100% of animals up to 21 days of the study after PDT (E = 150 J/cm^2 and Ps = 0.25 W/cm^2) with PS LBC, was 5.0 mg/kg body weight (Table 2) [24, 25].

Melanoma B16

Melanoma is one of the most aggressive tumors. A large number of experiments were performed with different doses of PS, different parameters of laser radiation and appropriate control in each experimental series. The results were not stable, especially at low PS doses and low values of laser radiation parameters. With an increase in the PS dose and laser radiation parameters, the most pronounced and stable tumor regression was observed (Fig. 4).

Таблица 2
Динамика роста перевивных опухолей после ФДТ с ФС ЛБХ в разных дозах и при различных параметрах лазерного излучения
Table 2
Dynamics of the growth of transplanted tumors after PDT with liposomal boronated chlorin e6 at different doses and at different parameters of laser radiation

№	Схема опыта / The experiment scheme	1. Коэффициент прироста опухоли (К) / The coefficient of tumor growth (K); 2. Торможение роста опухоли (ТРО,%) / Tumor growth inhibition (TGI,%); 3. Процент животных с полной регрессией опухоли (ПР, %) / The percentage of animals with complete tumor regression (CR,%) 4. Процент излеченных животных (90 сут)/ Percentage of animals healed (90 days)					
		Время исследования после ФДТ / Time of research after PDT					
		3 сут / 3 days	7 сут / 7 days	10 сут / 10 days	14 сут / 14 days	21 сут / 21 days	90 сут / 90 days
Саркома М–1 крыс / rat Sarcoma M-1 (E=150 Дж/см² / J/cm²; Ps=0,25 Вт/см² / W/cm²)							
1.	0,75 мг/кг/ 0,75 mg/kg	ПР/CR=100%				K / K=0,19±0,57* ТРО / TGI=94,0% ПР / CR=85,7%	ПР/CR= 71,4%
2.	1,25 мг/кг/ 1,25 mg/kg	ПР/CR=100%					ПР/CR= 90,0%
3.	2,5 мг/кг / 2,5mg/kg	ПР/CR=100%					
Контроль(К)/ Control (K)		K/K=1,28 ±0,23	K/K=6,97 ±1,25	K/K= 12,82±2,41	K/K=20,36±3,65	K/K= 52,54±10,56	**
Альвеолярный рак печени РС–1 крыс / rat Alveolar liver cancer RS–1 (E=150 Дж/см² / J/cm²; Ps=0,25 Вт/см²/ W/cm²)							
1.	2,5 мг/кг / 2,5 mg/kg	ПР/CR=100%			K / K=0,57±0,31* ТРО / TGI=98,1% ПР / CR=85,7%	K / K= 2,74±1,99* ТРО / TGI=91,4% ПР / CR=78,4%	**
2.	5,0 мг/кг / 5,0 mg/kg	ПР/CR=100%					**
Контроль (К) / Control (K)		K/K=0,48 ± 0,10	K/K=1,94 ± 0,40	K/K=4,96 ± 0,84	K/K=10,41 ±1,62	K/K=23,25 ±6,26	**
Меланома В16 мышей / mouse Melanoma B16							
1.	10,0 мг/кг; 150 Дж/см²; 0,25 Вт/см² / 10,0 mg/kg; 150 J/cm²; 0,25 W/cm²	ПР/CR=100%		K/K=0,68±1,19 ТРО/TGI=87,8% ПР/CR=80,0 %	K/K=1,76±1,76 ТРО/TGI=88,4% ПР/CR=70,0%	K/K=6,44±6,64* ТРО/TGI=89,0% ПР/CR=70,0%	**
2.	10,0 мг/кг; 300 Дж/см²; 0,44 Вт/см² / 10,0 mg/kg; 300 J/cm²; 0,44 W/cm²	ПР/CR=100%					**
Контроль (К) / Control (K)		K/K=0,57 ±0,20	K/K=2,01 ±0,54	K/K=4,74 ±1,02	K/K=12,89±4,58	K/K= 53,17±13,06	**
Карцинома Эрлиха мышей (интраперитонеальное введение) / mouse Ehrlich carcinoma (intraperitoneal administration) (E=100 Дж/см² / J/cm²)							
1.	1,25 мг/кг 150 Дж/см²/ 1,25 mg/kg 150 J/cm²	ПР/CR=100%					**
2.	2,5 мг/кг 100 Дж/см²/ 2,5 mg/kg 100 J/cm²	ПР/CR=100%				K/K=0,72 ± 0,39* ТРО/TGI=92,4% ПР/CR=66,7%	**
Контроль (К) / Control (K)		K/K=2,44 ± 0,64	K/K=4,43 ± 0,96	K/K=7,87 ± 1,83	K/K=15,41±5,03	K/K=32,32±12,55	**

№	Схема опыта / The experiment scheme	1. Коэффициент прироста опухоли (К) / The coefficient of tumor growth (K); 2. Торможение роста опухоли (ТРО,%) / Tumor growth inhibition (TGI,%); 3. Процент животных с полной регрессией опухоли (ПР, %) / The percentage of animals with complete tumor regression (CR,%) 4. Процент излеченных животных (90 сут)/ Percentage of animals healed (90 days)					
		Время исследования после ФДТ / Time of research after PDT					
		3 сут / 3 days	7 сут / 7 days	10 сут / 10 days	14 сут / 14 days	21 сут / 21 days	90 сут / 90 days
	Карцинома Эрлиха мышей (внутривенное введение) / mouse Ehrlich carcinoma (intravenous administration) (Ps=0,51 Вт/см^2 / W/cm^2)						
	1,25 мг/кг 150 Дж/см^2/ 1,25 mg/kg 150 J/cm^2	ПР/CR=100%					**
	2,5 мг/кг 100 Дж/см^2/ 2,5 mg/kg 100 J/cm^2	ПР/CR=100%				K/K=0,72 ± 0,39* ТРО/TGI=92,4% ПР/CR=66,7%	**
	Контроль (К) / Control (K)	K/K=2,44 ± 0,64	K/K=4,43 ± 0,96	K/K=7,87 ± 1,83	K/K=15,41±5,03	K/K=32,32±12,55	**

Примечание:

* – коэффициент абсолютного прироста опухоли в опыте достоверно ниже по сравнению с контролем (p <0,05);

** – нет данных (замеры объемов опухолей заканчивали на 21 сутки после ФДТ, поскольку на этот срок в контроле начинался падеж животных и дальнейшее сравнение перестает быть корректным. На 90 сутки выживших животных во всех контрольных группах не было, соответственно расчет ТРО не проводили).

Notes:

* – the coefficient of absolute tumor growth in the experiment is significantly lower than in the control (p < 0,05);

** – no data (measurements of tumor volumes were done on day 21 after PDT, since at this time the deaths of animals started in the control and further comparison ceased to be correct. On day 90, there were no surviving animals in any control groups, so the calculation of TGI was not performed.)

Таблица 3
Динамика роста саркомы М–1 крыс после ФДТ с интраперитонеальным введением борированного хлорина е6
Table 3
Dynamics of M-1 sarcoma growth in rats after PDT with intraperitoneal injection of boronated chlorin e6

№	Схема проведения ФДТ / PDT scheme	1. Коэффициент прироста опух. (К) / The coefficient of tumor growth (K); 2. Торможение роста опухоли (ТРО, %) / Tumor growth inhibition (TGI,%); 3.Процент животных с полной регрессией опухоли (ПР, %) / The percentage of animals with complete tumor regression (CR,%)				
		Время исследования после ФДТ / Time of research after PDT				
		3 сут / 3 days	7 сут / 7 days	10 сут / 10 days	14 сут / 14 days	21 сут / 21 days
	(E=150 Дж/см^2 / J/cm^2; Ps=0,25 Вт/см^2 / W/cm^2)					
1.	1,25 мг/кг / 1,25 mg/kg	K / K = –0.88±0,12* ТРО / TGI = 99,4% ПР / CR = 95,0%	K / K= –0.76±0,17* ТРО / TGI = 98,2% ПР / CR = 90,0%	K / K = 0,14±0,60* ТРО / TGI = 96,5% ПР / CR = 80,0%	K / K = 3.14±1,99* ТРО / TGI = 93,3% ПР / CR = 75.0%	K / K = 25,86±11,10* ТРО / TGI = 86,1% ПР / CR = 50,0%
1.	2,5 мг/кг / 2,5 mg/kg	ПР/CR=100%				K / K = 0,30 ± 1,30* ТРО / TGI = 99,4% ПР / CR = 92,0%
2.	5,0 мг/кг / 5,0 mg/kg	ПР/CR=100%				
	Контроль (К) / Control (K)	K / K = 1,42 ± 0,18	K / K = 8,20 ± 1,14	K / K = 16,44 ±2,22	K / K = 26,96 ±3,91	K / K = 61,54 ± 9,73

According to the results of pharmacokinetic studies, the optimal time for laser irradiation was established: 2.0 hours after the introduction of the PS. The maximum antitumor effect was obtained with a PS dose of 10.0 mg/kg body weight and laser radiation parameters E = 300 J/cm^2 and Ps = 0.44 W/cm^2 (Table 2) [26].

Ehrlich's carcinoma

Intraperitoneal administration. The optimal time for laser irradiation after the introduction of PS LBC at a dose of 2.5 mg/kg of body weight occurs after 1.5 hours. The antitumor effect depends on the PS dose and the parameters of laser radiation (Fig. 5).

The maximum antitumor effect, leading to the CR of Ehrlich carcinoma in 100% of animals up to 21 days after PDT, is manifested with the intraperitoneal injection of PS at a dose of 2.5 mg/kg of body weight and the parameters of laser radiation E=100 J/cm^2 and Ps=0.51 W/cm^2. On the 90th day after therapy, a high level of recovery of animals was obtained (80% of cases) (Table 2).

Intravenous administration. The optimal time for laser irradiation after intravenous administration of PS LBC at a dose of 2.5 mg/kg of body weight occurs in 30 - 75 minutes (Fig. 6).

The maximum efficiency of therapy for animals with Ehrlich's carcinoma was observed at a PS dose of 1.25 mg/kg of body weight, a light dose of 150 J/cm^2 and a power density of 0.51 W/cm^2 of laser radiation. With an increase in the PS dose to 2.5 mg/kg of body weight and a decrease in the light dose of laser radiation to 100 J/cm^2, a significant antitumor effect was obtained, consisting in TPO in 92.4% of observations at 66.7% CR (Table 2) [27].

Table 2 shows the results of studies of the antitumor response of PS LBC e6 in the experimental groups.

Previously, we analyzed the response of boronated chlorin in the model of rat M–1 sarcoma [28] (Table 3).

Comparison of the study results of the two drug forms revealed a clear advantage of PS LBC in comparison with the non-liposomal form. The maximum efficiency of PDT with PS LBC against sarcoma M–1 was observed at a dose of 1.25 mg/kg of body weight, which is 4 times lower than the dose of boronated chlorin (5.0 mg/kg), leading to the same antitumor response, other conditions being equal.

Conclusion

Based on the results of our studies, it has been shown that the domestic photosensitizer PS LBC has a high antitumor activity *in vivo*. By adjusting the PS doses and laser exposure parameters, the maximum inhibitory effect was obtained (CR in 100% of animals up to 21 days after PDT) on all tumor models used: sarcoma M–1 and alveolar liver cancer PC–1 in rats, melanoma B16 and Ehrlich's carcinoma in mice.

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