ORIGINAL ARTICLES

DOXORUBICIN ENHANCES THE ANTITUMOR EFFICACY OF SONODYNAMIC THERAPY WITH PHOTOSENSITIZER PHOTOLON IN AN *IN VIVO* EXPERIMENT

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Abstract

The antitumor effectiveness of sonodynamic therapy (SDT) with a chemotherapeutic drug and a photosensitizer (PS) of the chlorine series was studied in an *in vivo* experiment. The work was performed on 60 white nonlinear rats, divided into 2 series of 30 individuals each. Pliss lymphosarcoma, transplanted subcutaneously, was used as a tumor strain. Photolon was administered intravenously in a single dose of 2.5 mg/kg 2.5-3 hours before ultrasound exposure, and doxorubicin was administered intraperitoneally in a single dose of 5 mg/kg 0.5 hours before ultrasound exposure performed using the «Phyaction U» apparatus, generating radiation with a frequency of 1.04 MHz, intensities of 0.5 and 1.5 W/cm^2 and lasting 5 minute. The study groups in each series included 5 rats: control, ultrasound, doxorubicin, photolon + ultrasound, doxorubicin + ultrasound, photolon + doxorubicin + ultrasound. To assess antitumor effectiveness, criteria generally accepted in experimental oncology were used: average volume of tumors ($V_{av.}$, cm^3), absolute tumor growth rate (K, units), tumor growth inhibition coefficient (TGI, %), frequency of complete tumor regressions (CR, %), the average life expectancy of rats (ALE, days), the coefficient of increase in the average life expectancy of rats (%) and the median overall survival (days). Differences were considered statistically significant at a significance level of $p<0.05$. In the first and second series of experiments, the most effective modes were the use of photolon, doxorubicin and ultrasound with a frequency of 1.04 MHz and intensities of 0.5 and 1.5 W/cm^2, respectively. The proposed combination of therapeutic interventions made it possible to statistically significantly ($p<0.05$) increase the indicators of TGI, PR and ALE compared to the control and each of the components of the method separately. SDT methods developed and tested in *in vivo* experiments are characterized by high antitumor efficacy.

Keywords: Pliss lymphosarcoma, doxorubicin, photolon, ultrasound, sonodynamic therapy.

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СОНОДИНАМИЧЕСКАЯ ТЕРАПИЯ С ДОКСОРУБИЦИНОМ И ФОТОСЕНСИБИЛИЗАТОРОМ ФОТОЛОН В ЭКСПЕРИМЕНТЕ *IN VIVO*

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Резюме

Исследована противоопухолевая эффективность сонодинамической терапии (СДТ) с химиотерапевтическим лекарственным средством (ХЛС) и фотосенсибилизатором (ФС) хлоринового ряда в эксперименте *in vivo*. Работа выполнена на 60 белых нелинейных крысах, распределенных на 2 серии по 30 особей в каждой. В качестве опухолевого штамма использовали лимфосаркому Плисса, перевиваемую подкожно. Фотолон вводили внутривенно однократно в дозе 2,5 мг/кг за 2,5-3 ч до ультразвукового воздействия, а доксорубицин – внутрибрюшно однократно в дозе 5 мг/кг за 0,5 ч до ультразвукового воздействия, осуществляемого с помощью аппарата «Phyaction U», генерирующего излучение с частотой 1,04 МГц, интенсивностями 0,5 и 1,5 Вт/см^2 и продолжительностью 5 мин. Группы исследования в каждой из серий включали по 5 крыс: контроль, ультразвук, доксорубицин, фотолон + ультразвук, доксорубицин + ультразвук, фотолон + доксорубицин + ультразвук. Для оценки противоопухолевой эффективности использовались общепринятые

в экспериментальной онкологии критерии: средний объем опухолей ($V_{ср}$, см^3), коэффициент абсолютного прироста опухолей (К, отн. ед.), коэффициент торможения роста опухолей (ТРО, %), частота полных опухолевых регрессий (ПР, %), средняя продолжительность жизни крыс (СПЖ, сут), коэффициент увеличения средней продолжительности жизни крыс (УПЖ, %) и медиана общей выживаемости (сут). Различия считали статистически значимыми при уровне значимости $p<0,05$. В первой и второй сериях экспериментов наиболее эффективными режимами было применение фотолон, доксорубицин и ультразвука с частотой 1,04 МГц и интенсивностями 0,5 и 1,5 Вт/см^2 соответственно. Предложенное сочетание терапевтических воздействий позволило статистически значимо ($p<0,05$) увеличить показатели ТРО, ПР и УПЖ по сравнению с контролем и каждым из компонентов метода в отдельности. Разработанные и апробированные в экспериментах *in vivo* методики СДТ характеризуются высокой противоопухолевой эффективностью.

Ключевые слова: лимфосаркома Плисса, доксорубицин, фотолон, ультразвук, сонодинамическая терапия.

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Inrtoduction

The search for and testing of new methods for treating malignant neoplasms at the preclinical stage of research with their subsequent implementation in practical healthcare is a key aspect of experimental and clinical oncology. It is well known that the gold standard for treating patients with malignant neoplasms is surgery, radiation therapy and chemotherapy. A number of alternative methods based on the influence of physical factors on tumor cells and tissues also have a certain therapeutic potential. Such methods primarily include laser therapy, cryotherapy, hyperthermia, high-intensity focused ultrasound, etc. From a scientific point of view, it is relevant to study the possibility of combined use of ultrasound radiation (US) and various classes of drugs.

According to a number of authors, the effect of ultrasound with a pulse frequency of 0.5-3 MHz and an intensity of 0.5-5 W/cm^2 can increase the cytotoxicity of various chemotherapeutic drugs. Researchers associate this fact with an increase in the permeability of cell membranes and the implementation of the effects of cavitation, hyperthermia and sono-induced free-radical oxidation of the biological structures of the tumor cell [1, 2]. A new direction in experimental oncology is called "sonodynamic therapy" (SDT), and the drugs used are called sonosensitizers (SS). The SS class primarily includes radiosensitizers (dimexide, metronidazole) and a number of chemotherapeutic agents (bleomycin, adriamycin, cisplatin, etoposide, 5-fluorouracil, etc.) [3].

At the same time, in the late 80s of the 20th century, Japanese scientists led by T. Yumita conducted preclinical studies on tumor cell cultures, proving the presence of sonosensitizing properties in another class of drugs, such as photosensitizers (PS), which until then had been actively used for photodynamic therapy. In 1990, the first results were published confirming the high antitumor efficacy of SDT with hematoporphyrin [4].

In recent years, the results of numerous *in vitro* and *in vivo* studies have been published, indicating the high antitumor efficacy of SDT with PS in the treatment of a number of nosological forms of malignant neoplasms (breast cancer, lung, intestine, pancreas, soft tissue sarcoma, skin melanoma, osteosarcoma, leukemia, glioma) [5, 6, 7, 8]. A relevant and promising area of scientific research is the combined use of SDT with drugs that have fundamentally different mechanisms for implementing an antitumor response when activated by ultrasound, namely, PS and chemotherapeutic drugs [9, 10, 11, 12, 13].

There are a small number of publications in the foreign literature devoted to the study of this issue, most of which were carried out in *in vitro* experiments. So, H.J. Gao et al. (2010) (Department of Clinical Oncology, Guangzhou General Hospital of Guangzhou Command, People's Republic of China) assessed the effectiveness of SDT with chlorine e6 in doses from 0.05 to 1.6 mg/ml and adriamycin in doses from 0.1 to 0.4 g/ml on human breast cancer cell culture *MDA-MB-231*. The use of US with a frequency of 1 MHz and intensities from 0.5 to 2 W/cm^2 after preliminary addition of PS to the cell culture followed by the addition of adriamycin after sonodynamic exposure caused a statistically significant reduction in the number of viable tumor cells compared to each of the components of the proposed scheme ($p<0.05$) [9].

L. Liang et al. (2013) (State Key Laboratory of Bioreactor Engineering, Shanghai Key Laboratory of Chemical Biology, People's Republic of China) in an experiment on a culture of human cholangiocarcinoma tumor cells *QBC939* proved a dose-dependent increase in the cytotoxicity of hematoporphyrin and doxorubicin monomethyl ester when combined with US. Doxorubicin was used in doses of 0.0625; 0.125; 0.25; 0.5; 1; 2 and 4 μg/ml, and PS - 5, 10, 20, 40 and 80 μg/ml. Local ultrasonic influence on tumor cells, sensitized with the help of these drugs, was carried out with a frequency of 1.2 MHz and intensities from 0.5 to 2 W/cm^2. The authors used various combinations of drugs and ultrasound modes. The results obtained indicate a statistically significant increase in the number of non-viable tumor cells due

to the toxic effect of a significantly higher percentage of reactive oxygen species and significant activation of cell apoptosis when using ultrasound with high doses of both drugs. In addition, the expression of *p53*, *Fas*, *Bax* and *caspase-3* were significantly upregulated in cells exposed to the combination therapy [10].

T. Osaki et al. (2016) (Joint Department of Veterinary Clinical Medicine, Japan) studied the sonodynamic properties of 5-aminolevulinic acid (5-ALA) and bleomycin in combination with US with frequencies of 1 and 3 MHz, and intensities from 1 to 3 W/cm^2 in an experiment on mouse mammary cell culture. Thus, when using US with a frequency of 1 MHz and intensities of 1, 2 and 3 W/cm^2, the number of viable tumor cells decreased by 34.30%, 50.90% and 60.16%, respectively. When bleomycin was added to the cell culture with its further insonation, it led to a significant reduction in this indicator: 0.09%, 0.32% and 0.17%, respectively. Additionally, the authors report that the use of US with a frequency of 3 MHz, regardless of the radiation intensity and exposure modes, did not lead to positive results [11].

The same team of authors obtained similar results in an experiment on cell culture and laboratory animals with intestinal carcinoma of *Colon-26* mice with the combined use of bleomycin, aluminum disulfonate phthalocyanine and low-intensity US. PS was used in doses of 1, 5 and 10 μg/ml, bleomycin - in doses of 1, 5 and 10 μg/ml. Ultrasonic exposure was carried out with a frequency of 3 MHz and intensities from 1 to 3 W/cm^2, the frequency of ultrasonic pulses, depending on the series of experiments, varied from 5% to 100%. The number of viable cells in the «Bleomycin + SDT» group was statistically significantly lower compared to that in groups with different doses of PS (1, 5 and 10 μg/ml) when used in combination with US ($p=0.0498$; $p=0.0405$ and $p=0.0219$; respectively). The studied indicator in the combination therapy group «PS + bleomycin + SDT» was statistically lower than in the groups «PS + SDT» and «Bleomycin + SDT» ($p<0.05$) [12].

In the *in vivo* part of the study, performed on linear BALB/c mice with a subcutaneous model of intestinal carcinoma *Colon-26*, the high antitumor efficacy of combination therapy was proven. The study design included the following groups: intact control; PS; bleomycin; US; PS + US; bleomycin + US and PS + bleomycin + US (combination therapy). PS was administered once intraperitoneally at a dose of 20 mg/ml 18 hours before ultrasound exposure of transplanted tumors. Bleomycin – once intraperitoneally at a dose of 25 mg/ml 0.5 hours before ultrasound exposure of transplanted tumors. The SDT session was carried out with an ultrasound frequency of 3 MHz (50%) and intensity of 3 W/cm^2. The criteria for assessing effectiveness were the volume of tumors, calculated on the 12th day after the start of therapeutic interventions, and histological examination data. The authors registered a statistically significant inhibition of the growth of transplanted tumors in the group of combination therapy using drugs in the indicated doses compared with each component of the treatment regimen ($p<0.05$) and the control. According to a morphological study, this fact was associated with both a direct cytotoxic effect and lethal damage to tumor cells and tissues due to disruption of the blood supply to tumors due to disruption of the integrity and patency of the blood vessels feeding them ($p<0.05$) [12].

In a study by R. Xu et al. (2020) (Department of Breast Surgery, The First Affiliated Hospital, People's Republic of China) studied the antitumor efficacy of the combined use of high-intensity US with an intensity of 4 W/cm^2 in combination with silicon nanoparticles with doxorubicin and chlorin e6. In the *in vivo* experiment, we used linear BALB/c nude mice with subcutaneously inoculated human breast cancer *MDA-MB-231*. The data obtained indicate a significant inhibition of growth and a decrease in the weight of experimental tumors when using a combination treatment regimen compared with each of its components ($p<0.05$) [13].

The purpose of this work is to study the antitumor effectiveness of the SDT method with photolon and doxorubicin using low- and high-intensity US.

Materials and methods

Laboratory animals

The pilot study was performed on 60 white nonlinear outbred male rats obtained from the vivarium of N.N. Alexandrov National Cancer Centre of Belarus, with a body weight from 150 to 300 g, aged 2.5-3 months. The duration of quarantine before inclusion in the experiment was 14 days. The rats were kept under standard conditions of food and drink rations *ad libitum*, with 12-hour illumination, at a temperature of 18–22°C and a humidity of 55–60% in individual cages, 5 individuals in each. The conditions for keeping rats in the laboratory, as well as humidity, temperature, and lighting in the room, complied with the current sanitary rules for the design, equipment and maintenance of vivariums [14, 15, 16].

Tumor strain

Pliss lymphosarcoma (PLS) obtained as a cell culture (Russian Collection of Cell Cultures, Institute of Cytology RAS, St. Petersburg, Russian Federation) was used as a tumor strain.

Tumor model

PLS cell culture was inoculated subcutaneously in rats. Subcutaneous inoculation of the experimental study included the introduction under the skin of the left inguinal region of 0.5 ml of a suspension of tumor cells in 20% Hanks solution, obtained after taking and homogenizing tumor pieces from a donor rat. PLS is one of the rapidly growing tumors with a short latent period. In this regard, rats with PLS were included in the

experiment on the 5th day after transplantation, when the diameter of the tumor node, on average, was 5 mm.

Ethical aspects

Experimental studies were carried out in accordance with international legislation and the regulatory legal acts in force in the Republic of Belarus for conducting experimental studies with laboratory animals [17, 18, 19].

The nature of the studies performed was consistent with the principles of «3Rs» developed by W.M. Russell and R.L. Berch (1959) [20].

The study was approved by the Ethics Committee of the N.N. Alexandrov National Cancer Center of Belarus (protocol № 180).

Before irradiation, rats were anesthetized (neuroleptanalgesia: 0.005% fentanyl solution + 0.25% droperidol solution, in a ratio of 2:1, 0.2 ml per 100 g of body weight, intramuscularly). After the end of the observation period, the rats were sacrificed using generally accepted methods of euthanasia (*aether pro narcosi*) in compliance with the humane methods of handling laboratory animals.

Chemotherapy drug

The injection form of lyophilized doxorubicin powder (DOX) (RUE «Belmedpreparaty», Minsk, Republic of Belarus) was used as a chemotherapeutic drug. The DOX were dissolved in a 0.9% sodium chloride solution and administered on the 5th day after tumor transplantation with LSP once, intraperitoneally, at a dose of 5 mg/kg.

Photosensitizer

As a drug, an injectable form of PS based on chlorin e6 photolon (RUE «Belmedpreparaty», Minsk, Republic of Belarus, registration number 16/11/886 dated November 08, 2016, 100 mg) was used. Before use, PS powder was diluted with 0.9% sodium chloride solution and administered once by intravenous infusion into the tail vein of a rat in a darkened room at a dose of 2.5 mg/kg.

Sonodynamic therapy

A session of local ultrasound therapy (US) was carried out once using a device («Phyaction U», Gymna Yniphy, Belgium) using an applicator with a emitting surface area of 5 cm^2 with a frequency of 1.04 MHz, intensities of 0.5 and 1.5 W/cm^2 and lasting 5 minutes in continuous mode after preliminary hair depilation and surface treatment experimental tumor with a special gel. The ultrasound session began 2.5–3 hours after photolon administration, and intraperitoneal administration of DOX was carried out 0.5 hours before irradiation.

Study design

All therapeutic effects were carried out on the 5th day after LSP grafting, upon reaching the diameter of the tumor node of at least 5 mm. The control group consisted of rats with a transplanted tumor, which were not administered PS, DOX and were not irradiated (control). All rats were randomly distributed at each stage of the study into 6 groups of 5 individuals each (Tables 1, 2).

Таблица 1
Дизайн экспериментального исследования (серия 1)
Table 1
Experimental study design (series 1)

Наименование группы Groups	Количество крыс в группе, n Number of rats in the group, n
Контроль Control	5
ДОКС 5 мг/кг DOX 5 mg/kg	5
УЗТ 1,04 МГц; 0,5 Вт/см^2 UT 1.04 MHz; 0.5 W/cm^2	5
ДОКС 5 мг/кг + УЗТ 1,04 МГц; 0,5 Вт/см^2 DOX 5 mg/kg + US 1.04 MHz; 0.5 W/cm^2	5
ФС 2,5 мг/кг + УЗТ 1,04 МГц; 0,5 Вт/см^2 PS 2.5 mg/kg + US 1.04 MHz; 0.5 W/cm^2	5
ФС 2,5 мг/кг + ДОКС 5 мг/кг + УЗТ 1,04 МГц; 0,5 Вт/см^2 PS 2.5 mg/kg + DOX 5 mg/kg + US 1.04 MHz; 0.5 W/cm^2	5

*ДОКС – доксорубицин; УЗТ – ультразвуковая терапия; ФС – фотосенсибилизатор.
*DOX – doxorubicin; US – ultrasound therapy; PS – photosensitizer.

Таблица 2
Дизайн экспериментального исследования (серия 2)
Table 2
Experimental study design (series 2)

Наименование группы Groups	Количество крыс в группе, n Number of rats in the group, n
Контроль Control	5
ДОКС 5 мг/кг DOX 5 mg/kg	5
УЗТ 1,04 МГц; 1,5 Вт/см^2 UT 1.04 MHz; 1.5 W/cm^2	5
ДОКС 5 мг/кг + УЗТ 1,04 МГц; 1,5 Вт/см^2 DOX 5 mg/kg + US 1.04 MHz; 1.5 W/cm^2	5
ФС 2,5 мг/кг + УЗТ 1,04 МГц; 1,5 Вт/см^2 PS 2.5 mg/kg + US 1.04 MHz; 1.5 W/cm^2	5
ФС 2,5 мг/кг + ДОКС 5 мг/кг + УЗТ 1,04 МГц; 1,5 Вт/см^2 PS 2.5 mg/kg + DOX 5 mg/kg + US 1.04 MHz; 1.5 W/cm^2	5

*ДОКС – доксорубицин; УЗТ – ультразвуковая терапия; ФС – фотосенсибилизатор.
*DOX – doxorubicin; US – ultrasound therapy; PS – photosensitizer.

Criteria for evaluating antitumor efficacy

The antitumor efficacy of the interventions was assessed according to the indicators generally accepted in experimental oncology, which characterize the dynamics of changes in the average tumor volume ($V_{av.}$, cm^3), as well

as the change in the coefficient of absolute tumor growth (K) and the index of tumor growth inhibition (TGI, %). The growth dynamics of transplanted tumors was recorded starting from the 6th day after transplantation of the PLS tumor strain for 2 weeks with an interval of 2–3 days.

Tumor volume was calculated using the following Shreks formula (1):

$$V = \frac{\pi}{6} \times d1 \times d2 \times d3\ , \qquad (1)$$

where:

d1,2,3 – three mutually perpendicular tumor diameters (in cm);

π/6 = 0.52 – a constant value;

V – the volume of the tumor (in cm^3).

The coefficient of absolute tumor growth (K) was calculated by the following formula (2):

$$K = \frac{Vt - V_0}{V_0}, \qquad (2)$$

where:

V_0 – the initial volume of the tumor (before exposure);

Vt – the tumor volume for a certain period of observation.

The coefficient of tumor growth inhibition (TGI) was calculated by the following formula (3):

$$TGI = \frac{Vcontrol - Vexpeirence}{Vcontrol} \times 100\%, \qquad (3)$$

where:

$V_{control}$ – the average volume of the tumor in the control group (in cm^3);

$V_{experience}$ – the average volume of the tumor in the main group (in cm^3).

The minimally significant criterion demonstrating the effectiveness of the treatment of transplanted tumors was considered TGI > 50%.

The frequency of complete tumor regressions (CR) was assessed 60 days after the end of exposure by the absence of visual and palpatory signs of tumor growth.

$$CR\ frequency = \frac{Number\ of\ animals\ without\ tumors}{Number\ of\ animals\ in\ group} \times$$

$$\times\ 100\%. \qquad (4)$$

The evaluation of the antitumor effect by increasing the lifespan was carried out at the end of the experiment and the death of all rats. The average life expectancy (ALE, days) in the groups was determined and the indicators of life expectancy increase (LEI, %) were calculated using the formula [5]:

$$LEI = \frac{ALEexperiment - ALEcontrol}{ALEcontrol} \times 100\%, \qquad (5)$$

where:

LEI – an indicator of the increase in the life expectancy of dead rats (in %);

$ALE_{experiment}$ – the average life expectancy of dead rats in the experimental groups (per day);

$ALE_{control}$ – the average life expectancy of dead rats in the control group (per day).

The minimum significant indicator of LEI demonstrating the effectiveness of treatment of transplanted tumors was considered to be 50%.

Statistical processing of the obtained data

Statistical processing of data ($V_{av.}$, coefficients K and TGI) was carried out using application packages Excel (version 2010), Origin Pro (version 7.0) and Statistica (version 10.0). Data were presented as M±m (mean ± error of the mean). To assess the significance of differences, the nonparametric *Mann-Whitney U test* was used. Comparative analysis of survival rates was carried out using a nonparametric *log-rank test*. Differences were considered statistically significant at a significance level of $p<0.05$.

Results and discussion

The effectiveness of the interventions proposed in the Design was assessed based on the analysis of indicators characterizing the change in the dynamics of the linear sizes of transplanted tumors ($V_{av.}$, coefficients K and TGI).

Information about the dynamics of changes in $V_{av.}$ and, accordingly, the coefficients K and TGI associated with this indicator, when using the above effects in rats with LSP in series 1 of the experiment are presented in Table 3.

Combination therapy, including a single intra-abdominal injection of DOX at a dose of 5 mg/kg, followed 0.5 hours later by an US session with a frequency of 1.04 MHz and an intensity of 0.5 W/cm^2 according to the $V_{av.}$ Criterion was statistically significantly more effective compared to US alone (p=0.001; $V_{av.}$ in the group was 4.59 times less) and tended to increase efficiency compared to chemotherapy without radiation (p=0.86; $V_{av.}$ in the group 1.2 times less).

In turn, combination therapy, including a single intravenous injection of photolon at a dose of 2.5 mg/kg 2.5–3 hours and intraperitoneal administration of DOX at a dose of 5 mg/kg 0.5 hours before the ultrasound session with a frequency of 1.04 MHz and intensity 0.5 W/cm^2 according to the $V_{av.}$ criterion was statistically significantly more effective compared to the combined use of PS and US (p=0.004; $V_{av.}$ in the group was 9.84 times less) and tended to increase efficiency compared to the DOX + US regimen (p=0.61; $V_{av.}$ in the group was 1.94 times less).

When assessing long-term results in terms of the incidence of CR of tumors, high efficiency was noted both in the DOX group and in the combination therapy groups (DOX + US and PS + DOX + US). It is worth noting that in the PS + DOX + US group the most optimistic results were recorded according to the criteria for

lifespan and lifespan compared to the control group (ALE 52.0±5.1 days versus 18.0±1.0 days; LEI = 188.89%; p=0.00004). When comparing data on survival rates in this group with the results of groups in which the effects are components of a «triple» scheme, statistical significance was obtained for the differences in life

Таблица 3
Данные о динамике изменения линейных размеров опухолей в эксперименте на крысах с ЛСП

Table 3
Data on the dynamics of changes in the linear sizes of tumors in an experiment on rats with LSP

Наименование группы Groups	Критерии оценки противоопухолевого эффекта Criteria for assessing the antitumor effect					
	Средний объем опухолей ($V_{ср.}$), в см³ (M±m) 95% ДИ Average tumor volume ($V_{av.}$), cm³ (M±m) 95% CI					
	Коэффициент абсолютного прироста опухолей (К), в отн.ед. Absolute tumor growth rate (K), units					
	Коэффициент торможения роста опухолей (ТРО), в % Tumor growth inhibition coefficient (TGI), %					
	p vs. контроль p vs. control					
	сутки после перевивки days after transplantation					
	5	7	10	12	14	17
Контроль Control	0,03±0,01	1,61±0,53 0,6-2,6	11,23±2,05 7,2-15,2	19,92±3,04 14,0-25,9	41,91±4,08 33,9-49,9	57,89±4,45 49,2-66,6
	–	52,67	373,33	663,00	1396,00	1928,67
	–	–	–	–	–	–
	–	–	–	–	–	–
ДОКС DOX	0,02±0,01	0,90±0,42 0,1-1,7	2,03±1,48 0,9-4,9	3,04±2,59 2,0-8,1	5,19±4,02 2,7-13,1	9,17±7,05 4,6-23,0
	–	44,00	100,50	151,00	258,50	457,50
	–	44,10	81,92	84,74	87,62	84,16
	>0,05	0,32	0,0039	0,0014	0,00005	0,0001
УЗТ US	0,03±0,01	0,89±0,39 0,1-1,7	8,64±2,24 4,2-13,0	14,25±3,29 7,8-20,7	22,89±4,42 14,2-31,6	34,98±2,26 30,6-39,4
	–	28,67	287,00	474,00	762,00	1165,00
	–	44,72	23,06	28,46	45,38	39,58
	>0,05	0,29	0,41	0,23	0,009	0,0008
ДОКС + УЗТ DOX + US	0,03±0,01	0,33±0,06 0,2-0,4	1,33±1,27 1,2-3,8	4,15±4,07 3,8-12,1	5,91±5,09 4,1-15,9	7,62±5,88 3,9-19,1
	–	10,00	43,33	137,33	196,00	253,00
	–	79,50	88,16	79,17	85,89	86,84
	>0,05	0,035	0,0017	0,01	0,00018	0,00003
ФС + УЗТ PS + US	0,02±0,01	1,26±0,38 0,5-2,0	8,52±1,57 5,4-11,6	17,93±2,75 12,5-23,3	22,71±3,39 16,1-29,4	38,67±8,68 21,7-55,7
	–	62,00	425,00	895,50	1134,50	1932,50
	–	21,74	24,13	9,99	45,81	33,20
	>0,05	0,60	0,32	0,64	0,004	0,075
ФС + ДОКС + УЗТ PS + DOX + US	0,03±0,01	0,08±0,05 0,0-0,2	0,65±0,65 0,6-1,9	1,77±1,77 1,7-5,2	2,19±2,19 2,1-6,5	3,93±3,93 3,8-11,6
	–	1,67	20,67	58,00	72,00	130,00
	–	95,03	94,21	91,11	94,77	93,21
	>0,05	0,015	0,0005	0,0003	0,000003	0,000002

*ДОКС – доксорубицин; УЗТ – ультразвуковая терапия; ФС – фотосенсибилизатор.
*DOX – doxorubicin; US – ultrasound therapy; PS – photosensitizer.

expectancy rates and median overall survival (52.0±5.1 days and 49 days versus 19.0±0.0 days and 19 days for DOX + US (p=0.00005), and 20.4±0.6 day and 21 days for PS + US (p=0.00007), respectively) (Table 4).

Information about the dynamics of changes in $V_{av.}$ and, accordingly, the coefficients K and TGI associated with this indicator, when using the above effects in rats with LSP in series 2 of the experiment are presented in Table 5.

When carrying out combined therapy of transplanted LSP tumors in rats with a single intra-abdominal injection of DOX at a dose of 5 mg/kg, followed 0.5 hours later by an US session with a frequency of 1.04 MHz and an intensity of 1.5 W/cm^2, a moderately pronounced inhibition of tumor growth was noted in compared with US in mono mode ($V_{av.}$ in the group is 3.35 times less, p=0.0014) and with chemotherapy without radiation ($V_{av.}$ in the group was 1.19 times less, p=0.74).

Combination therapy based on a single intravenous injection of photolon at a dose of 2.5 mg/kg for 2.5–3 hours and intraperitoneal administration of DOX at a dose of 5 mg/kg 0.5 hours before an ultrasound session with a frequency of 1.04 MHz and intensity 1.5 W/cm^2, was characterized by greater antitumor efficacy in comparison with the combined use of PS and US ($V_{av.}$ in the group is 6.70 times less, p=0.0078) and DOX + US ($V_{av.}$ in the group is 2.34 times less, p=0.087).

60 days after the implementation of therapeutic interventions in the second series of experiments, the highest incidence of CR (40%) was noted in the combination therapy group (PS + DOX + US). When assessing the survival rates of dead rats, there was no statistical difference with the control in the groups, with the exception of DOX + US (p=0.043), (p<0.05) (Table 6).

In recent years, the interest of researchers in studying the possibility of using methods based on the action of physical factors in the schemes of combined, complex and multicomponent treatment of malignant neoplasms has significantly increased. Such methods include photodynamic therapy [21, 22, 23, 24], cryotherapy [25, 26], hyperthermia [27] and others. A promising direction of scientific research is to study the possibility of combined use of ultrasound and different classes of drugs (sonodynamic therapy, SDT).

The SDT methods with various classes of drugs developed in our study and tested *in vivo* experiments have high antitumor efficacy. The first and second series of experiments showed that two modes are the most effective: the use of photolon, DOX and US with a frequency of 1.04 MHz and intensities of 0.5 and 1.5 W/cm^2, respectively. The proposed combination of therapeutic effects made it possible to significantly inhibit the growth of transfused tumors (p<0.05), increase the frequency of CR (p<0.05) and optimize the survival rates of rats (p<0.05) with transfused tumors compared with the control and each of the components of the method separately.

The closest analogue to our study is the work of P. Xu (2020) (The First Affiliated Hospital, People's Republic of China), in which the authors studied the antitumor efficacy of high-intensity US with an intensity of 4 W/cm^2 in combination with silicon nanoparticles with doxorubicin and e6 chloride on a human breast

Таблица 4
Информация об отдаленных результатах исследования

Table 4
Information about long-term results of the study

Наименование группы Groups	Критерии оценки противоопухолевого эффекта Criteria for assessing the antitumor effect			p vs. контроль p vs. control
	Частота полных регрессий, % Frequency complete regressions, %	СПЖ, сут 95 ДИ, % Average life expectancy, days 95 CI, %	УПЖ, % Average life expectancy increase rate, days	
Контроль Control	0	18,0±1,0 16,0-20,0	–	–
ДОКС DOX	40	26,0±7,0 12,3-39,7	44,44	0,28
УЗТ US	0	19,0±0,0 19,0-19,0	5,55	0,33
ДОКС + УЗТ DOX + US	60	19,0±0,0 19,0-19,0	5,55	0,33
ФС + УЗТ PS + US	0	20,4±0,6 19,2-21,6	13,33	0,06
ФС + ДОКС + УЗТ PS + DOX + US	40	52,0±5,1 42,1-62,1	188,89	0,00004

*ДОКС – доксорубицин; УЗТ – ультразвуковая терапия; ФС – фотосенсибилизатор; СПЖ – средняя продолжительность жизни; УПЖ – коэффициент увеличения средней продолжительности жизни.
*DOX – doxorubicin; US – ultrasound therapy; PS – photosensitizer.

Таблица 5
Данные о динамике изменения линейных размеров опухолей в эксперименте на крысах с ЛСП

Table 5
Data on the dynamics of changes in the linear sizes of tumors in an experiment on rats with LSP

Наименование группы Groups	Критерии оценки противоопухолевого эффекта Criteria for assessing the antitumor effect					
	Средний объем опухолей ($V_{ср.}$), в см^3 (M±m) 95% ДИ Average tumor volume ($V_{av.}$), cm^3 (M±m) 95% CI					
	Коэффициент абсолютного прироста опухолей (K), в отн.ед. Absolute tumor growth rate (K), units					
	Коэффициент торможения роста опухолей (ТРО), в % Tumor growth inhibition coefficient (TGI), %					
	p vs. контроль p vs. control					
	сутки после перевивки days after transplantation					
	5	7	9	12	14	16
Контроль Control	0,012±0,002	1,63±0,22 1,2-2,1	11,43±1,42 8,6-14,2	21,81±1,63 18,6-25,0	54,19±2,95 48,4-60,0	66,11±2,89 60,4-71,8
	–	134,83	951,50	1816,50	4514,83	5508,17
	–	–	–	–	–	–
	–	–	–	–	–	–
ДОКС DOX	0,014±0,001	0,99±0,21 0,6-1,4	3,41±0,27 2,9-3,9	6,56±0,72 5,1-8,0	8,66±1,19 6,3-11,0	9,75±1,42 7,0-12,5
	–	69,71	242,57	467,57	617,57	695,43
	–	39,26	70,17	69,92	84,02	85,25
	>0,05	0,059	0,0002	0,000003	0,00000	0,00000
УЗТ US	0,012±0,002	1,03±0,19 0,7-1,4	3,93±0,83 2,3-5,6	15,02±2,86 9,4-20,6	26,47±3,54 19,5-33,4	27,51±4,00 19,7-35,4
	–	84,83	326,50	1250,67	2204,83	2291,50
	–	36,81	65,62	31,13	51,15	58,39
	>0,05	0,063	0,0008	0,063	0,00009	0,00008
ДОКС + УЗТ DOX + US	0,011±0,001	0,32±0,07 0,2-0,5	0,85±0,22 0,4-1,3	5,05±1,49 2,1-8,0	7,19±1,96 3,3-11,0	8,22±2,18 3,9-12,5
	–	28,09	76,27	458,10	652,64	746,27
	–	80,34	92,56	76,85	86,73	87,57
	>0,05	0,0001	0,00001	0,00001	0,00000	0,00000
ФС + УЗТ PS + US	0,011±0,002	0,65±0,25 0,2-1,1	2,54±0,72 1,1-4,0	12,12±3,67 4,9-19,3	21,36±5,92 9,8-33,0	23,53±6,04 11,7-35,4
	–	58,09	229,91	1100,82	1940,82	2138,09
	–	60,12	77,78	44,43	60,58	64,41
	>0,05	0,013	0,0002	0,034	0,0004	0,00005
ФС + ДОКС + УЗТ PS + DOX + US	0,011±0,002	0,33±0,08 0,2-0,5	0,83±0,29 0,3-1,4	2,14±0,67 0,8-3,5	3,41±1,12 1,2-5,6	3,51±1,24 1,1-5,9
	–	29,00	74,45	193,55	309,00	318,09
	–	79,75	92,74	90,18	93,71	94,69
	>0,05	0,0002	0,00002	0,00000	0,00000	0,00000

*ДОКС – доксорубицин; УЗТ – ультразвуковая терапия; ФС – фотосенсибилизатор.
*DOX – doxorubicin; US – ultrasound therapy; PS – photosensitizer

Таблица 6
Информация об отдаленных результатах исследования
Table 6
Information about long-term results of the study

Наименование группы Groups	Критерии оценки противоопухолевого эффекта Criteria for assessing the antitumor effect			p vs. контроль p vs. control
	Частота полных регрессий, % Frequency complete regressions, %	СПЖ, сут 95 ДИ, % Average life expectancy, days 95 CI, %	УПЖ, % Average life expectancy increase rate, days	
Контроль Control	0	19,0±0,0 19,0-19,0	–	–
ДОКС DOX	20	21,3±1,3 18,8-23,8	12,11	0,11
УЗТ US	0	19,0±0,0 19,0-19,0	0,00	1,00
ДОКС + УЗТ DOX + US	0	22,2±1,4 19,7-25,1	16,84	0,043
ФС + УЗТ PS + US	20	20,0±0,0 20,0-20,0	5,26	1,00
ФС + ДОКС + УЗТ PS + DOX + US	40	20,0±0,0 20,0-20,0	5,26	1,00

*ДОКС – доксорубицин; УЗТ – ультразвуковая терапия; ФС – фотосенсибилизатор; СПЖ – средняя продолжительность жизни; УПЖ – коэффициент увеличения средней продолжительности жизни.
*DOX – doxorubicin; US – ultrasound therapy; PS – photosensitizer.

cancer tumor model *MDA-MB-231*. The data obtained indicate a significant inhibition of growth and a decrease in the mass of experimental tumors when using the combined treatment regimen compared with each of its components ($p<0.05$) [13]. Nevertheless, the use of higher US intensities in this study may be associated with a greater risk of serious adverse reactions and the predominant realization of the effects of hyperthermia rather than sonochemical reactions.

Systematization and analysis of the above publications of foreign researchers allowed us to conclude that the use of PS and chemotherapy drugs in combination with US radiation (sonodynamic effect) was characterized by a synergistic increase in the antitumor effectiveness of combined therapy compared with each of its components, which was confirmed by a statistically significant increase in the number of non-viable tumor cells and inhibition of the growth of transfused tumors in laboratory animals. The mechanism of action of chemotherapy drugs (doxorubicin, adriamycin, bleomycin) used in the listed *in vitro/in vivo* studies consists in interaction with DNA, formation of free radicals and direct action on cell membranes with suppression of nucleic acid synthesis. Ultrasound exposure to tumor cells sensitized by these drugs can significantly increase their active therapeutic concentration by affecting the permeability of tumor cell membranes. In the case of sonodynamic activation of PS, sonochemical reactions mediated by the influence of ultrasound occur in the structures of tumor cells due to the toxic effects of significantly increasing amounts of reactive oxygen species formed during sonodynamic modification of PS molecules. An important role is played by the effects of ultrasound itself, such as cavitation and local hyperthermia, leading to both direct and indirect damage to tumors due to disruption of the functional state and integrity of blood vessels that feed tumor tissues.

Considering that in the available literature sources we have found only a few publications devoted to the study of the effectiveness of the combined use of PS and chemotherapy drugs in combination with ultrasonic radiation, the implementation of further research in this area is an urgent and promising area of scientific work. It is advisable to perform experiments on a larger number of tumor models (subcutaneous, orthotopic). Equally important is the study of the antitumor effectiveness of SDT methods with a large number of PS and chemotherapy drugs, as well as the implementation of studies aimed at exploring the possibilities of using subtherapeutic doses of drugs in order to reduce the frequency and severity of adverse reactions.

CONCLUSION

The methods of SDT with various classes of drugs developed in our study make it possible to expand the range of therapeutic options in experimental and clinical oncology.

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