EXPERIMENTAL IN VIVO STUDIES OF THE ANTITUMOR EFFICACY OF PHOTODYNAMIC AND RADIODYNAMIC THERAPY AND THEIR COMBINATIONS

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Abstract

The authors studied the antitumor efficacy of photodynamic therapy (PDT) in combination with radiodynamic therapy (RDT) in an in vivo experiment. The study was approved by the Ethics Committee of the N.N. Alexandrov National Cancer Center of Belarus (protocol dated February 25, 2022, № 180). The work was performed on 26 white non-linear rats weighing 200 ± 50 g. Pliss lymphosarcoma (PLS) was used as a tumor model, which was transplanted subcutaneously. Photosensitizer (PS) «Photolon» (RUE «Belmedpreparaty», Belarus) was administered intravenously at a dose of 2.5 mg/kg of body weight. The RDT session was performed by the contact method (CRT) once 2.5–3 times after the end of the infusion of the PS on the «microSelectron-HDR V3 Digital apparatus» (Elekta, Sweden) using γ-radiation (^{192}Ir) in a single focal dose 6 Gy. A PDT session was performed once immediately after exposure to ionizing radiation using a «PDT diode laser» (LTD Imaf Axicon, Belarus, λ=660±5 nm) at an exposure dose of 100 J/cm^2 with a power density of 0.2 W/cm^2 and a power of 0.353 watts. All rats were divided into 4 groups of 6–7 animals each: intact control (IC), PS + PDT, PS + CRT, PS + CRT + PDT. The criteria for evaluating antitumor efficacy were: the average volume of tumors (V_{av}, cm^3), the coefficient of absolute growth of tumors (K, in RU), the coefficient of tumor growth inhibition (TGI, %), the frequency of complete tumor regressions (CR, %), the proportion of cured rats (%), an increase in the average duration of dead rats (%). Differences were considered statistically significant at $p<0.05$. On the 18th day of the experiment, V_{av} in groups was 63.25±2.76 cm^3; 29.03±6.06 cm^3 ($p=0.0002$); 22.18±5.94 cm^3 ($p<0.0001$); 11.76±3.29 cm^3 ($p=0.0000$), respectively. Coefficients K – 4516.86 RU; 2638.09 RU; 2024.45 RU; 979.00 RU. TGI coefficients – 54.10% (PS + PDT); 64.93% (PS + CRT); 81.41% (PS + CRT + PDT). An increase in the average duration of dead rats indicator – 48.57% (PS + PDT); 60.00% (PS + CRT); 97.71% (PS + CRT + PDT). On the 60th and 90th days of the experiment, the frequency of PR and the proportion of cured rats were the same and amounted to 0%; 16.7%; 14.3%, and 28.6%, respectively. The results obtained indicate the prospects and relevance of further research in this scientific direction.

Key words: experimental research, rats, transplanted tumors, photodynamic therapy, radiodynamic therapy, photosensitizer.

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ЭКСПЕРИМЕНТАЛЬНЫЕ ИССЛЕДОВАНИЯ IN VIVO ПРОТИВООПУХОЛЕВОЙ ЭФФЕКТИВНОСТИ ФОТОДИНАМИЧЕСКОЙ И РАДИОДИНАМИЧЕСКОЙ ТЕРАПИИ, А ТАКЖЕ ИХ СОЧЕТАНИЯ

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Резюме

В рамках пилотного исследования авторами изучена противоопухолевая эффективность фотодинамической терапии (ФДТ) в комбинации с радиодинамической терапией (РДТ) в эксперименте *in vivo* на подкожно перевитой опухолевой модели лимфосаркомы Плисса

(ЛСП) у крыс. Фотосенсибилизатор (ФС) на основе хлорина е6 вводили внутривенно в дозе 2,5 мг/кг массы тела. Сеанс РДТ проводили на установке для контактной лучевой терапии (КЛТ) однократно через 2,5–3 ч после окончания введения ФС с использованием γ-излучения (^{192}Ir) в разовой очаговой дозе 6 Гр. Сеанс ФДТ осуществляли однократно непосредственно после воздействия ионизирующим излучением с помощью полупроводникового лазера «PDT diode laser» (ООО «Imaf Axicon», Беларусь, λ=660±5 нм) со световой дозой 100 Дж/см^2 с плотностью мощности 0,2 Вт/см^2 и мощностью 0,353 Вт. Все крысы были разделены на 4 группы по 6–7 особей в каждой: интактный контроль (ИК), ФС + ФДТ, ФС + КЛТ, ФС + КЛТ + ФДТ. Критерии оценки противоопухолевой эффективности: средний объем опухолей ($V_{ср}$., см^3), коэффициент абсолютного прироста опухолей (К, в относительных единицах (ОЕ), показатель торможения роста опухолей (ТРО, %), частота полной регрессии опухоли (ПР, %), доля излеченных крыс (%), показатель увеличения продолжительности жизни (УПЖ, %). Различия считались статистически значимыми при уровне значимости p<0,05. На 18-е сутки эксперимента $V_{ср}$. в группах составил 63,25±2,76 см^3; 29,03±6,06 см^3 (p=0,0002); 22,18±5,94 см^3 (p<0,0001); 11,76±3,29 см^3 (p=0,0000), соответственно. Коэффициенты К – 4516,86 ОЕ; 2638,09 ОЕ; 2024,45 ОЕ; 979,00 ОЕ. Показатель ТРО – 54,10% (ФС + ФДТ); 64,93% (ФС + КЛТ); 81,41% (ФС + КЛТ + ФДТ). Показатель УПЖ – 48,57% (ФС + ФДТ); 60,00% (ФС + КЛТ); 97,71% (ФС + КЛТ + ФДТ). На 60-е и 90-е сутки эксперимента частота ПР и доля излеченных крыс были одинаковыми и составили в группах 0%; 16,7%; 14,3% и 28,6%, соответственно. Полученные результаты свидетельствуют о перспективности и актуальности дальнейших исследований в данном научном направлении.

Ключевые слова: экспериментальное исследование, крысы, перевивные опухоли, фотодинамическая терапия, радиодинамическая терапия, фотосенсибилизатор.

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Introduction

Photodynamic therapy (PDT) is a method for the treatment of precancerous diseases and malignant neoplasms, the effectiveness of which has been proven and confirmed by the results of numerous preclinical studies on cell cultures and laboratory animals with transplanted tumors, as well as clinical studies, i.a. multicenter randomized studies including a significant number of patients with various nosological forms of oncological pathology [1, 2]. PDT is based on the use of special drugs – photosensitizers (PS), the activation of which in pathologically altered tissues is realized by exposure to laser radiation with a certain wavelength [3, 4, 5]. However, in recent years, scientific projects have actively explored the possibility of using other physical factors, such as ultrasound ("sonodynamic therapy"), hyperthermia ("thermodynamic therapy"), electric fields ("electrodynamic therapy"), and ionizing radiation ("radiodynamic therapy") as ways to launch complex physicochemical reactions at the molecular and cellular levels, leading to the transition of PS molecules from the ground state to an excited state, similar to PDT, followed by the destruction of tumor cells, in particular, and tumor death, in general [6, 7, 8].

In order to increase the antitumor efficacy of PDT, it is advisable to use the method in combination with traditional approaches in the treatment of malignant neoplasms, in particular, with radiation therapy (RT) [9, 10]. The combined use of PDT and RDT makes it possible to use subtherapeutic modes of laser and ionizing radiation. Such modes lead to an increase in the effect of each of the therapeutic methods due to a synergistic effect with a significant reduction in the risk of several adverse reactions that occur when high doses of these physical factors are used, primarily, of RT.

Materials and methods

Laboratory animals

The pilot study was performed on 26 white nonlinear outbred male rats obtained from the vivarium of N. N. Alexandrov National Cancer Centre of Belarus, with a body weight of 200±50 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 20–22°C and a humidity of 50–60% in individual cages, 6–7 individuals in each. The conditions for keeping rats in the laboratory, as well as indicators of humidity, temperature, and illumination in the room, corresponded to the current sanitary rules for the arrangement, equipment, and maintenance of vivariums (Sanitary rules and regulations 2.1.2.12-18-2006 "Arrangement, equipment and maintenance of experimental biological clinics (vivariums)", Decree of the Chief State Sanitary Doctor of the Republic of Belarus, dated October 31, 2006 No. 131) and Interstate standards: State Standard 33216-2014 ("Guidelines for keeping and caring for laboratory animals. Rules for keeping and caring for laboratory rodents and rabbits" and State Standard 33215-2014 "Guidelines for the maintenance and care of laboratory animals. Rules for the equipment of premises and organization of procedures", approved by the Resolution of the Interstate Council for Standardization, Metrology and Certification, a protocol of December 22, 2014, No. 73-P).

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 and maintained by passivation *in vivo*. 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 6th day after transplantation, when the diameter of the tumor node, on average, was 3–5 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, namely:

1. European Convention for the Protection of Vertebrate Animals used for Experimental or Other Scientific Purposes (Strasbourg, France, of 18.03.1986), as amended in accordance with the provisions of the Protocol (ETS No. 170 of 02.12.2005).

2. Directive 2010/63/EU of the European Parliament and the European Union on the protection of animals used for scientific purposes (dated 22.09.2010).

3. Technical Code of Common Practice No 125-2008 "Good Laboratory Practice" (GLP) (Decree of the Ministry of Health of the Republic of Belarus No. 56 dated March 28, 2008).

The nature of the studies performed was consistent with the principles of "3Rs" developed by W.M. Russell and R.L. Berch (1959), namely:

1) "Reduction" – reduction in the number of laboratory animals used in the experiment.

2) "Refinement" – improvement of the methodology of the experiment through the use of painkillers and non-traumatic methods.

3) "Replacement" – replacement (transition from animal research to methods that do not use living beings).

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.

The study was approved by the Ethics Committee of N. N. Alexandrov National Cancer Centre of Belarus (extract from the protocol dated February 25, 2022 No. 180).

Photo- and radiosensitizer

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.

Radiodynamic therapy

The irradiation of inoculated tumors was carried out by the contact method (contact radiation therapy, CRT) using a microSelectron-HDR V3 Digital apparatus (Elekta, Sweden) using γ-radiation (^{192}Ir). The source had a high activity (at the beginning of the experiments it was 5.2 *Ci*), which determined the high dose rate and short duration of irradiation sessions required for rats in a state of drug sleep. To conduct CRT on the area of the inoculated tumor, a Leipzig applicator was used, which was fixed on the surface of the tumor with soft rubber holders. Irradiation was performed once at a single focal dose (SFD) of 6 Gy, which is equivalent to 10.8 Gy at $\alpha/\beta = 3$, 2.5–3 hours after the end of the infusion. The time of the irradiation session was calculated using the Oncentra Brachy v4.5.2 planning system (Elekta, Sweden) on an empty series of images using the TG-43 algorithm without taking into account the reflection and scattering of radiation inside the applicator. The CRT technique was used with normalization to a point located at a distance of 5 mm from the therapeutic surface of the applicator, in accordance with the size of the target and the recommendations of GEC-ESTRO ACROP and others. The used method of irradiation made it possible to apply the planned SFD to transplanted tumors in rats without over-irradiation of normal tissues surrounding the tumor.

Photodynamic therapy

PDT sessions were performed once right after exposure to ionizing radiation (IRT) using a PDT diode laser (LTD Imaf Axicon, Minsk, Republic of Belarus) with a wavelength of 660 ± 5 nm. Irradiation of grafted tumors was started 2.5–3 hours after the end of PS infusion with a light dose of 100 J/cm^2 with a power density of 0.2 W/cm^2 and a power of 0.353 W. The duration of exposure was 8 minutes.

Study design

All exposures were performed on the 6th day after PLS inoculation when the diameter of the tumor node was at least 3–5 mm. All rats, 26 individuals (males), included in the study, were randomly distributed into 4 groups of 6–7 individuals in each. Rats with transplanted

tumors, which were not injected with PS and did not undergo any irradiation, acted as controls (intact control, IC) (Table 1).

Таблица 1
Дизайн экспериментального исследования
Table 1
Experimental study design

Наименование группы Study groups	Число крыс в группе, n Number of rats in the group, n
ИК Intact control	6
ФС 2,5 мг/кг + КЛТ РОД 6 Гр PS 2.5 mg/kg + CRT SFD 6 Gy	7
ФС 2,5 мг/кг + ФДТ 100 Дж/см^2 0,2 Вт/см^2 PS 2.5 mg/kg + PDT 100 J/cm^2 0.2 W/cm^2	6
ФС 2,5 мг/кг + КЛТ РОД 6 Гр+ ФДТ 100 Дж/см^2 0,2 Вт/см^2 PS 2.5 mg/kg + CRT SFD 6 Gy + PDT 100 J/cm^2 0.2 W/cm^2	7

* ФС – фотосенсибилизатор; КЛТ – контактная лучевая терапия; РОД – разовая очаговая доза; ФДТ – фотодинамическая терапия.
* PS – photosensitizer; CRT – contact radiotherapy; SFD – single focal dose; PDT – photodynamic therapy.

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 formula (1):

$$V = \frac{1}{6}\pi \times d_1 \times d_2 \times d_3$$

where

$d_{1,2,3}$ – three mutually perpendicular tumor diameters (in cm);

$\pi/6 = 0.52$ – 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):

$$K = \frac{V_t - V_0}{V_0}$$

where

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

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

The value of the index K > 0 (V at the corresponding period of observation exceeded its initial value) was regarded as continued tumor growth; -1 < K < 0 (V at the corresponding observation period was less than its initial value) was regarded as inhibition of tumor growth; and K = -1 – as complete tumor regression.

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

$$TGI\% = \frac{Vcontrol - Vexperience}{Vcontrol} * 100\%$$

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.

The proportion of cured rats in the groups was determined 90 days after the end of exposure by the absence of visual and palpatory signs of tumor growth.

Quantitative criteria for assessing the inhibitory effect on grafted tumors in rats were as follows (Table 2) [11]:

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 (4):

$$LEI\% = \frac{ALEexperiment - ALEcontrol}{ALEcontrol} * 100\%$$

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).

Statistical processing of the obtained data

Statistical processing of the results ($V_{av.}$, K, and TGI) was performed using Excel, Origin Pro (version 7.0), and

Таблица 2
Критерии оценки противоопухолевой эффективности по коэффициенту торможения роста опухоли и частоте полных регрессий
Table 2
Criteria for evaluating antitumor efficacy in terms of the coefficient of tumor growth inhibition and the frequency of complete regressions

Критерии противоопухолевой эффективности Criteria of antitumor efficacy	Значения эффективности Values efficiency
ТРО < 20% TGI < 20%	0
ТРО < 20–50% TGI < 20–50%	±
ТРО < 51–80%/ TGI < 51–80%	+
ТРО < 81–90% TGI < 81–90%	++
ТРО < 91–100% + < 50% ПР/ TGI < 91–100% + CR < 50%	+++
ТРО > 91–100% + > 50% ПР/ TGI > 91–100% + CR > 50%	++++

* ТРО – коэффициент торможения роста опухоли; ПР – полная регрессия.
* TGI – tumor growth inhibition; CR – complete regression.

Statistica (version 10.0) software packages. Data are presented as M±m (mean ± error of the mean). To assess the significance of differences, the Mann-Whitney U test was used. Overall survival was assessed using the non-parametric Kaplan-Meier method. The date of tumor inoculation was taken as point 0, the death of a rat was considered an event, and the end of observation was the death of all rats in the experimental group. Comparative data analysis was performed using a nonparametric log-rank test. Differences were considered statistically significant at $p<0.05$.

Results

The inoculation of the tumor strain was 100% (26 out of 26 rats had visual and palpatory signs of tumor growth at the time of the start of therapeutic interventions, on the 6th day after inoculation).

Adverse reactions and complications associated with intravenous administration of PS, as well as PDT and CRT sessions, were not registered.

In the experiment, the antitumor efficacy of the method of combined therapy of transplantable tumors was evaluated, including systemic (intravenous) administration of a PS based on chlorin e6, followed by a single exposure to ionizing radiation in the SFD of 6 Gy and laser radiation with a light dose of 100 J/cm^2 with a power density of 0.2 W/cm^2 in comparison with each of the components of the method (PS + CRT, PS + PDT) and IC.

As can be seen from Table 3, during the entire period of evaluation of indicators characterizing the change in the growth dynamics of transplanted tumors (from 6 to 18 days after therapeutic exposure), its statistically significant inhibition was noted both in the combination therapy group and in the groups of rats that were treated in monomodes (PS + PDT and PS + CRT), compared with the IC group ($p<0.05$).

On the 18th day of the experiment, $V_{av.}$ in the combination therapy group was statistically significantly less: 5.38 times compared with IC ($p=0.00001$), 2.47 times compared with PS + PDT ($p=0.025$), and tended to decrease compared with the PS + CRT group (1.89 times; $p=0.15$).

Antitumor effectiveness of impacts on a semi-quantitative scale of assessment [11] is presented in Table 4.

Table 5 presents data on the survival rates of dead rats in this series of experiments. The results obtained testify to the high antitumor efficacy of the developed method of combined therapy: a statistically significant LEI was achieved in comparison with IC and a tendency to optimize the studied parameters was noted in comparison with each of the components of the method ($p=0.12$ – PS + PDT and $p=0.24$ – PS + CRT).

Thus, the developed method of combined therapy, which includes intravenous administration of a PS based on chlorin e6 at a dose of 2.5 mg/kg of body weight, followed, after 2.5–3 hours, by a single session of CRT in the SFD of 6 Gy and PDT with a light dose of 100 J/cm^2 with a power density of 0.2 W/cm^2 demonstrated high antitumor efficacy. On the 18th day after the session of treatment of animals, the coefficient K was 979.00 RU; the value of TGI, compared with the IC was 81.41%. On the 60th and 90th days, the CR and cure rates were 28.6% and 28.6%, respectively. ALE and LEI indicators were 34.60±3.75 days and 97.71%, respectively. The effectiveness of the impact on a semi-quantitative scale of assessment was "+++".

Discussion

As already mentioned, in recent years, the possibility of using such physical factors as ultrasound, hyperthermia, electric fields, etc., as trigger mechanisms for the activation of the PS molecule in pathologically altered cells and tissues has been actively studied [6, 7, 8]. One of the most relevant areas of scientific research in experimental and clinical oncology is radiodynamic therapy (RDT) – a method of treating malignant neoplasms based on the combined use of PS and their derivatives and ionizing radiation with certain param-

eters. PS traditionally used for PDT may have radiosensitizing properties, and in this case, they can be considered as radiosensitizing agents that increase the antitumor efficacy of RT. It is well known that tumor physiology is characterized by low oxygen tension (hypoxia, anoxia), low glucose and high lactate levels, interstitial hypertension, and extracellular acidosis. The vascular network of the tumor is characterized by the pronounced proliferation of endotheliocytes, which leads to the development of structural defects and functional failure of microcapillaries, as a result of which the intratumoral blood flow becomes chaotic with the presence of areas of insufficient vascularization. Hypoxic tumor cells have an increased resistance to ionizing radiation

Таблица 3
Данные о динамике роста перевивных опухолей в эксперименте на крысах с ЛСП
Table 3
Data on the growth dynamics of transplanted tumors in an experiment on rats with LSP

Наименование группы Groups	Сутки после перевивки Days after tumors transplantation ИССЛЕДУЕМЫЕ КРИТЕРИИ: Средний объем, в см³ (M±m) Коэффициент абсолютного прироста опухолей (К), в ОЕ Коэффициент торможения роста опухолей (ТРО), в % Уровень значимости различий по отношению к интактному контролю RESEARCH CRITERIA: Vav., cm³ (M±m) Coefficient of absolute tumor growth (K), relative units (RU) Coefficient of tumor growth inhibition (TGI), % P vs. intact control					
	6	9	11	13	15	18
ИК IC	0,014±0,001	1,23±0,19	10,29±0,71	19,85±0,65	47,19±0,74	63,25±2,76
	–	86,86	734,00	1416,86	3369,71	4516,86
	–	–	–	–	–	–
	–	–	–	–	–	–
ФС + КЛТ PS + CRT	0,011±0,002	0,33±0,13	1,46±0,51	3,88±1,15	15,92±4,58	22,18±5,94
	–	29,00	131,73	351,73	1446,27	2024,45
	–	73,17	85,81	80,45	66,26	64,93
	>0,05	0,0018	0,00000	0,00000	0,00001	0,00002
ФС + ФДТ PS + PDT	0,011±0,002	0,62±0,20	3,02±0,62	7,76±2,01	22,69±5,43	29,03±6,06
	–	55,36	273,55	704,45	2061,73	2638,09
	–	49,59	70,65	60,91	51,92	54,10
	>0,05	0,046	0,000002	0,00007	0,0005	0,0002
ФС + КЛТ + ФДТ PS + CRT + PDT	0,012±0,001	0,17±0,03	1,15±0,46	3,88±1,13	11,14±3,42	11,76±3,29
	–	13,17	94,83	322,33	927,33	979,00
	–	86,18	88,82	80,45	76,39	81,41
	>0,05	0,00008	0,00000	0,00000	0,00000	0,00000

* ФС – фотосенсибилизатор; КЛТ – контактная лучевая терапия; ИК – интактный контроль; ФДТ – фотодинамическая терапия.
* PS – photosensitizer; CRT – contact radiotherapy; IC – intact control; PDT – photodynamic therapy.

and require the use of high doses of radiation, leveling this effect, which, as a result, can lead to the development of radiation reactions and damage to normal tissues surrounding the tumor. The key to preventing this situation is the use of radiosensitizers that modify the antitumor efficacy of RT (in particular, PS) or a combination of RT with other therapeutic options (for example, PDT) using reduced doses of radiation [8, 9, 10, 12].

When interpreting the main mechanisms underlying tumor cell damage with the combined use of PS and ionizing radiation, the authors conclude that the key link in the realization of the antitumor effect of RDT is free radical oxidation, which develops as a result of exposure to radiation on the water in the cell with subsequent transfer of PS molecules from the ground state to the excited state and the formation of a significant amount of free radi-

Таблица 4
Критерии оценки противоопухолевой эффективности по коэффициенту торможения роста опухоли и частоте полных регрессий

Table 4
Criteria for evaluating antitumor efficacy in terms of the coefficient of tumor growth inhibition and the frequency of complete regressions

Наименование группы Groups	Критерии оценки эффективности Criteria for evaluating effectiveness		
	Показатель торможения роста опухолей (ТРО, %) Tumor growth inhibition coefficient TGI, %	Частота полных регрессий, % Frequency of complete regressions, %	Эффективность Efficacy
ИК IC	–	0,0	0
ФС + ФДТ PS + PDT	54,10	16,7	+++
ФС + КЛТ PS + CRT	64,93	14,3	+++
ФС + КЛТ + ФДТ PS + CRT + PDT	81,41	28,6	+++

* ФС – фотосенсибилизатор; КЛТ – контактная лучевая терапия; ИК – интактный контроль; ФДТ – фотодинамическая терапия.
* PS – photosensitizer; CRT – contact radiotherapy; IC – intact control; PDT – photodynamic therapy.

Таблица 5
Показатели выживаемости крыс после комбинированного лечения

Table 5
Survival rates of rats after combined treatment

Наименование группы Groups	Критерии оценки эффективности Criteria for evaluating effectiveness		
	Средняя продолжительностьжизни, сут Average life expectancy, days	Увеличение средней продолжительности жизни, % Increase in average life expectancy, %	p относительно ИК p vs. IC
ИК IC	17,50±2,16	–	–
ФС + ФДТ PS + PDT	26,00±3,48	48,57	0,058
ФС + КЛТ PS + CRT	28,00±3,86	60,00	0,034
ФС + КЛТ + ФДТ PS + CRT + PDT	34,60±3,75	97,71	0,0017

* ФС – фотосенсибилизатор; КЛТ – контактная лучевая терапия; ИК - интактный контроль; ФДТ – фотодинамическая терапия.
* PS – photosensitizer; CRT – contact radiotherapy; IC - intact control; PDT – photodynamic therapy.

cals (reactive oxygen species – ROS) [13, 14]. Absorbing radiation, the PS molecule enters into a cascade of reactions, which leads to the formation of a hydroxyl radical, superoxide anion, and singlet oxygen in the cell, which are also accumulated due to the radiation radiolysis of water. Later, lethal damage to cellular components (cytoplasmic membranes, granular endoplasmic reticulum, mitochondria, DNA, etc.) occurs at the level of physicochemical processes. Possessing a high oxidative potential, ROS interact with membrane lipids of tumor cell organelles with the formation of oxidation products, destabilization, and subsequent destruction of the cell as a whole. The consequence of the above reactions to the combined effect is an oxidative stress syndrome that induces apoptosis [15].

In the available literature, there are few publications devoted to the study of the radiodynamic activity of PS based on protoporphyrin IX, hematoporphyrin and its derivatives in experiments in vitro/in vivo (gliomas c6 and U-373 MG, gliosarcoma 9L; squamous cell carcinoma of the human esophagus OE-21, adenocarcinoma human esophagus OE-33, human bladder carcinoma RT4, and colon adenocarcinoma HT-29) [13, 14, 16 17, 18]. The authors report a statistically significant reduction in the number of viable tumor cells and inhibition of the growth of grafted tumors in the combination therapy groups compared with RT alone.

Thus, American researchers (Panetta J.V. et al.) from the Fox Chase Cancer Center (USA) presented the results of the use of RDT with protoporphyrin IX in mice with an orthotopic model of human prostate carcinoma PC-3. 5-aminolevulinic acid (5-ALA), which causes the formation of endogenous PS protoporphyrin IX, was administered orally at a dose of 100 mg/kg 4 hours before the start of irradiation of subcutaneously transplanted tumors, which was carried out once at a dose of 4 Gy. The authors reported that after 7 and 14 days from the start of therapeutic interventions in the RDT group, the average tumor volume was 24±9% and 21±8% less compared to the RT group in monomode, respectively ($p<0.05$) [19].

In their later study, D.M. Yang *et al.* (Fox Chase Cancer Center, USA) proved the presence of radiosensitizing properties in protoporphyrin IX in an experiment on C57BL/6 linear mice with a subcutaneously transplanted small cell lung cancer tumor KP1. 5-ALA was administered orally at a dose of 100 mg/kg 4 hours before the start of irradiation of subcutaneously transplanted tumors, which was carried out once in the SFD of 4 Gy. After 14 days from the start of treatment in the RDT group, inhibition of the growth of grafted tumors by 52.1%, 48.1% and 57.9% was registered compared with the groups of 5-ALA ($p<0.001$), RT in monomode ($p<0.001$) and intact control ($p<0.001$), respectively [20].

Another study by Takahashi J. et al. (Health and Medical Research Institute, Japan) presents the results of RDT with protoporphyrin IX human glioblastomas U251MG and U87MG in BALB/c nu/nu mice. 5-ALA was administered orally at doses of 60 and 120 mg/kg 4 h before the start of irradiation of subcutaneously transplanted tumors, which was carried out at SFD of 2 Gy 5 times a week for 6 weeks until a SFD of 60 Gy was reached. The authors report that the proposed method of irradiation had a pronounced inhibitory effect on the growth of both models of transplanted tumors during the entire observation period (42 and 70 days, respectively), causing the development of irreversible damage in the tumor tissue, registered according to a morphological study [12].

Y. Matsuyama et al. (Mie University Graduate School of Medicine, Japan) in their *in vivo* experiments studied the effect of ionizing radiation on the antitumor properties of a photosensitizing substance, acridine orange (AO). As objects of study, the authors used C3H/HeSlc and BALB/cSlc-nu/nu linear mice with transplanted tumors: LM8 mouse osteosarcoma, PC-3 human prostate cancer, and MDA-MB-231 human breast cancer. AO was injected subcutaneously along the perimeter of tumors at a dose of 1 μg/mL. Irradiation was performed once per SFD of 5 Gy. The authors reported that RDT with AO showed a pronounced cytostatic effect against all types of tumors. On the 14th day after the start of therapeutic effects, the average volume of LM8 tumors in the control group was 890 mm^3, AO – 780 mm^3, RT SFD of 5 Gy – 120 mm^3 and AO + RT SFD of 5 Gy – 42 mm^3 ($p<0.05$); for MDA-MB-231 – 1060, 620, 1010 and 29 mm^3 ($p<0.05$), and for PC-3 – 530, 200, 45 and 14 mm^3 ($p<0.05$), respectively [21].

And finally, C. Dupin et al. (Bordeaux Institute of oncology, France) presented the experience of using the RDT method in an experiment on immunodeficient RAGγ2C–/– mice with an orthotopic model of human glioblastoma P3. 5-ALA was used as a photosensitizing agent and was administered intraperitoneally at a dose of 100 mg/kg. Irradiation of transplanted tumors was carried out 3 times a week in the following modes: 3×2 Gy, 5×2 Gy, and 5×3 Gy; 2.55 Gy/min. Based on the analysis of the obtained results according to the criterion of survival, the optimal effect was fractionated irradiation in the mode of 5 × 3 Gy 3 times a week (73–83 days) *vs.* control (without exposure) (15-24 days), RT 3×2 Gy (41-47 days) and RT 5×3 Gy (48-62 days) ($p<0.05$). In a comparative aspect, there was a tendency to optimize survival rates in the 5-ALA + RT 5×2 Gy group (53-67 days) to the RT 5×2 Gy group ($p=0.24$) [22].

Several clinical trials have been initiated in large cohorts of patients to evaluate the safety and tolerability of RDT. Thus, the clinical trial "A Phase I Dose Finding Study Of Low-dose Radiation With Sensitization Using 5-aminolevulinic Acid In Advanced Malignancies", which is based on the determination of optimal doses of RT and PS in patients with various nosological forms of malignant neoplasms (solid tumors of the head and neck, chest and abdominal cavities, small pelvis) was launched

by Fox Chase Cancer Center (USA) in July 2020. The study is planned to include 130 patients. As a PS, 5-ALA is used in 3 doses. Irradiation is carried out fractionally, the course of therapy is carried out once and is 21 days. In the future, patients are under dynamic observation for 56 days to assess the frequency and severity of adverse reactions, as well as preliminary data on the antitumor efficacy of the method [23].

A clinical trial "Phase I/II Dose Escalation Trial of Radiodynamic Therapy (RDT) With 5-Aminolevulinic Acid in Patients With First Recurrence of Glioblastoma" led by Prof. Stummer W. (University Hospital Münster, Germany) started in October 2022. It is planned to include 34 patients with a recurrent form of gioblastoma (the first recurrence after combined or complex treatment). 5-ALA is used as a PS. Irradiation will be fractionated, and the aim of the study will be to determine the maximum tolerated doses of PS and RT, as well as the optimal number of RDT sessions. Patient survival rates (overall 6-month survival, 6-month progression-free survival, etc.) will be studied as criteria for antitumor efficacy [24].

The analyzed data testify to the significant prospects of this direction in experimental oncology. The results obtained in experiments *in vitro/in vivo* allow to conclude that several PS have radiosensitizing properties, which creates prerequisites for optimizing and further improving the combined and complex therapy of patients with malignant neoplasms of various localizations.

Conclusion

PDT is a method of therapy for precancerous diseases and malignant neoplasms, demonstrating high antitumor efficacy against these diseases in experimental and clinical oncology [25, 26, 27]. Nevertheless, to optimize the use of PDT, it is advisable to use it in combination with a number of other methods of therapy. Pilot data obtained on the basis of an analysis of the immediate and long-term results of an experimental study on transplantable tumors in rats indicate a pronounced trend towards a higher antitumor effect of combined treatment, including the use of PS followed by RDT and PDT sessions with a single irradiation regimen compared to RDT and PDT in monomodes. No publications devoted to the study of the effectiveness of the combined use of PS of the chlorin series and these methods of therapy, demonstrating positive results, were found in the available literature sources, which allows to conclude that it is necessary and promising to develop deeper research in this direction.

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