ANTITUMOR EFFICIENCY OF CONTACT RADIOTHERAPY IN COMBINATION WITH A CHLORIN-BASED PHOTOSENSITIZER IN EXPERIMENT

Tzerkovsky D.A., Protopovich Ya.L., Kozlovsky D.I., Suslova V.A.
N.N. Alexandrov National Cancer Centre of Belarus, Lesnoy, Republic of Belarus

Abstract

Authors have studied the antitumor efficacy of contact radiation therapy (CRT) in combination with a chlorin-based photosensitizer (PS) in an experiment on laboratory animals with transplanted tumors. The experimental study was performed in 50 white outbred rats weighing 250±50 g. Subcutaneously transplanted Pliss lymphosarcoma (PLS) and alveolar liver cancer PC1 (PC1) were used as tumor models. Chlorin-based PS photolon (RUE «Belmedpreparaty», Republic Belarus) was injected intravenously at a dose of 2.5 mg/kg. The radiation sessions were carried out 2.5–4 hours (depending on the tumor model) after the administration of the PS using the device «microSelectron HDR V3 Digital» («Nucletron», Netherlands) with a 192-Ir radiation source in single focal doses 5 and 10 Gy. All laboratory animals (for PLS and PC1) were subdivided into 5 groups of 5 animals each: intact control, CRT 5 Gy, CRT 10 Gy, PS + CRT 5 Gy, PS + CRT 10 Gy. For the PLS tumor model – on the 14th day from the beginning of the experiment V_{av}. in groups were 26.31±5.81; 22.45±6.97; 18.99±4.86; 10.75±5.18 and 28.06±2.85 cm^3, respectively ($p<0.05$). The coefficients of tumor growth inhibition in the experimental groups were 14.67%, 27.82%, 59.14% and 6.65%, respectively. The frequency of complete tumor regressions 60 days after the start of the experiment was 0%, 20%, 20%, 60%, and 20%, respectively. On PC1 tumor model – on the 14th day from the beginning of the experiment V_{av}. in groups were 4.48±1.03; 0.80±0.21; 0.29±0.09; 0.19±0.07 and 0.32±0.08 cm^3, respectively ($p=0.009$). The coefficients of tumor growth inhibition in the experimental groups were 82.14%, 93.53%, 95.76% and 92.86%, respectively. The frequency of complete tumor regressions 60 days after the start of the experiment was 0%, 0%, 20%, 0%, and 0%, respectively. Systemic administration of chlorin-based PS before the CRT session increases the antitumor efficacy of radiation therapy in animals with transplantable tumors of different histological structure and growth patterns. The data obtained indicate that further studies of the radiosensitizing properties of PS are promising.

Key words: experimental study, laboratory animals, transplanted tumors, contact radiotherapy, photosensitizer.

For citation: Tzerkovsky D.A., Protopovich Ya.L., Kozlovsky D.I., Suslova V.A. Antitumor efficiency of contact radiotherapy in combination with a chlorin-based photosensitizer in experiment // *Biomedical Photonics*, 2021, T. 10, No. 2, pp. 25–33 (in Russian). doi: 10.24931/2413–9432–2021–10–2–25–33

Contacts: Tzerkovsky D.A., tzerkovsky@mail.ru

ПРОТИВООПУХОЛЕВАЯ ЭФФЕКТИВНОСТЬ КОНТАКТНОЙ ЛУЧЕВОЙ ТЕРАПИИ В КОМБИНАЦИИ С ФОТОСЕНСИБИЛИЗАТОРОМ ХЛОРИНОВОГО РЯДА В ЭКСПЕРИМЕНТЕ

Д.А. Церковский, Е.Л. Протопович, Д.И. Козловский, В.А. Суслова
Республиканский научно-практический центр онкологии и медицинской радиологии им. Н.Н. Александрова, Лесной, Республика Беларусь

Резюме

Авторами изучена противоопухолевая эффективность контактной лучевой терапии (КЛТ) в комбинации с фотосенсибилизатором (ФС) хлоринового ряда в эксперименте на лабораторных животных с перевивными опухолями. Работа выполнена на 50 лабораторных животных (белые беспородные крысы) с массой тела 250±50 г. В качестве опухолевых моделей использовали лимфосаркому Плисса (ЛСП) и альвеолярный рак печени РС (РС1), перевитые подкожно. ФС хлоринового ряда фотолон (РУП «Белмедпрепараты», Беларусь) вводился внутривенно капельно в дозе 2,5 мг/кг массы тела. Сеанс КЛТ проводили через 2,5 – 4 ч (в зависимости от опухолевой модели) после введения ФС с использованием аппарата «microSelectron HDR V3 Digital» («Nucletron», Нидерланды) с источником излучения 192–Ir в разовых очаговых дозах (РОД) 5 и 10 Гр. Все лабораторные животные, как в подгруппе с ЛСП, так и в подгруппе с РС1, были разделены на 5 групп по 5 особей в каждой: интактный контроль, КЛТ РОД 5 Гр, КЛТ РОД 10 Гр, ФС + КЛТ РОД 5Гр, ФС + КЛТ РОД 10 Гр. На модели ЛСП на 14–е сутки от начала воздействий средний объем опухоли ($V_{ср}$) в группах составил 26,31±5,81; 22,45±6,97; 18,99±4,86; 10,75±5,18 и 28,06±2,85 см^3, соответственно ($p<0,05$). Коэффициент торможения роста опухоли (ТРО) в опытных группах

составил 14,67%; 27,82%; 59,14% и - 6,65%, соответственно. Частота полных регрессий опухолей через 60 суток после начала эксперимента составила 0%, 20%, 20%, 60% и 20%, соответственно. На модели РС1 на 14–е сутки от начала воздействий $V_{ср}$. в группах составил 4,48±1,03; 0,80±0,21; 0,29±0,09; 0,19±0,07 и 0,32±0,08 см^3, соответственно (p=0,009). Коэффициент ТРО в опытных группах составил 82,14%; 93,53%; 95,76% и 92,86%, соответственно. Частота полных регрессий опухолей через 60 суток после начала эксперимента составила 0%, 0%, 20%, 0% и 0%, соответственно. Результаты исследования показали, что введение ФС хлоринового ряда перед сеансом КЛТ увеличивает противоопухолевую эффективность лучевой терапии у животных с различными по гистологической структуре и характеру роста перевивными опухолями. Полученные данные свидетельствуют о перспективности дальнейших исследований радиосенсибилизирующих свойств ФС.

Ключевые слова: экспериментальное исследование, лабораторные животные, перевивные опухоли, контактная лучевая терапия, фотосенсибилизатор.

Для цитирования: Церковский Д.А., Протопович Е.Л., Козловский Д.И., Суслова В.А. Противоопухолевая эффективность контактной лучевой терапии в комбинации с фотосенсибилизатором хлоринового ряда в эксперименте // Biomedical Photonics. – 2021. – Т. 10, № 2. – С. 25–33. doi: 10.24931/2413–9432–2021–10–2-25–33

Контакты: Церковский Д.А., tzerkovsky@mail.ru

Introduction

Radiomodifiers are used in clinical oncology in the treatment of patients with malignant tumors in order to selectively enhance the antitumor effect of radiation therapy (RT) or to weaken its negative impact on normal tissues. The radiomodifying agents used are electron acceptor compounds (metronidazole, mesonidazole, etc.), hyperthermia (general and local), and artificial hyperglycemia. The use of radiomodifiers makes it possible to increase the radiosensitivity of tumor cells located in hypoxic zones of the tumor, without increasing the degree of radiation damage to normal oxygenated cells [1].

At the end of the twentieth century, the results of the first clinical studies were published, which demonstrated that the combined use of RT with antimetabolites (5–fluorouracil, methotrexate) significantly improves the results of treatment of patients with squamous cell carcinoma of head and neck. Cytostatic drugs, ultrasound and laser radiation in low–intensity modes, magnetic and electric fields are used as physico-chemical factors that modify the radioresistance of tumors [1].

In recent years, the method of photodynamic therapy (PDT) has been increasingly used in clinical practice [2-8]. Of particular interest are studies into the radiosensitizing properties of photosensitizers (PS) used in photodynamic therapy. The first PSs whose radiosensitizing activity was proved in experimental studies *in vitro* and *in vivo* were hematoprophyrin and photofrin II [9-11].

The main scientific idea of this study is to examine and prove new properties of the photolon chloride series PS in an *in vivo* experiment on laboratory animals with inoculated tumours. The paper studies the possibility of enhancing radiation damage to inoculated tumors due to the combined use of ionizing radiation and PS as a radiomodifier.

It is for the first time that research in this direction is conducted in the Republic of Belarus and in the CIS countries. In the available literature sources, there are only a few publications (by author groups from Germany, Lithuania, and Japan) on the study of the radiosensitizing effect of PS (hematoporphyrin, photofrin II, 5–aminolevulinic acid) [10-13].

There are no works devoted to the treatment of animals with induced tumors using photolon chloride series PS, which makes this study relevant and promising for experimental oncology.

Materials and methods

Laboratory animals

The pilot study was performed on 50 white mongrel rats of both sexes, obtained from the vivarium of the N. N. Alexandrov RRPC of OMR, with a body weight of 250±50 g, aged 2.5-3 months. The duration of quarantine before the inclusion in the experiment was 14 days. Laboratory animals were kept in standard conditions in terms of food and drinking rations, with a 12-hour lighting mode, at a temperature of 20-22° C and a humidity of 50-60% in cages with 5 individuals in each. The indicators of humidity, temperature, and illumination in the room complied with the current sanitary rules for the device, equipment and maintenance of vivariums [14].

Tumor strains

The study used tumor strains (Pliss lymphosarcoma, PLS) [15] and PC alveolar liver cancer (PC1) [16] obtained from the Russian collection of cell cultures (Institute of Cytology of the Russian Academy of Sciences, St. Petersburg, Russian Federation).

Tumor model

The tumor model in laboratory animals was created by subcutaneous passivation *in vivo*. Subcutaneous grafting included the introduction of 0.5 ml of a 10% suspension of tumor cells in a 0.6% Hanks' solution subcutaneously in the left inguinal region. Laboratory animals with

PLS were included in the experiment on the 7th day after the transfer, and those with PC1, on the 21st day after the inoculation.

Ethical aspects

The experimental studies were conducted in accordance with the international legislation and the regulatory acts in force in the Republic of Belarus on conducting experimental studies with laboratory animals, namely:

1. The European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, France, dated 18.03.1986), as amended in accordance with the provisions of the Protocol (SED No. 170 of 02.12.2005).
2. Directive 2010/63/EU of the European Parliament and of the European Union on the protection of animals used for scientific purposes (dated 22.09.2010).
3. TPC 125-2008 «Good Laboratory Practice» (GLP) (Resolution of the Ministry of Health of the Republic of Belarus No. 56 of 28.03.2008).

Laboratory animals were put under anesthesia (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 for laboratory animals, they were put to death with generally accepted methods of euthanasia (*aether pro narcosi*) with the observance of humane methods of laboratory animals treatment.

The radiosensitizer

Photolon (RUE «Belmedpreparaty», Minsk, Republic of Belarus), which is a trisodium salt of e6 chlorine with povidone K17, was used as a radiosensitizing agent. The PS was a lyophilized powder for the preparation of intravenous solution in the form of a porous mass of greenish-black color, 100 mg (registration number 16/11/886 of 08.11.2016). The PS was introduced by intravenous infusion in a darkened room at a dose of 2.5 mg/kg of body weight (according to the instructions for medical use).

The contact radiation therapy session

The animals were exposed to ionizing radiation once, with «microSelectron HDR V3 Digital device (the Netherlands) with 192-Ir microSelectron V2 radiation source. Irradiation was performed in single focal doses (SFD) of 5 and 10 Gy. The activity of the radiation source at the beginning of the experiments was 5.2 Ci. The irradiation time was 73.1 seconds and 146.2 seconds, respectively. Irradiation was started 2.5-3 hours after the end of the PS infusion (on the PLS model) and 3.5-4 hours after the infusion (on the PC1 model). The time between the completion of the PS infusion and the beginning of the irradiation sessions was determined in previous studies, which showed exactly these time intervals for inoculated tumors as the time to reach the maximum concentration of PS in the tumor tissue.

The design of the research

All the treatments were performed after the tumor node reached the diameter of at least 4-5 mm: on the 7th day after PLS transplantation and on the 21st day for PC1. The study was performed on 25 laboratory animals randomly distributed into groups of 5 animals each (for each of the tumor strains). The characteristics of the experimental groups are presented in Table 1.

Таблица 1
Дизайн исследования
Table 1
Study design

№	Группа исследования Study groups
1	Интактный контроль Intact control
2	КЛТ, РОД 5 Гр CRT, single focal doses 5 Gy
3	КЛТ, РОД 10 Гр CRT, single focal doses 10 Gy
4	ФС фотолон 2,5 мг/кг + КЛТ, РОД 5 Гр PS photolon 2.5 mg/kg + CRT, single focal doses 5 Gy
5	ФС фотолон 2,5 мг/кг + КЛТ, РОД 10 Гр PS photolon 2.5 mg/kg + CRT, single focal doses 10 Gy

Criteria for evaluating antitumor efficacy

The antitumor effectiveness of exposure was evaluated according to the indicators characterizing the dynamics of volume changes (*V*), the coefficient of absolute tumor growth (*K*) and the coefficient of tumor growth inhibition (*TGI*).

The volume of tumors was calculated by the formula (1):

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

$d_{1, 2, 3}$ are three mutually perpendicular diameters of the tumor (in cm);
$\pi/6 = 0.52$ is a constant value;
V is the volume of the tumor (in cm^3).

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

$$K = \frac{V_t - V_0}{V_0}, \text{ where}$$

V_0 is the initial volume of the tumor (before the introduction of the chemotherapy drug);
V_t is the volume of the tumor at a certain period of observation.

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

$$TGI\% = (V_{control} - V_{experiment}) / V_{control} * 100$$, where

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

The quantitative criteria for evaluating the inhibitory effect on inoculated tumors in laboratory animals were as follows (Table 2) [17].

The dynamics of the growth of inoculated tumors was recorded starting from day 7 after the inoculation of the tumor strain of PLS and from day 21 for PC1, for 2 weeks with an interval of 2-3 days.

The frequency of complete regressions (CR) was estimated 60 days after the performed exposures. In each group, the share of animals (%) with no visual and palpatory signs of tumor growth was evaluated [17].

Statistical data processing

Statistical processing of experimental data and graphical representation of the results were carried out with Excel 2010, Origin Pro (version 7.0) and Statistica (version 8.0) software. The results are presented in the form M±m, where M is the arithmetic mean and m is the error of the mean. To assess the reliability of the differences, the Mann–Whitney *U* criterion was used. The differences were considered statistically significant at the significance level of $p<0.05$.

Results

The inoculation rate of both tumor models (PLS and PC1) in laboratory animals was 100%.

The study compared the antitumor effectiveness of CRT as an independent therapy and the combination of CRT with the use of chlorin-type PS as a radiosensitizer. The results of the CRT effectiveness evaluation after systemic administration of photolon on the PLS model are presented in Fig. 1 and in Table 3.

As can be seen from the presented data, CRT in the SFD 5 Gy caused a slight inhibition of the growth of inoculated tumors, compared with the intact control group. An increase in the SFD to 10 Gy enhanced the effect of CRT, but the differences in the average tumor volumes (V_{aver}) in the groups of animals exposed to radiation at doses of 5 Gy or 10 Gy were statistically insignificant ($p=0.69$).

Intravenous administration of photolon at a dose of 2.5 mg/kg of body weight, followed by CRT in the SFD of 5 Gy, increased the antitumor effect of the radiation exposure, compared with CRT with the same SFD without a sensitizer. Thus, on the 14th day after the irradiation session in the group of rats receiving combined treatment, V_{aver} was 2 times lower than in the group of animals subjected to only CRT (10.75±5.18 cm^3 and 22.45±6.97 cm^3, respectively), although this difference did not reach a statistically significant level ($p=0.19$).

Intravenous administration of photolon preceding CRT with the SFD of 10 Gy did not lead to an increase in the antitumor effect of radiation exposure, compared with CRT with SFD of 10 Gy without a sensitizer.

Thus, the optimal treatment regimen for the PLS model was intravenous administration of photolon at a dose of 2.5 mg/kg of body weight, followed, after 2.5-3

Таблица 2
Критерии оценки противоопухолевой эффективности
Table 2
Evaluation criteria for antitumor efficacy

Критерии Criteria	Эффективность Efficacy
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% + ПР*<50% TGI <91–100% + CR<50%	+++
ТРО<91–100% + ПР>50% TGI <91–100% + CR>50%	++++

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

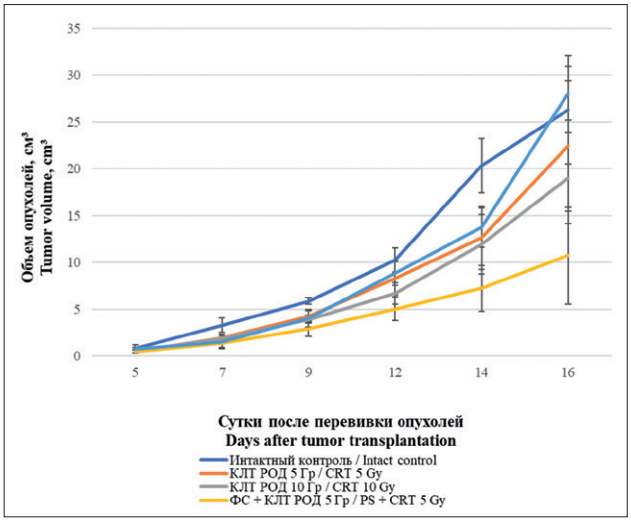

Рис. 1. Динамика роста перевивных опухолей модели ЛСП при исследуемых схемах терапевтического воздействия
Fig. 1. Dynamics of growth of transplanted tumors of the PLS model under the studied therapeutic regimens

Таблица 3
Эффективность лечения лабораторных животных с перевивными опухолями ЛСП
Table 3
Effectiveness of treatment of laboratory animals with transplantable PLS tumors

Наименование группы Groups	Показатели Indicators				
	$V_{ср.}$ опухоли до начала эксперимента, см³ Average tumor volume before experiments, cm³	$V_{ср.}$ опухоли на 14-е сутки эксперимента, см³ Average tumor volume 14 days after the start of experiments, cm³	K коэффициент абсолютного прироста опухолей Coefficient of absolute tumor growth	ТРО, % TGI, %	ПР, % CR, %
Интактный контроль Intact control	0,83±0,12	26,31±5,81	30,70	–	0
КЛТ РОД 5 Гр CRT 5 Gy	0,48±0,19	22,45±6,97	45,77	14,67	20
КЛТ РОД 10 Гр CRT 10 Gy	0,51±0,13	18,99±4,86	36,24	27,82	20
ФС + КЛТ РОД 5 Гр PS + CRT 5 Gy	0,46±0,15	10,75±5,18	22,37	59,14	60
ФС + КЛТ РОД 10 Гр PS + CRT 10 Gy	0,75±0,48	28,06±2,85	36,41	–6,65	20

hours, by a single exposure to ionizing radiation with the SFD of 5 Gy. On the 14th day after the treatment of animals, the K coefficient was 22.37%, the value of TGI, compared with the intact control group, was 59.14%, and the frequency of CR was 60%. The effectiveness of the treatment corresponded to «+» on a semi-quantitative assessment scale (Table 2).

The evaluation of the effectiveness of the combined use of CRT and photolon as a radiosensitizer on the PC1 model in rats showed the following.

As can be seen from the data presented in Fig. 2 and in Table 4, CRT in the SFD of 5 Gy caused a statistically significant inhibition of the inoculated tumors growth compared with the intact control: on day 14 of observation, V_{aver} in rats in the control group was 4.48±1.03 cm³, while in the group of animals after a session of CRT in the SFD 5 Gy it was 0.80±0.21 cm³. An increase in SFD to 10 Gy led to an increase in the antitumor effectiveness of exposure, and the difference in the values of V_{aver} in the groups of animals receiving CRT with the SFD of 5 Gy and 10 Gy approached the level of statistical significance (p=0.053).

Intravenous administration of photolon at a dose of 2.5 mg/kg of body weight, preceding radiation exposure, significantly potentiated the antitumor effect of CRT in the SFD 5 Gy: on the 14th day after the irradiation session, V_{aver} in this group of animals was 0.19±0.07 cm³. was 0.19±0.07 cm³. The differences in the values of V_{aver} in the group of rats that were injected with photolon before CRT, and in the group of animals that received only CRT with the SFD 5 Gy, are statistically significant (p=0.022).

As in the PLS model, in rats with an inoculated PC1 tumor, intravenous administration of photolon, preceding CRT with the SFD of 10 Gy, did not lead to an increase in the therapeutic effectiveness of radiation exposure.

Thus, the optimal combined treatment regimen for the PC1 rat model was intravenous administration of PS at a dose of 2.5 mg/kg of body weight, followed, after 3.5-4 hours, by a single exposure to ionizing radiation with the SFD of 5 Gy. On the 14th day of the follow-up after the treatment session, the K coefficient was 5.33%, and the value of TGI, compared with the control group of animals, was 95.76%. The CR of tumors in rats with an inoculated PC1 tumor after

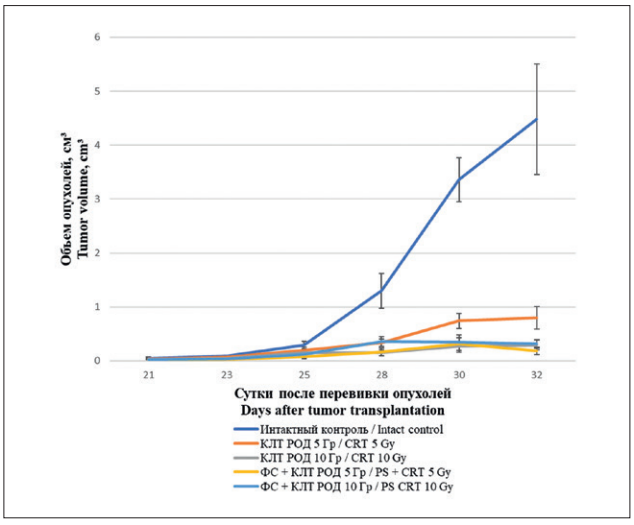

Рис. 2. Динамика роста перевивных опухолей модели PC1 при исследуемых схемах терапевтического воздействия
Fig. 2. Dynamics of growth of transplanted tumors of the PC1 model under the studied therapeutic regimens

Таблица 4
Эффективность лечения лабораторных животных с перевивными опухолями РС1
Table 4
Effectiveness of treatment of laboratory animals with transplantable PC1 tumors

Наименование группы Groups	Показатели Indicators				
	$V_{ср.}$ опухоли до начала экспери-мента, см³ Average tumor volume before experiments, cm³	$V_{ср.}$ опухоли на 14-е сутки эксперимента, см³ Average tumor volume 14 days after the start of experiments, cm³	К коэффициент абсолютного прироста опухолей Coefficient of absolute tumor growth	ТРО, % TGI, %	ПР, % CR, %
Интактный контроль Intact control	0,05±0,02	4,48±1,03	88,60	–	0
КЛТ РОД 5 Гр CRT 5 Gy	0,04±0,02	0,80±0,21	19,00	82,14	0
КЛТ РОД 10 Гр CRT 10 Gy	0,03±0,01	0,29±0,09	8,67	93,53	20
ФС + КЛТ РОД 5 Гр PS + CRT 5 Gy	0,03±0,01	0,19±0,07	5,33	95,76	0
ФС + КЛТ РОД 10 Гр PS + CRT 10 Gy	0,03±0,01	0,32±0,08	9,67	92,86	0

treatment was not registered. The effectiveness of the impact corresponded to « ++ » on the semi-quantitative assessment scale.

Discussion

Studies into the radiosensitizing properties of various classes of PS are currently relevant and promising. The overwhelming number of publications devoted to the consideration of this area of therapy, in experimental studies *in vitro* and *in vivo*, use hematoprophyrin and photofrin II as PS [9-11].

The main mechanisms of the antitumor response in the combined use of RT and PS have not been sufficiently studied. According to Shaffer M. et al., on the one hand, PS (for example, photophrine II), when exposed to ionizing radiation, can enhance the radiolytic effect due to oxygen species formed in the tumor cell under the influence of radiation itself [18]. On the other hand, RT leads to sublethal and lethal damage to tumor cells. In the future, sublethal changes are usually reversible based on the mechanisms for restoring the functions of the tumor cell. In the case of activation of photofrin II by ionizing radiation, the oligomeric components of this PS, interacting with intermediate free radicals (hydroxyl radicals) formed in the tumor cell during irradiation, prevent the development of these processes and, consequently, this combination creates antitumor effects [18, 19].

The results of experimental studies of the radiosensitizing effect of photosensitizing agents are presented in a number of research publications by other authors.

For instance, the study Kulka U. et al., performed on the cell lines of bladder cancer RT4 and glioblastoma U-373 MG, evaluated the effectiveness of the combined use of ionizing radiation with SFD from 2 to 8 Gy and photophrine. The maximum antitumor effect, expressed in a statistically significant decrease in the number of viable tumor cells, was noted when using PS and irradiation with SFD of 6 and 8 Gy. The percentage of viable U-373 MG tumor cells in the groups «PS + SFD 6 Gy» and «PS + SFD 8 Gy» was 2.7±1.1% and 0.2±0.1%, respectively, and was statistically significantly lower than when irradiated with the same parameters without adding PS to the nutrient medium (3.9±1.1% and 0.5±0.2%, respectively; $p<0.05$). The percentage of viable RT4 tumor cells in the groups «PS + SFD 6 Gy» and «PS + SFD 8 Gy» was 4.7±2.3% and 0.9±0.5%, respectively, and was statistically significantly lower than when irradiated with the same parameters without adding PS to the nutrient medium (6.7±1.1% and 1.7±0.7%, respectively; $p<0.05$). The authors came to the conclusion that irradiation of tumors sensitized by PS and in maximum concentrations adsorbed in mitochondria leads to the formation of a large number of reactive oxygen species and, as a result, the initiation of oxidative stress, which causes lethal and sublethal cell damage by apoptosis [20].

Shaffer M. et al., based on the results obtained in *in vivo* experiments on linear mice with a subcutaneous model of bladder cancer RT4, concluded that the combined use of photophrine and ionizing radiation (10 Gy) allows to increase the time of tumor volume doubling from 6.2 to 10.9 days compared with single-mode irradiation ($p<0.05$) [21].

Rutkovskienė L. et al. studied the radiosensitizing properties of hematoporphyrin (1 mcg/ml) and temoporphin (0.1 mcg/ml) derivatives on glioma C6 cell culture. The irradiation of the cell culture in the monolayer

was carried out with gamma rays using cobalt-60 (dose rate: 1.1 Gy/min) with a variation of SFD from 2 to 8 Gy. The authors showed that the use of PS without irradiation did not have a toxic effect on glioma C6. Irradiation without PS with the SFD of 2 Gy reduced the number of viable cells by 20%, and with the TFD of 4 Gy, by 50%. Radiosensitization with a hematoporphyrin derivative in combination with irradiation with TFD of 2-8 Gy significantly reduced this indicator compared to the single-mode irradiation group ($p<0.05$). Temoporphine did not show radiosensitizing properties [22].

In the research of Schaffer M. et al., attempts are made to test the treatment regimens developed in the experiment in patients with malignant neoplasms [10, 23-25].

In 2002, the results of treatment of 2 patients with unresectable recurrent bladder cancer were published. Photofrin II was used as PS, and irradiation (remote RT) was carried out with TFD of 44.8+14 Gy 24 hours after the introduction of PS at a dose of 1 mg/kg of body weight. The method used made it possible to reduce the volume of tumors by 35% and 40%, respectively, and perform surgery at the end of the RT course [23].

In 2006, Schaffer M. et al. presented the experience of clinical use of photofrin II in combination with RT in 12 patients (7 with unresectable solid pelvic tumors, 3 with malignant gliomas, 1 with a relapse of oropharyngial carcinoma, 1 with a relapse of sphenoidal sinus adenocarcinoma). Irradiation (remote RT) was performed in TFD 30-50.4 Gy 24 hours after intravenous administration of PS at a dose of 1 mg/kg of body weight. The median follow-up time was 12.9 months. No serious adverse events were observed. The frequency of CR was in 33.3% cases (4 patients), a decrease in the volume of the tumor by 45% or more was in 33.3% (4 patients), and the stabilization of the process in 33.3 % (4 patients). Only in 1 patient, 5 months after treatment, the occurrence of a local relapse of the disease was observed [10].

In 2013, a group of scientists published the results of treatment of a patient with grade III astrocytoma using remote RT (TFD 60 Gy) with a preliminary intravenous infusion of photofrin II at a dose of 1 mg/kg of body weight. The PS was administered 24 hours before the start of irradiation. The authors note a long progression-free follow-up period (106 months), the absence of adverse events and phenomena [24].

In 2019, Schaffer P. et al. presented the results of treatment of a patient with cervical carcinoma (FIGO IIIb deg.). Photofrin II was used as PS, and irradiation (remote RT) was carried out 24 hours after the introduction of PS at a dose of 1 mg/kg of body weight, with TFD 50.4 + 14 Gy (fractionated; SFD of 1.8–2 Gy daily, 5 times a week). According to the authors, a local relapse of the disease was detected 30 months after the end of the course of treatment (hysterectomy was performed) [25].

It is worth noting that the overwhelming majority of studies is aimed at studying the antitumor effectiveness of the combined use of PS and remote RT. In the available literary sources, we found only one publication dedicated to the use of contact RT. A. Morandi et al. presented the results of the combined use of photofrin II at a dose of 3 mg/kg of body weight and intratissual RT. The model used was a solid form of mammary adenocarcinoma in linear BALB/c mice. Exposure to ionizing radiation was carried out 24 hours after the completion of PS infusion, with SFD of 5 and 10 Gy. The results obtained indicate an increase in the antitumor effectiveness of intratissual RT when it is used with PS [26].

Conclusion

The pilot data obtained from the analysis of the immediate and long-term results of an experimental study on various models of inoculated tumors in rats indicate a pronounced tendency to a higher antitumor effect of combined treatment, including the use of PS followed by CRT sessions at certain radiation doses, compared with CRT alone. No experimental studies were found in the available literature sources that examine the effectiveness of the combined use of chlorine-type PS and ionizing radiation and demonstrating positive results, which brings us to the conclusion that more in-depth research in this direction is necessary and will be promising.

Experimental studies of the effects of combined treatment on laboratory animals with inoculated tumors will be continued in order to further optimize the CRT regimens with the use of chlorin-series PS as a radiosensitizer.

The work was carried out with the financial support of the Belarusian Republican Foundation for Fundamental Research of the National Academy of Sciences of Belarus (grant No. M19M-137, 2019-2021).

REFERENCES

1. Tzerkovsky D.A., Protopovich E.L. Radiosensitizing effect of photosensitizers (review of the results of experimental and clinical studies). *Oncological Journal*, 2018, Vol. 12(2), pp. 110–115 (in Russian).
2. Sokolov V.V., Filonenko E.V., Telegina L.V. et al. Combination of fluorescent imaging and local spectrophotometry in fluorescent

ЛИТЕРАТУРА

1. Церковский Д.А., Протопович Е.Л. Радиосенсибилизирующий эффект фотосенсибилизаторов (обзор результатов экспериментальных и клинических исследований) // Онкологический журнал. – 2018. – Т. 12, № 2. – С. 110–115.
2. Sokolov V.V., Filonenko E.V., Telegina L.V., Bulgakova N.N., Smirnov V.V. Combination of fluorescent imaging and local spectrophotom-

diagnostics of early cancer of larynx and bronchus. *Quantum Electronics*, 2002, Vol. 32(11), pp. 963–969 (in Russian).
3. Sokolov Victor V., Chissov V.I., Filonenko E.V. et al. Photodynamic therapy of cancer with the photosensitizer PHOTOGEM. *Proceedings of SPIE - The International Society for Optical Engineering*, 1995, Vol. 2325, pp. 367–374.
4. Filonenko, E.V. The history of development of fluorescence diagnosis and photodynamic therapy and their capabilities in oncology. *Russian Journal of General Chemistry*, 2015,Vol. 85(1), pp. 211–216.
5. Sokolov Victor V., Chissov V.I., Filonenko E.V., Yakubovskaya R.I. et al. First clinical results with a new drug for PDT. *Proceedings of SPIE - The International Society for Optical Engineering*, 1995, Vol. 2325, pp. 364–366.
6. Semyonov D.Yu., Vasil'ev Yu.L., Dydykin S.S. et al. Antimicrobial and antimycotic photodynamic therapy (review of literature). *Biomedical Photonics*, 202, Vol.10(1), pp. 25–31 (in Russian). https://doi.org/10.24931/2413–9432–2021–10–1-25–31
7. Filonenko E.V., Trushina O.I., Novikova E.G. et al. Photodynamic therapy in the treatment of intraepithelial neoplasia of the cervix, vulva and vagina. *Biomedical Photonics*, 2020, Vol. 9(4), pp. 31–39 (In Russian.) https://doi.org/10.24931/2413–9432–2020–9-4–31–39
8. Rakhimzhanova R.I., Shanazarov N.A., Turzhanova D.E. Photodynamic therapy of intradermal metastatic breast cancer (literature review). *Biomedical Photonics*, 2019, Vol. 8(3), pp. 36–42 (In Russian.) https://doi.org/10.24931/2413–9432–2019–8-3–36–42
9. Kulka U. et al. Radiosensitization of tumours by porphyrins. *Cancer Lett*, 2006, Vol. 235, pp. 40–47.
10. Schaffer M. et al. Feasibility of photofrin II as a radiosensitizing agent in solid tumors – preliminary results. *Onkologie*, 2006, Vol. 29, pp. 514–519.
11. Schaffer M. et al. Radiation therapy combined with photofrin or 5-ALA: effect on Lewis sarcoma tumor lines implanted in mice. Preliminary results. *Tumori*, 2002, Vol. 88, pp. 407–410.
12. Bloznelytë-Plëðnienë L. and Rutkovskiene L. Radiosensitized treatment of primary or metastatical malignant brain tumors with hematoporphyrin derivative. *Electron. Electric. Engineer*, 2006, Vol. 4(68), pp. 83–86.
13. Yamamoto J. et al. Radiosensitizing effect of 5-aminolevulinic acid-induced protoporphyrin IX in glioma cells in vitro. *Oncol. Rep*, 2012, Vol. 27, pp. 1748–1752.
14. Sanitarnye pravila i normy 2.1.2.12–18–2006 «Ustrojstvo, oborudovanie i soderzhanie jeksperimental'no-biologicheskih klinik (vivariev)», *Postanovlenie ot 31.10.2006 g. № 131, g. Minsk, Respublika Belarus'* [Resolution No. 131 of 31.10.2006, Minsk, Republic of Belarus] (in Belarus).
15. Limfosarkoma Plissa [Electronic source]. http://www.bionco.ru/methods/exp_chemotherapy/strains/char_strains/LSP/. – Accessed: 15.03.2021 (in Russian).
16. Holangioma (al'veolyarnyj rak pecheni RS1) [alveolar liver cancer PC1] [Electronic source]. – Rezhim dostupa: http://www.bionco.ru/methods/exp_chemotherapy/strains/char_strains/rs1/. – Accessed: 15.03.2021 (in Russian).
17. Metodicheskie ukazanija po izucheniju protivoopuholevoj aktivnosti farmakologicheskih veshhestv / Treshhalina E.M. i dr. // V kn.: Rukovodstvo po jeksperimental'nomu (doklinicheskomu) izucheniju novyh farmakologicheskih veshhestv / pod obshhej red. chlen-korr. RAMN prof. R.U. Habrieva. – *2 izd., pererab. i dop.* – *M.: OAO izd. «Medicina»* [2nd ed., reprint. and add. - M.: JSC ed. "Medicine"], 2005, Vol. 832 S, pp. 637–651 (in Russian).
18. Schaffer M. et al. The application of photofrin II® as a sensitizing agent for ionizing radiation – a new approach in tumor therapy? *Current Med. Chem*, 2005, Vol. 12, pp. 1209–1215.
19. Schaffer M. et al. Effect of Photofrin II as a radio-sensitizing agent in two different oesophageal carcinoma cell lines. *J. Porphyrins Phthalocyanines*, 2005, Vol. 9, pp. 470–475.
20. Kulka U. et al. Photofrin as a radiosensitizer in an in vitro cell surv

etry in fluorescent diagnostics of early cancer of larynx and bronchus // Quantum Electronics. – 2002. – Vol. 32(11) . – P. 963–969.
3. Sokolov Victor V., Chissov V.I., Filonenko E.V., Sukhin Garry M.,Yakubovskaya R.I., Belous T.A., Zharkova Natalja N., Kozlov Dmitrij N., Smirnov V.V. Photodynamic therapy of cancer with the photosensitizer PHOTOGEM // Proceedings of SPIE - The International Society for Optical Engineering. – 1995. – Vol. 2325. – P. 367–374.
4. Filonenko, E.V. The history of development of fluorescence diagnosis and photodynamic therapy and their capabilities in oncology // Russian Journal of General Chemistry. – 2015. – Vol. 85(1) . – P. 211–216.
5. Sokolov Victor V., Chissov V.I., Filonenko E.V., Yakubovskaya R.I., Sukhin D.G., Galpern Maria G., Vorozhtsov Georgij N., Gulin A.V., Zhitkova M.B., Zharkova Natalja N., Kozlov Dmitrij N., Smirnov V.V. First clinical results with a new drug for PDT // Proceedings of SPIE - The International Society for Optical Engineering. – 1995. – 2325. – P. 364–366.
6. Семенов Д.Ю., Васильев Ю.Л., Дыдыкин С.С., Странадко Е.Ф., Шубин В.К., Богомазов Ю.К., Морохотов В.А., Щербюк А.Н., Морозов С.В., Захаров Ю.И. Антимикробная и антимикотическая фотодинамическая терапия (обзор литературы) // Biomedical Photonics. – 2021. – Т. 10(1) . – С. 25–31. https://doi.org/10.24931/2413–9432–2021–10–1-25–31
7. Филоненко Е.В., Трушина О.И., Новикова Е.Г., Зароченцева Н.В., Ровинская О.В., Иванова-Радкевич В.И., Каприн А.Д. Фотодинамическая терапия в лечении интраэпителиальных неоплазий шейки матки, вульвы и влагалища. // Biomedical Photonics. – 2020. – Т. 9(4) . – С.31–39. https://doi.org/10.24931/2413–9432–2020–9-4–31–39
8. Рахимжанова Р.И., Шаназаров Н.А., Туржанова Д.Е. Фотодинамическая терапия внутрикожных метастазов рака молочной железы (обзор литературы) // Biomedical Photonics. – 2019. – Т. 8(3) . – С. 36–42. https://doi.org/10.24931/2413–9432–2019–8-3–36–42
9. Kulka U. et al. Radiosensitization of tumours by porphyrins // Cancer Lett. – 2006. – Vol. 235. – P. 40–47.
10. Schaffer M. et al. Feasibility of photofrin II as a radiosensitizing agent in solid tumors – preliminary results // Onkologie. – 2006. – Vol. 29. – P. 514–519.
11. Schaffer M. et al. Radiation therapy combined with photofrin or 5-ALA: effect on Lewis sarcoma tumor lines implanted in mice. Preliminary results // Tumori. – 2002. – Vol. 88. – P. 407–410.
12. Bloznelytë-Plëðnienë L. and Rutkovskiene L. Radiosensitized treatment of primary or metastatical malignant brain tumors with hematoporphyrin derivative // Electron. Electric. Engineer. – 2006. – Vol. 4(68). – P. 83–86.
13. Yamamoto J. et al. Radiosensitizing effect of 5-aminolevulinic acid-induced protoporphyrin IX in glioma cells in vitro // Oncol. Rep. – 2012. – Vol. 27. – P. 1748–1752.
14. Санитарные правила и нормы 2.1.2.12–18–2006 «Устройство, оборудование и содержание экспериментально-биологических клиник (вивариев)», Постановление от 31.10.2006 г. № 131, г. Минск, Республика Беларусь.
15. Лимфосаркома Плисса [Электронный ресурс]. – Режим доступа: http://www.bionco.ru/methods/exp_chemotherapy/strains/char_strains/LSP/. – Дата доступа: 15.03.2021.
16. Холангиома (альвеолярный рак печени PC1) [Электронный ресурс]. – Режим доступа: http://www.bionco.ru/methods/exp_chemotherapy/strains/char_strains/rs1/. – Дата доступа: 15.03.2021.
17. Методические указания по изучению противоопухолевой активности фармакологических веществ / Трещалина Е.М. и др. // В кн.: Руководство по экспериментальному (доклиническому) изучению новых фармакологических веществ / под общей ред. член-корр. РАМН проф. Р.У. Хабриева. – 2 изд., перераб. и доп. – М.: ОАО изд. «Медицина», 2005 г. – 832 С. – С. 637–651.
18. Schaffer M. et al. The application of photofrin II® as a sensitizing agent for ionizing radiation – a new approach in tumor therapy? //

vival assay. *Biochem. Biophys. Res. Commun*, 2003, Vol. 311, pp. 98–103.
21. Shaffer M. et al. Photofrin as a radiosensitizing agent for tumors: studies in comparison to other porphyrins, in an experimental in vivo model. *J. Photochem. Photobiol*, 2002, Vol. 66(3), pp. 157–164.
22. Rutkovskienė L. et al. Sensitization of rat C6 glioma cells to ionizing radiation by porphyrins. *Acta Medica Lituanica*, 2011, Vol. 18(2), pp. 56–62.
23. Shaffer M. et al. Application of Photofrin II as a specific radiosensitizing agent in patients with bladder cancer – a report of two cases. *Photochem. Photobiol. Sci,* 2002, Vol. 1(9), pp. 686–689.
24. Schaffer M. et al. Treatment of astrocytoma grade III with Photofrin II as a radiosensitizer. A case report. *Strahlenther Onkol*, 2013, Vol. 189(11), pp. 972–976.
25. Shaffer P. et al. Treatment of cervix carcinoma FIGO IIIb with Photofrin II as a radiosensitizer: a case report. *Photochem. Photobiol. Sci*, 2019, Vol. 18(5), pp. 1275–1279.
26. Moradi A. et al. In vivo evaluation of photofrin II radiosensitivity for the treatment of adenocarcinoma tumors in balb/C mice using brachytherapy. *IFMBE Proceedings*, 2009, Vol. 25(1), pp. 141–143.

Current Med. Chem. – 2005. – Vol. 12. – P. 1209–1215.
19. Schaffer M. et al. Effect of Photofrin II as a radio-sensitizing agent in two different oesophageal carcinoma cell lines // J. Porphyrins Phthalocyanines. – 2005. – Vol. 9. – P. 470–475.
20. Kulka U. et al. Photofrin as a radiosensitizer in an in vitro cell survival assay // Biochem. Biophys. Res. Commun. – 2003. – Vol. 311. – P. 98–103.
21. Shaffer M. et al. Photofrin as a radiosensitizing agent for tumors: studies in comparison to other porphyrins, in an experimental in vivo model // J. Photochem. Photobiol. B. – 2002. – Vol. 66(3). – P. 157–164.
22. Rutkovskienė L. et al. Sensitization of rat C6 glioma cells to ionizing radiation by porphyrins // Acta Medica Lituanica. – 2011. – Vol. 18(2). – P. 56–62.
23. Shaffer M. et al. Application of Photofrin II as a specific radiosensitizing agent in patients with bladder cancer – a report of two cases // Photochem. Photobiol. Sci. – 2002. – Vol. 1(9). – P. 686–689.
24. Schaffer M. et al. Treatment of astrocytoma grade III with Photofrin II as a radiosensitizer. A case report // Strahlenther Onkol. – 2013. – Vol. 189(11). – P. 972–976.
25. Shaffer P. et al. Treatment of cervix carcinoma FIGO IIIb with Photofrin II as a radiosensitizer: a case report // Photochem. Photobiol. Sci. – 2019. – Vol. 18(5). – P. 1275–1279.
26. Moradi A. et al. In vivo evaluation of photofrin II radiosensitivity for the treatment of adenocarcinoma tumors in balb/C mice using brachytherapy // IFMBE Proceedings. – 2009. – Vol. 25(1). – P. 141–143.