REVISTA INCLUSIONES

UNIVERSIDAD E INVESTIGACIÓN: AL SERVICIO DEL ORBE

Revista de Humanidades y Ciencias Sociales

Volumen 7 . Número Especial
Octubre / Diciembre
2020
ISSN 0719-4706

REVISTA
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REVISTA DE HUMANIDADES
Y CIENCIAS SOCIALES

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REVISTA INCLUSIONES M.R.
REVISTA DE HUMANIDADES Y CIENCIAS SOCIALES

CUADERNOS DE SOFÍA EDITORIAL

ISSN 0719-4706 - Volumen 7 / Número Especial / Octubre – Diciembre 2020 pp. 507-524

MORPHOLOGICAL STUDY OF THE COMBINED LASER AND PHOTODYNAMIC EFFECT WITH RADACHLORIN ON THE STRUCTURE OF EXPERIMENTAL EHRLICH SARCOMA

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Fecha de Recepción: 09 de junio de 2020 – **Fecha Revisión**: 20 de junio de 2020

Fecha de Aceptación: 28 de septiembre 2020 – **Fecha de Publicación**: 01 de octubre de 2020

Abstract

The relevance of the problem studied is due to the social importance of introduction of highly effective methods of malignant tumors treatment. The article analyzes processing methods of laser radiation used and its antineoplastic activity. The experimental method of radochlorin effect on the Ehrlich sarcoma laboratory model was used as a basic method for the study of this problem. The main results of the research are fundamental and describe new, previously unknown aspects of intraoperative photodynamic therapy (using radachlorin) combined with high-intense laser radiation and its effect on Ehrlich sarcoma tumor tissue. Laser photodynamic therapy (PDT) was followed not only by typical circulatory disturbance, but also by suppressed growth factor expression of tumor angiogenesis. Captose-3-dependent apoptosis was proved activate under the influence of photosensitizer of the second generation. Apoptotic index significantly increases during the first week after laser PDT session in a dose of 200-400 J/cm^2, and laser PDT dose increase results in the lower expression of blc-2 oncoprotein and tumor cell proliferative activity at all stages of the experiment. The results of the study may be of great importance in developing new clinical methods for intraoperative laser radiation effect in treatment of malignant tumors.

Keywords

Experimental Ehrlich sarcoma – Photodynamic therapy – Laser

Para Citar este Artículo:

Rostovtsev, Nikolay M.; Zhukovskaya, Elena V.; Pasternak, Alexey E.; Kotlyarov, Alexander N. y Mustakimov, Boris H. Morphological study of the combined laser and photodynamic effect with radachlorin on the structure of experimental ehrlich sarcoma. Revista Inclusiones Vol: 7 num Especial (2020): 507-524.

Introduction

Development and introduction of laser technologies in oncology, their combination with photodynamic therapy and chemotherapy extends the scope of treatment and significantly improves quality of life and remote results[1]. Photodynamic therapy (PDT) is a method of malignancy treatment based on the ability of drugs (photosensitizers) to accumulate selectively in tumor tissue; local exposure of tumor to laser radiation with certain wavelength corresponding to photosensitizer peak absorption initiates photochemical reaction producing singlet oxygen and other active radicals that have cytotoxic effect on tumor cells[2]. In literature dealing with PDT its advantages over radiation therapy in treatment of cancer are obvious and associated with selective impact on tumor tissue and absence of systemic and severe local complications[3]. There is many unsolved technical problems preventing wide spread of laser methods in practical oncology[4]. At the same time, it is clear that PDT effectiveness depends not only on the properties and photosensitizer dose, but also on the density, power and wavelength of laser radiation. Like PDT, laser radiation involves radiation doses delivered to tumor, with the difference that in this case the cytotoxic factor is biological tissue heating with the absorbed laser radiation. As the way of radiation delivery to tumors is the same in these two methods, but the specifics of impact on tumor is different, the idea of combined application of PDT and laser radiation is very promising. Study of pathomorphosis of neoplastic process influenced by laser photodynamic therapy is of practical importance for improving the effectiveness of cancer treatment[5].

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[1] I. Y. Kubasova, “Fluorescent diagnosis and photodynamic therapy in treatment of malignant brain tumors”, Ros. bioterapevticheskiy zhurnal, Vol: 5 num 4 (2006): 54-63; I. S. Spichenkova, “Combined radiation and photodynamic therapy of experimental sarcoma M1 tumor in rats”. Ros. bioterapevticheskiy zhurnal, Vol: 2 num 4 (2003): 31-64; M. A. Biel, “Photodynamic therapy of head and neck”. Methods Mol. Biol, Vol: 635 (2010): 283- 293 y A. Juarranz, “Photodynamic therapy of cancer. Basic principles and applications”, Clin. Transl. Oncol, Vol: 10 num 3 (2008): 148-154.

[2] V. N. Zalesskij, “Apoptosis of gastrointestinal tumor cells at photodynamic therapy”, Voprosy onkologii, vol: 50 num 1 (2004): 9-19; M. A. Kortava, “Role of uptake factor in photodynamic therapy effectiveness in treatment of breast adenocarcinoma Ca-755 in mice with two pharmaceutical forms of photosensitizer”, Ros. bioterapevticheskiy zhurnal, Vol: 5 num 4 (2006): 64-67 y I. Y. Kubasova, “Fluorescent diagnosis and photodynamic therapy in treatment of malignant brain tumors”, Ros. bioterapevticheskiy zhurnal, Vol: 5 num 4 (2006): 54-63.

[3] N. V. Kudinova, “Photodynamic therapy of tumors: immunological aspect of treatment”, Ros. bioterapevticheskiy zhurnal, Vol: 9 num 1 (2010): 69-76; M. Fadela; N. Samyb; M. Nasrc; A. A. Alyoussefd & E. F. Stranadko, “Topical colloidal indocyanine green-mediated photodynamic therapy for treatment of basal cell carcinoma”. Pharmaceutical Development and Technology, Vol: 12. Published online: 19.02.2016; P. Mroz, “Stimulation of anti-tumor immunity by photodynamic therapy”, Expert Rev. Clin. Immunol, Vol: 7 num 1 (2011): 75-91 y A. E. O'Connor, “Mechanism of cell death mediated by a BF-2 chelated tetraacryl-azadipyrromethene photodynamic therapeutic: dissection of the apoptotic pathway in vitro and in vivo”, Int. J. Cancer, Vol: 130 num 3 (2012): 705-715.

[4] D. Nowis, “Direct tumor damage mechanisms of photodynamic therapy”, Acta Biol. Pol, vol: 52 num 2 (2005): 339-352.

[5] I. S. Davidenko, “Role of angiogenesis in treatment of metastatic breast cancer”, Ros. bioterapevticheskiy zhurnal, Vol: 4 num 6 (2007): 8-12; N. V. Kudinova, “Photodynamic therapy of tumors: immunological aspect of treatment”, Ros. bioterapevticheskiy zhurnal, Vol: 9 num 1 (2010): 69-76; E. V. Yaroslavtseva-Isaeva, “Development of photodynamic therapy method in treatment of experimental tumor (sarcoma M1) with local administration of photosensitizer”, Ros. bioterapevticheskiy zhurnal, Vol: 2 num 4 (2003): 19-22 y Lv. Ting, “Evolution of collagen alteration after topical photodynamic therapy (PDT) using second harmonic generation (SHG) microscopy in vivo study in a mouse model”, Photodiagnosis and photodynamic therapy, Vol: 9 num 2 (2012): 164-169.

The aim is to study the dynamics of morphological changes associated with using the increasing doses of laser radiation combined with photodynamic therapy involving radachlorin, partial tumorectomy and X-ray therapy. To describe changes in vessels and angiogenesis process in experimental Ehrlich sarcoma.

Materials and methods

The experiments were performed in CBA, C57BI and C3HA white outbred mice, F hybrids (CBA*C57BI) with Ehrlich ascites sarcoma transplanted in subcutaneous fat. The animals were divided into 5 groups: the intact group (control), group I – the tumor exposed to PDT with energy density of 100 J/cm^2, group II – the tumor exposed to PDT in a dose of 200 J/cm^2, group III – in a dose of 400 J/cm^2, group IV – Ehrlich carcinoma exposed to single X-ray irradiation in a dose of 5 Gy. In group V, half of the tumor volume was resected with intraoperative laser PDT (LPDT) in a dose 400 J/cm^2. The control group of animals (n = 48) did not receive any treatment. Second generation photosensitizer – chlorin e6 derivative – radachlorin (Russia) introduced intraperitoneally was used in the study. Photosensitizer dose was calculated by the formula:

$$V=\frac{K\times M\times C}{3500}$$

where К - mg/kg, M – mouse weight, C – potency.

Exposure to laser light during PDT session involved ether anesthesia. Laser apparatus “LAKHTA MILON” with the wavelength of 920 nm, maximum output power – 30 W was used as a source of laser radiation. The tumor-bearing animals were sacrificed on days 7, 14, 21, 28 after transplanting Ehrlich carcinoma by transcervical injection. The treatment started on day 7 after tumor transplantation. The excised tumor was measured and fixed in 10% neutral formalin. After histological preparation the tumor tissue specimens were embedded in paraffin, and the sections were stained with hematoxylin and eosin, Van Gieson’s picrofuchsin and Schiff’s reagent combined with alcian blue. The relative amount of tumor epithelium and necrotic zones was evaluated using eyepiece graticules by point count in random fields[6]. To evaluate necrosis rate in the tumor, necrotized and intact tumor parenchyma ratio (Vn/i coeffcient) was calculated.

Microslides were stained with hematoxylin and eosin. Tumor bloodstream was detected with marker for endothelial cell CD34 («DAKO», QBEnd-10, 1:50). Using stereometric method phases on the area unit were selected; then their measurement and calculation in histologic specimen images were performed, tumor bloodstream area using was determined using license program for micro-object image analysis “Videotest-Morfologiya 5.0” (“Videotest”, Russia). Relative volume of the tumor bloodstream was evaluated by point count[7]. To estimate tumor angiogenic activity, expression of Vascular Endothelial Growth Factor (VEGF, «DAKO», VG, 1:40) – angiogenesis stimulator was studied. VEGF results of immunohistochemical reactions were estimated using semiquantative method. Interpretation of VEGF expression was the following: negative – no cells are stained or less than 10% of tumor cells are stained, weak – 10-20% of tumor cells

[6] M.A. Kortava, “Role of uptake factor in photodynamic therapy effectiveness in treatment of breast adenocarcinoma Ca-755 in mice with two pharmaceutical forms of photosensitizer”, Ros. bioterapevticheskiy zhurnal, Vol: 5 num 4 (2006): 64-67.

[7] G. G. Avtandilov, Medical morphometry (Moscow: Meditsina, 1990).

are stained, moderate – 20-50% of tumor cells are stained, intensive – over 50% of tumor cells are stained.

Computer data processing involved parametric and non-parametric statistical methods. Statistical significance of differences in the compared characteristics in groups was estimated using Student's t-test and Mann-Whitney U test[8].

Carcinoma volume calculation was based on the following formula:

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

where d_1, d_2, d_3 – three mutually perpendicular tumor dimensions.

Tumor growth dynamics was estimated using the coefficient of absolute tumor growth rate (inhibition) (K) by the formula:

$$K=\frac{V_1 - Vv}{Vv}$$

where V_1 – tumor volume on the day of measurement, Vv - initial tumor volume. The dynamics of tumor growth rate coefficient (inhibition) was estimated using the following criteria:

- K>0 (tumor volume at the given time of observation was over its initial volume) was assessed as continued tumor growth;
- -1<K≤0 (tumor volume at the given time was less than or equal to its initial volume) was assessed as tumor growth inhibition;
- K=1 was regarded as a complete tumor regression.

Treatment effectiveness was estimated based on tumor growth inhibition within set period of time. Tumor growth inhibition (TGI) was calculated by the formula:

$$TGI=\frac{Vk - Vo}{Vk} \times 100\%$$

where Vk – median tumor volume in control group, Vo – median tumor volume in experimental group. Minimum significant criterion of tumor treatment effectiveness is at TGI ≥ 50%. Partial tumor resorption is decrease in tumor size by 50%, no effect - tumor size decrease by less than 50%, complete resorption - absence of tumor signs[9].

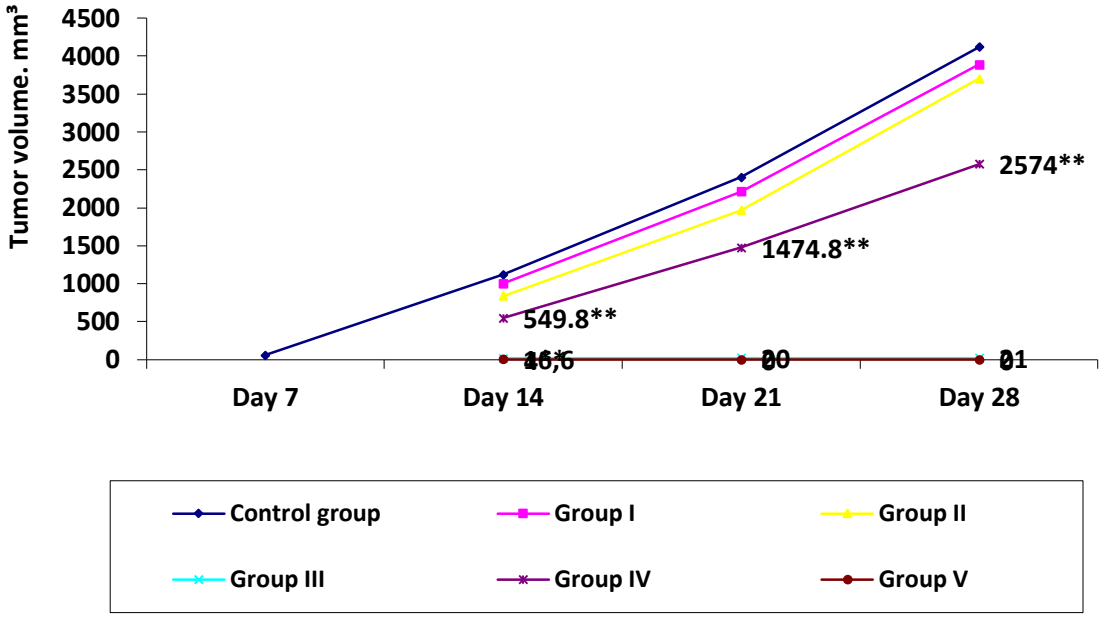
Results and discussion

Ehrlich tumor kinetics on day 7 after transplantation was characterized by 100% carcinoma growth in the control group; the mean tumor volume was 61.2±5.4 mm³. On day 14 (logarithmic growth period) the tumor size increased more than 18 times the initial tumor volume; on days 21, 28 it was twice as large as at the previous moment of measurement ($p<0,05$). Thus, at the final stage of observation the tumor volume was 4118.1±223.9 mm³.

[8] S. Glants, Medical-biological statistics (Moscow: Praktika, 1999).

[9] M. A. Kortava, "Role of uptake factor in photodynamic therapy effectiveness in treatment of breast adenocarcinoma Ca-755 in mice with two pharmaceutical forms of photosensitizer", Ros. bioterapevticheskiy zhurnal, Vol: 5 num 4 (2006): 64-67.

In the experimental group I at the exposure to 100 J/cm² laser radiation the post-PDT carcinoma growth dynamics was almost the same as the tumor growth rate in the control group. Light radiation dose increase up to 200 J/cm² (group II) resulted in significant decrease of tumor growth rate just only during the first week after PDT session (Fig.1). In comparison with these groups significantly lower tumor volume was observed after X-ray therapy ($p<0.05$) during all the periods of the study.

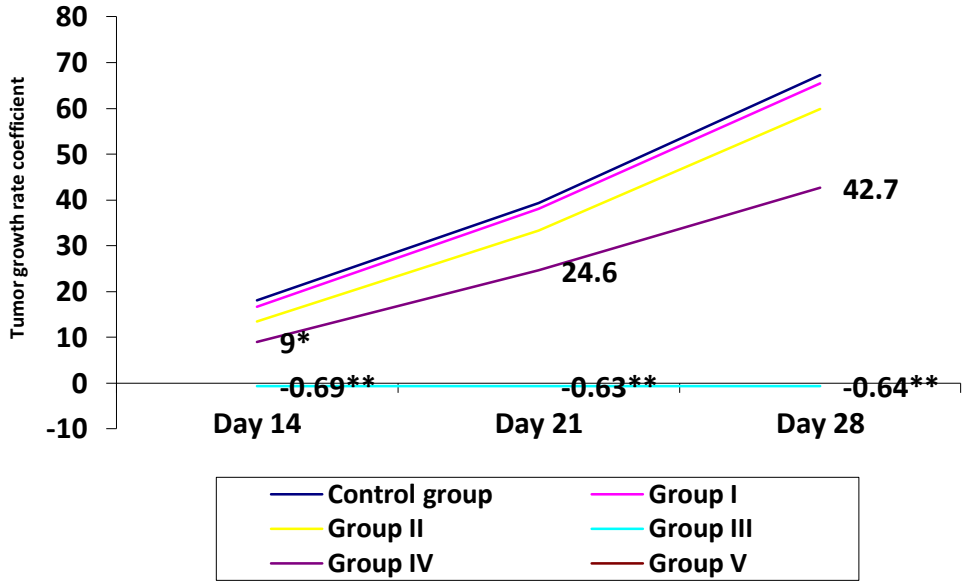


Note: * - $p<0.05$ in comparison with the control group and groups I, II; ** - $p<0.05$ in comparison with the control group and groups I, II, III; *** $p<0.05$ in comparison with the control group and groups I, II, III, IV.

Fig. 1
Dynamics of Ehrlich tumor growth

The tumor growth rate suppression which was the most expressed and persistent during the whole observation period was associated with PDT in a dose of 400 J/cm² (group III). On day 14 all the animals of this group had a sharply marginated crust located in the region exposed to PDT; under the crust there was tumor tissue with the volume of 19.6±2.2 mm³ and inhomogeneous appearance with whitish-yellow and dark red areas of softened tissue. In group V on day 7 after tumor resection combined with PDT there was tumor tissue with the volume of 4.0±0.7 mm³ located in the area of post-operative suture. At later dates, macroscopic and histological signs of tumor growth were not noticed in group V mice.

Coefficient values of the absolute tumor growth rate at low doses of laser radiation (100-200 J/cm²) were associated with the significant tendency to increase on days 21 and 28. Negative coefficient of absolute tumor growth rate at PDT in a dose of 400 J/cm² indicates Ehrlich tumor growth inhibition. If radiation therapy was used, this coefficient two times decreased in comparison with the control group during the first 7 days after exposure, and then the continued tumor growth was observed ($p<0.05$) (Fig.2).



Note: * - p<0.05 in comparison with the control group; ** - p<0.05 in comparison with the control group and groups I, II, IV.

Fig. 2
Dynamics of coefficient of absolute Ehrlich tumor growth rate (inhibition)

On day 7 after X-ray irradiation the TGI was 51% (partial resorption). However, during the next 7 days its value was decreasing; besides, half of tumor-bearing mice died on the background of the local radiation injuries. PDT effectiveness (dose of 400 J/cm²) was proved by high TGI values indicating tumor resorption at all stages of the experiment (Fig. 3).

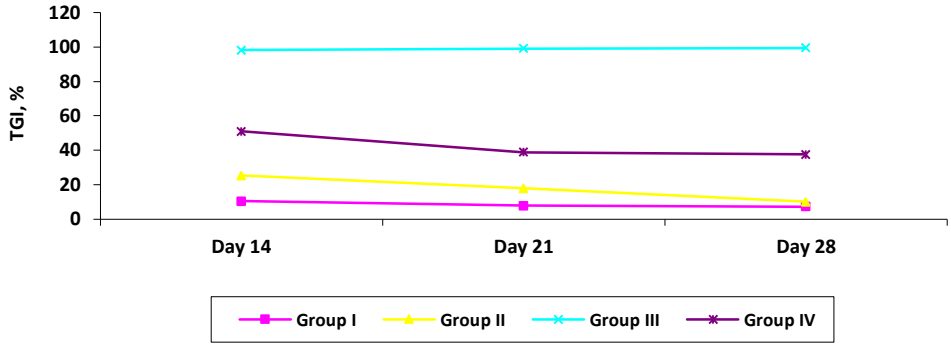


Fig. 3
Dynamics of Ehrlich tumor growth inhibition in the experimental groups.

The TGI value of 99.6% was maximum in group V on day 7 after treatment. In this group there was a complete resorption of the residual tumor in all the animals. Incomplete carcinoma regression in group III is probably associated with shielding of deep tumor layers at PDT.

Histologic examination on day 7 showed that in mice of the control group tumor tissue consisted of extensive layers of atypical polymorphic cells located in derma, forming glandular structures in several areas.

The tumor had numerous small necrotic zones in the form of oxyphil granular masses. Parenchyma volume on day 14 after Ehrlich carcinoma transplantation reached its maximum value – 40.1±1.69% ($p>0.05$) – with tumor invasion into papillary layer (Fig.4). Different tendency was observed in values of necrotic zone volume. From day 14 till day 21 there was significant increase of spontaneous necroses in the tumor ($p<0.05$). Thus, the dynamics of tumor process in the control group was associated with the early autonomic tumor progression, its marked invasive growth with the developed parenchymal component and spontaneous necrosis growth.

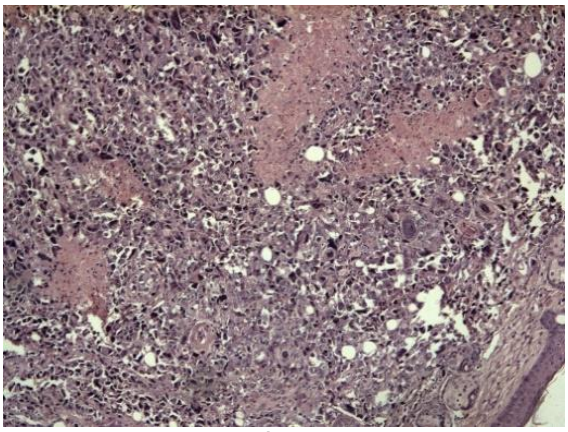


Fig. 4
Primitive glands formed by tumor cells; multiple necroses in the tumor tissue. Hematoxylin-eosin staining. Magnified x 200.

Relative parenchyma volume in group I significantly differed from values in the control group just on day 7 after PDT and laser exposure; on day 14 parenchyma volume increased up to 37.5±1.49% ($p<0.05$). In group II the volume of the intact parenchyma on day 7 was significantly less than in the control group and group I, and there was no significant increase of this value on days 14 and 21. Morphological examination performed in group III on day 7 showed prevalence of extensive zones of necrotic masses with micro bleeds in all the fields. Small tumor epithelial cells were located in deep layers of derma (Fig. 5). On days 14 and 21 parenchyma volume in this group was slightly over 2% which is significantly less than in the control group and in groups I, II, IV.

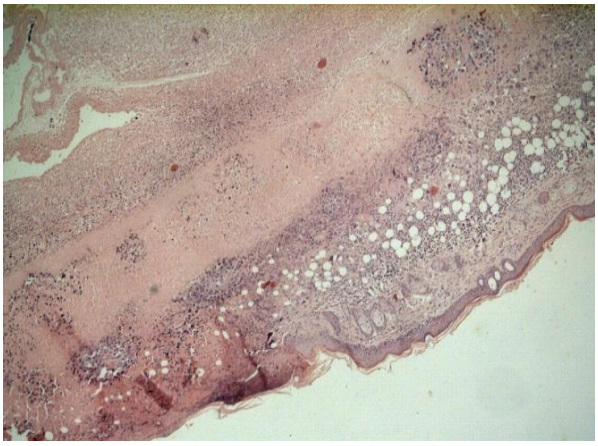


Fig. 5
Groups of tumor cells amid extensive necrotic fields. Hematoxylin-eosin stain. Magnified x 100

On days 7, 14, 21 after radiation therapy for carcinoma (group IV) the relative volume of vital parenchyma was significantly less than in groups I-II, but on day 14 the increase of parenchyma volume ($p<0.05$) was observed in group IV animals (Fig. 6).

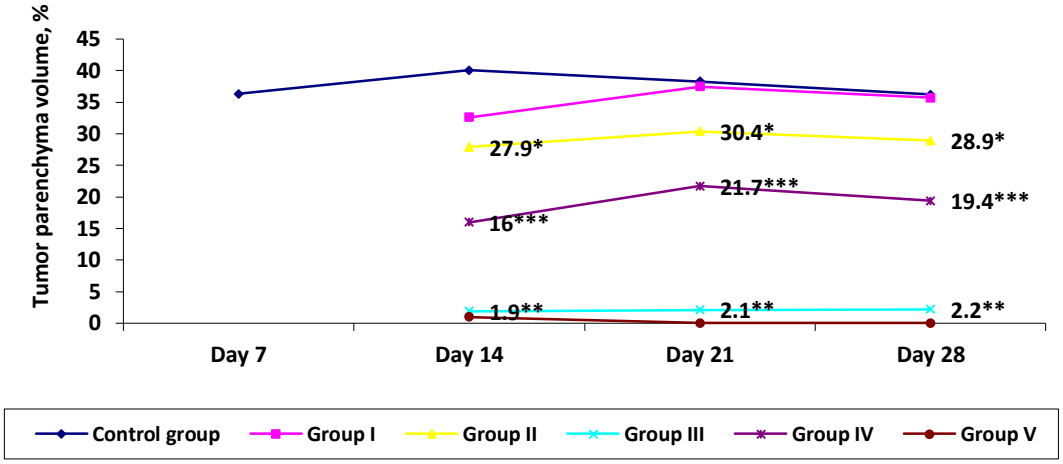


Fig. 6
Relative parenchyma volume in the control and experimental groups.

Note: * - $p<0.05$ in comparison with the control group and group I; ** - $p<0.05$ in comparison with the control group and groups I, II; *** $p<0.05$ in comparison with the control group and groups I, II, III.

The volume of necrotic zones in groups I-II was significantly higher than the same parameter in the control group only on day 7. Necrosis in groups III, IV on day 7 significantly prevailed over those in the control group and in groups I, II. The relative volume of necrosis by day 21 was 4.2% ($p<0.05$) in group III (Fig. 7).

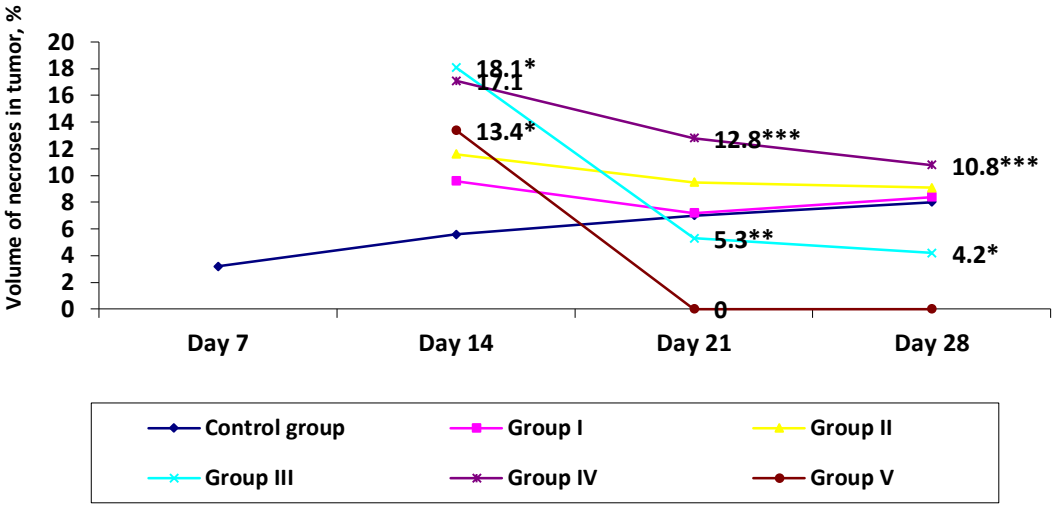


Fig. 7
Relative volume of necrosis in the control and experimental groups.

Note: * - $p<0.05$ in comparison with the control group and groups I, II; ** - $p<0.05$ in comparison with group II; *** $p<0.05$ in comparison with group III.

In group V volume of necrosis on day 7 was significantly higher than the same parameter in the control group and groups I, II, but was lower than in groups III and IV ($p<0.05$). Pathomorphological study showed that the volume of intact parenchyma presented as cell nests amid necrotic detritus masses was the lowest among all the examined groups ($p<0.05$). By day 14 there was granulation tissue in the area of photodynamic exposure and operative intervention, and by day 21 – cicatrix formed by the mature fibrous tissue without any signs of tumor growth (Fig. 8).

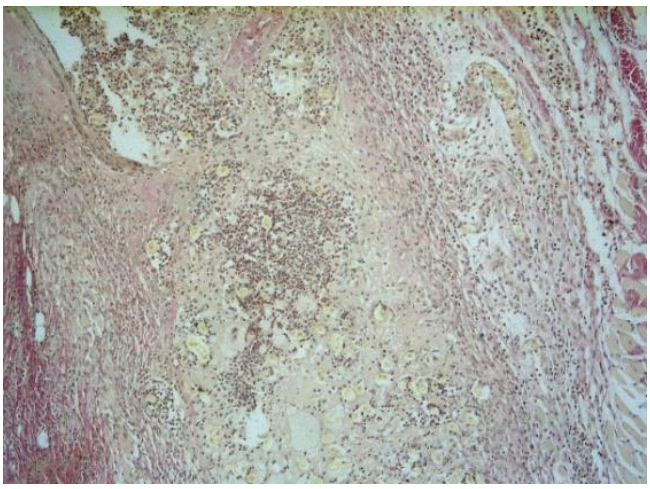


Fig. 8
Formation of mature collagenous picrofuchsinophilic fibers.
Van Gieson's picrofuchsin staining. Magnified x 100.

On day 7 Vn/i coefficient was not significant different from the values in the control group and groups I and II, but was significantly lower than in groups III and IV ($p<0.05$) (Table 1).

Groups	Days after treatment		
	7	14	21
Group I	0.42±0.06º	0.3±0.06	0.31±0.05
Group II	0.59±0.09	0.53±0.1º¹	0.4±0.07
Group III	1.64±0.29º¹²	0.29±0.1	0.62±0.18
Group IV	1.61±0.33º¹²	0.8±0.11º¹³	0.71±0.09º¹²
Group V	0.53±0.14³*		

Note: º p≤0.05 in comparison with the control group, ¹ p≤0.05 in comparison with group I, ² p≤0.05 in comparison with group II, ³ p≤0.05 in comparison with group III, * - in comparison with group IV.

Table 1
Vn/i coefficient dynamics in the experimental groups

Data presented in Table 1 show that maximum Vn/i coefficient values in groups III, IV were on day 7. By day 14 after PDT there was rejection and resorption of most necrotic masses, in contrast with animals that had received radiation therapy (Fig. 9, 10). Thus, tumor tissue in mice group IV had large necrotic areas due to higher ratio of parenchyma necrosis on days 14 and 21 (Table 2).

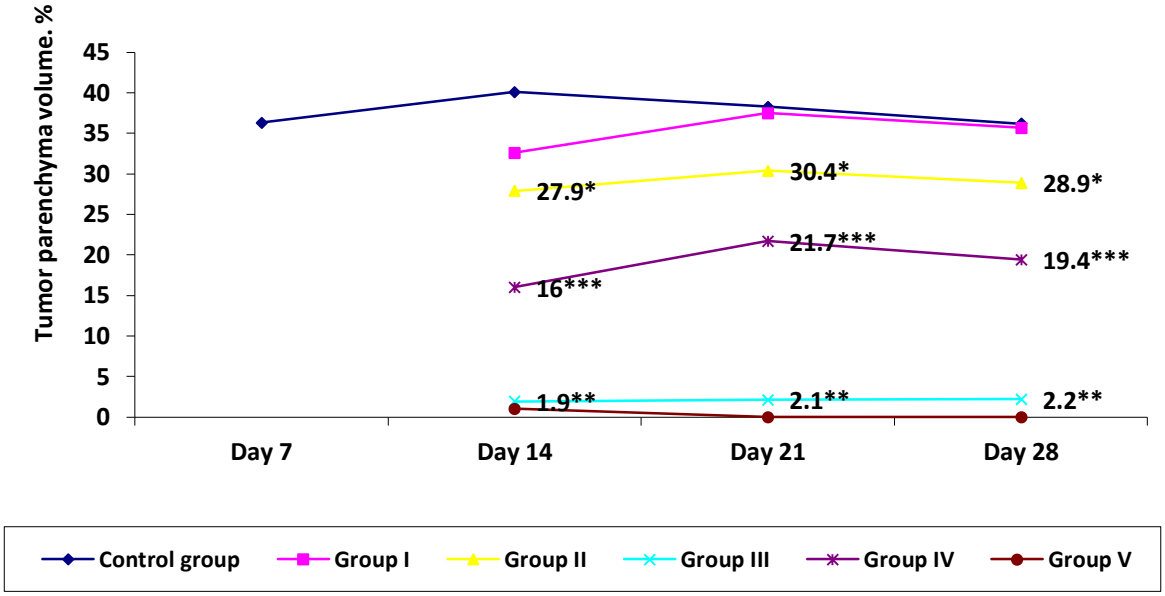
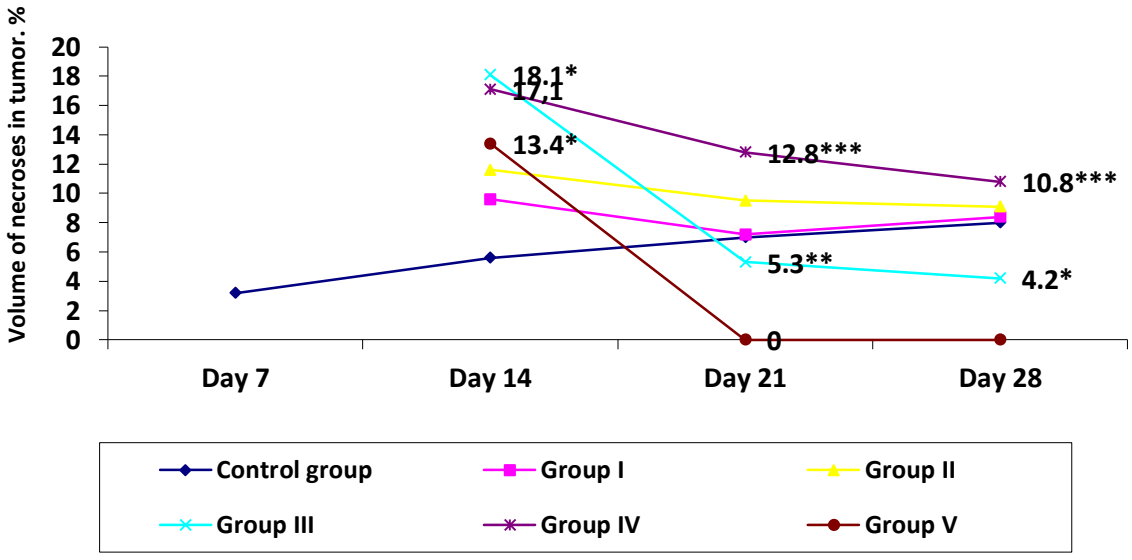


Fig. 9
Relative parenchyma volume in the control and experimental groups.

Note: * - p<0.05 in comparison with the control group and group I; ** - p<0.05 in comparison with the control group and groups I, II; *** p<0.05 in comparison with the control group and groups I, II, III.



Note: * - p<0.05 in comparison with the control group and groups I, II; ** - p<0.05 in comparison with group II; *** p<0.05 in comparison with group III.

Fig. 10
Relative volume of necroses in the control and experimental groups.

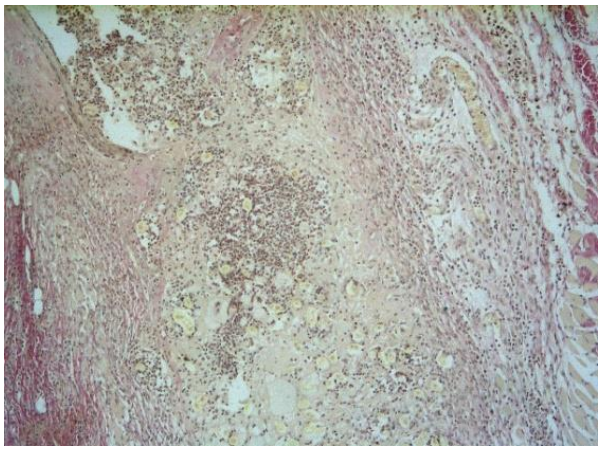


Fig. 11
Formation of mature collagenous picrofuchsinophilic fibers. Van Gieson's picrofuchsin staining. Magnified x 100.

Groups	Days after treatment		
	7	14	21
Group I	0.42±0.06^{o}	0.3±0.06	0.31±0.05
Group II	0.59±0.09	0.53±0.1^{o1}	0.4±0.07
Group III	1.64±0.29^{o12}	0.29±0.1	0.62±0.18
Group IV	1.61±0.33^{o12}	0.8±0.11^{o13}	0.71±0.09^{o12}
Group V	0.53±0.14^{3*}		

Note: p≤0.05 in comparison with the control group, ¹ p≤0.05 in comparison with group I, ² p≤0.05 in comparison with group II, ³ p≤0.05 in comparison with group III, * - in comparison with group IV.

Table 2
Vn/i coefficient dynamics in the experimental groups

Immunomorphological study performed in the intact tumor on day 7 after transplantation revealed a developed capillary network (Fig. 12).

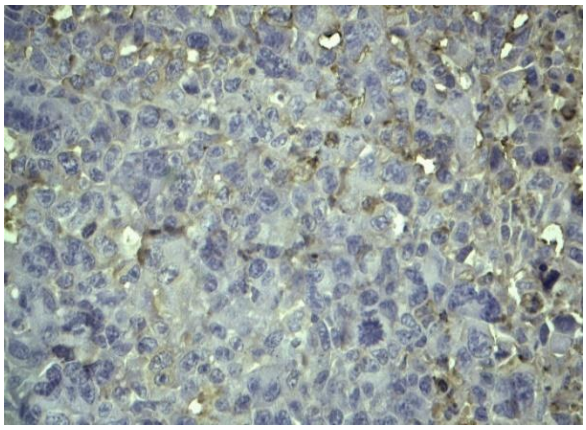


Fig. 12
Developed bloodstream in the intact Ehrlich carcinoma on day 7. Expression of endothelial cell CD34. Immunohistochemical method. Magnified x 400.

Tumor bloodstream volume in the control group reached its maximum values on day 14 after tumor transplantation. As experiment continued the area of vascularization was decreasing while the tumor was growing ($p>0.05$). After PDT in group I the relative volume of tumor bloodstream was decreasing in comparison with the intact carcinoma just during the first week of exposure. More expressed changes after PDT in group II were observed as areas of dilated and empty blood vessels with impaired innervation. At the same time, the values of vessel area in groups I and II did not differ significantly from the control group values. This fact is associated with the transient vasoconstriction and release of vasodilating mediators. The area of vascularization and the relative volume of vessels significantly decreased at PDT dose increase up to 400 J/cm² - in groups III and IV (Table 3).

Groups	Days after treatment					
	7		14		21	
	Relative volume of vessels, %	Vessel area, μm²	Relative volume of vessels, %	Vessel area, μm²	Relative volume of vessels, %	Vessel area, μm²
I	12.2±0.87 º	8674.9±1439.06	13.4±0.84	8951.9±1015.5	12.5±0.77	8793.4±1390.31
II	10.2±0.77 º	8251.0±1892.75	10.3±0.8º¹	8430.8±1269.5	9.3±0.76º¹	7842.0±932.0
V	0.6±0.13º¹²	456.5±70.54º¹²	0.7±0.14º¹²	538.3±67.99º¹²	0.7±0.13º¹²	376.2±75.44º¹²
	0.3±0.09º¹²³	154.6±116.58º¹²³				

Note: º $p\leq0.05$ in comparison with the control group, ¹ $p\leq0.05$ in comparison with group I, ² $p\leq0.05$ in comparison with group II, ³ $p\leq0.05$ in comparison with group III.

Table 3

Bloodstream relative volume and area in the experimental groups

At the same time, the development of typical extensive vascular disorders, such as thromboses, fibrinoid necrosis and vasculitis, formation of thrombotic masses with vessel obliteration was observed in the bloodstream (Fig.13). At the final stage of experiment, tumor endothelial cell proliferation and neovascularization were not revealed in group III animals.

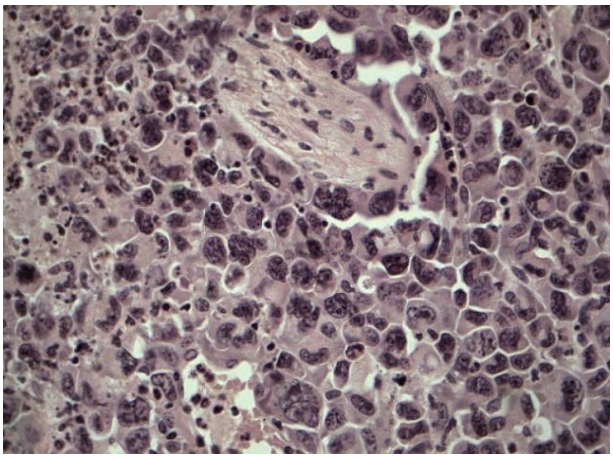


Fig. 13

Thrombus formation in a vessel, in tumor tissue – congestion and stases after PDT (400 J/cm²). Hematoxylin-eosin staining. Magnified x 400.

By day 14 granulation tissue formed in group IV in the area of exposure, which evolved into dense connective tissue by day 21. In the control group the maximum VEGF expression was observed on day 14 after tumor transplantation. VEGF was expressed mainly by grouped poorly differentiated cells of loose connective tissue.

In mice groups I and II, the number of VEGF-positive cells in tumor significantly decreased on days 14 and 21 after PDT, but no significant differences with the control group were found.

The most prominent decrease of VEGF expression was observed in groups III and IV; on day 7 only poorly expressed VEGF reaction in comparison with the intact group and groups I and II ($p<0.05$) were registered. Besides, VEGF expression was observed in the endothelial cells of the tumor vessels, and there was no positive immunohistochemical reaction in the tumor epithelium (Fig. 14).

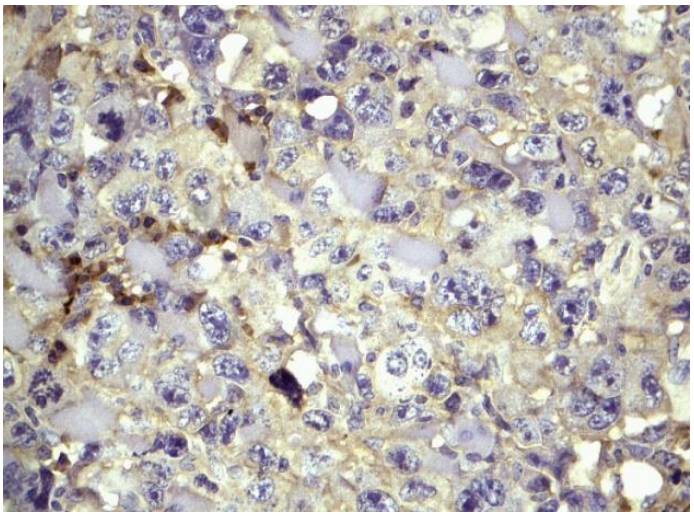


Fig. 14
Poor VEGF reaction in tumor endothelium on day 7 after PDT (400 J/cm²).
Immunohistochemical method. Magnified x 400.

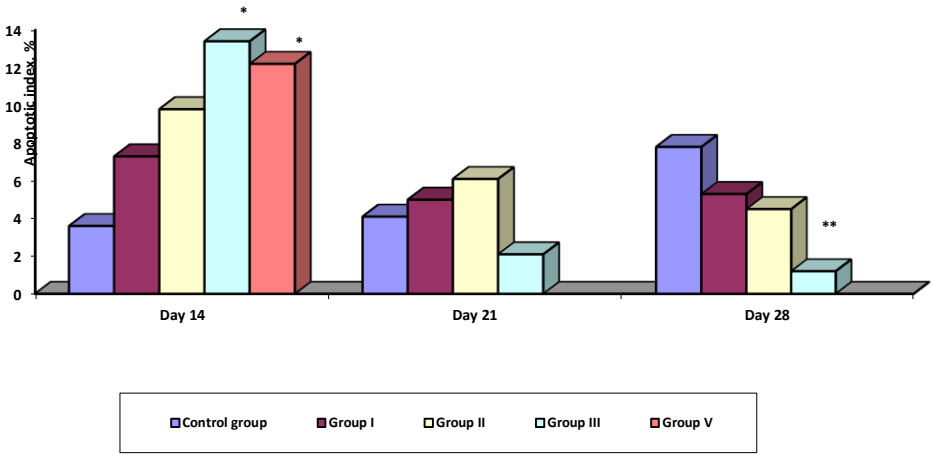
Mainly negative reaction for VEGF in the remaining tumor cells was observed on days 14 and 21 after PDT ($p<0.05$). Thus, the performed study shows that disturbed blood flow in tumor at PDT is one of the most profound effects of its photodynamic destruction.

Parameters of cell kinetics were studied on days 7, 14, and 21 after the treatment (days 14, 21, and 28 after tumor transplantation). Proliferative activity was assessed using Ki-67 index («DAKO», MIB-1, 1:100). The level of apoptosis inhibition was determined based on the expression of anti-apoptotic factor – bcl-2 oncoprotein («Cell margue», E17, 1:50). Expression of apoptosis was revealed with caspase-3, marker of the effector phase – Caspase 32 («Novo castra», JHM 62, 1:85). Having counted the cells with positive reaction of the stated antigens, apoptotic index (AI), apoptosis inhibition index (AII), and proliferation index (PI) were calculated by the formula:

$$\text{AI (AII, PI)} = \frac{\text{number of stained nuclei (cells)}}{\text{total number of cells}} \times 100\%$$

Immunohistochemical examination was conducted using streptavidin-biotin-peroxidase method on paraffin sections following the standard routine. It was found that AI

of intact Ehrlich tumor tended to increase as tumor mass and volume were growing (p>0.05). On day 7 AI in all the experimental groups was higher than in the control group, and statistical differences were not revealed only in comparison with group I (Fig. 15).



Note. Hereinafter: * - p<0.05 in comparison with the control group and group I; ** - p<0.05 in comparison with the control group and groups I and II

Fig. 15
Apoptotic index of tumor cells in the control and experimental groups

On day 7 the maximum AI of tumor was revealed in III and IV group animals (p<0.05 in comparison with group I). Morphological pattern was seen as massive cell apoptosis, and the remaining tumor cells were in a state of dystrophy and necrobiosis (Fig. 16). On days 14 and 21, the level of apoptosis in groups I-III decreased; in group III significant decrease of AI was observed.

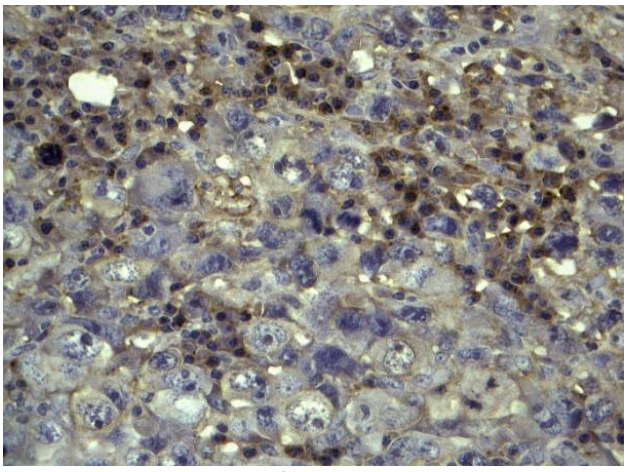


Fig. 16
Prominent expression of CPP32 in tumor cells.
Immunohistochemical method. Magnified x 400.

Index of bcl-2 expression in the control group reached its maximum on day 21 (p<0.05). This fact indicated that the pool of cells containing anti-apoptotic protein grew as experiment continued. On day 7, the AII was significantly lower in groups II, III, and IV than in the control group. Among the experimental groups the AII was the lowest in group IV – 0.7±0.14% (p<0.05 in comparison with groups I and III).

On days 14 and 21, the AII significantly differed in group III from the values of the intact group and groups I and II (Fig. 17).

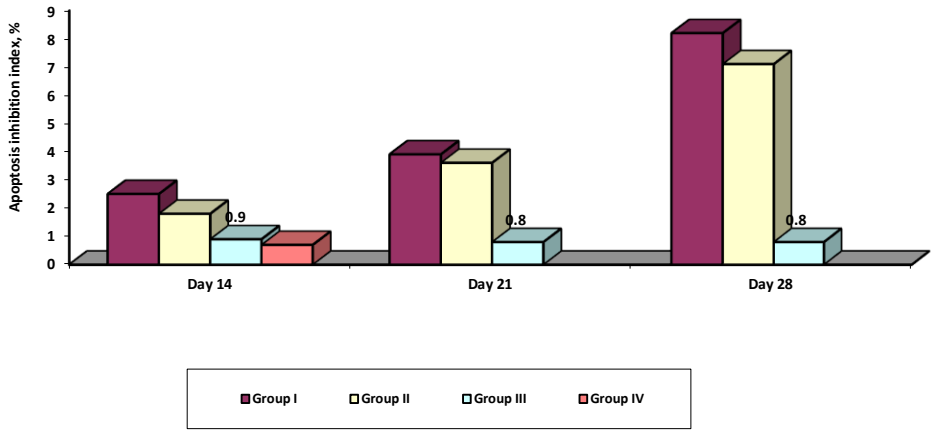


Fig. 17
Tumor cell apoptosis inhibition index in the control and experimental groups.

Consequently, the number of bcl-2-positive cells in progressing tumor still tended to grow when non-lethal volume of tumor cells were affected by PDT (in a dose of 100 and 200 J/cm²). Light radiation dose increase up to 400 J/cm² resulted in prominent suppression of bcl-2 protein in tumor tissue.

At the final stage of experiment the PI of tumor cells in the intact animals decreased almost by half – 6.0±1.33% ($p<0.05$). On day 7 the PI values in the control group and groups I and II were considerably higher than in groups III and IV ($p<0.05$). The lowest proliferative activity of cells was observed in group IV – 2.3±0.33% ($p<0.05$) (Fig. 18).

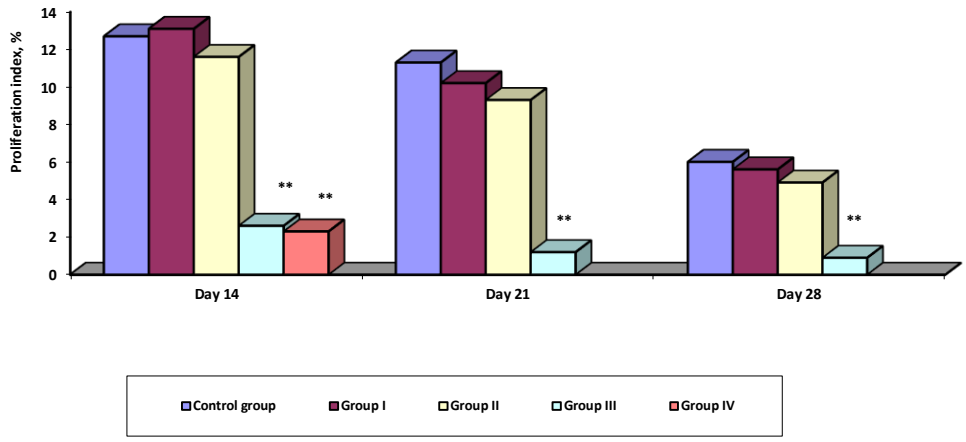


Fig. 18
Tumor cell proliferation index in the control and experimental groups.

At the later stages the tendency toward PI decrease was statistically significant only in group III ($p<0.05$ in comparison with the control group and groups I and II).

Conclusion

The obtained data show that after PDT the speed of reparative processes in the area of exposure is higher than after radiation therapy. This is mainly associated with the immunologic mechanism of PDT effect when inflammatory response conduces to the activation of local antitumor immunity. Besides, accelerated healing is associated with the stimulating effect of PDT on local collagen synthesis and with the absence of destructive effect on the collagenous fibers in the area of exposure. Circulatory disorders in tumor after PDT lead to consequent disorders of blood rheology (thrombosis, vasculitis, vessel obliteration), and also to transient changes in vessel filling (angiospasm, angioparesis). The expressed decrease of tumor vessel perfusion - decrease in bloodstream volume and area – is associated with the irreversible damage of blood rheology by PDT in a dose of 400 J/cm^2. PDT leads not only to typical circulatory disturbance, but also suppresses growth factor expression of tumor angiogenesis.

The prominent suppression of cell proliferation rate also resulted from laser PDT in a dose of 400 J/cm^2. Thus, the research confirms the data by other authors showing that exposure to second generation photosensitizers activates caspase-3-dependent apoptosis in tumor tissue[10]. The study of this protein expression has shown that apoptotic index significantly increases during the first week after laser PDT session (in a dose of 200 and 400 J/cm^2). Moreover, laser PDT dose increase results in the lower expression of blc-2 oncoprotein and tumor cell proliferative activity at all stages of the experiment[11].

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