ORIGINAL ARTICLES

THE EFFECT OF PHOTODYNAMIC THERAPY ON THE WOUND PROCESS DYNAMICS IN PATIENTS WITH PURULENT HAND DISEASES

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Abstract

Despite the progress in modern surgery, the number of patients with purulent finger and hand diseases keeps growing these days in the clinical practice of surgeons. In recent years, there has been a tendency to develop more severe forms of panaritium and phlegmon in an increasingly young contingent of patients. Increasingly, doctors refuse to use the classical method of managing a postoperative wound of the hand involving the installation of drainage tubes. This phenomenon is polygenic and calls for special attention due to the frequent deplorable consequences of a treatment failure. The high urgency of this issue in Moscow Hospital No. 4 has become a rationale to study the effect of photodynamic therapy (PDT) on the course of the wound process in patients with this pathology.

The purpose of this work is to develop a technique to advance the treatment outcomes for patients with purulent finger and hand diseases in case of open postoperative wound treatment.

This study includes a comparative analysis of the wound process dynamics in 49 (49.5%) patients who underwent a photodynamic therapy session in the postoperative period and in 50 (50.5%) patients who received an open wound treatment after the operation. Photodynamic therapy was performed on the second postsurgical day by a laser apparatus "Atkus-2" (wave length 661 nm) with a gel form of the chlorin-series photosensitizer photoditazin in the form of an application at the rate of 1 g ml per 3–5 cm^2 of the wound surface. The power density was chosen in the range of 0.1–1 W / cm^2, and the time of exposure to the wound varied from 30 to 400 seconds, depending on the area of the wound.

To assess the dynamics of the wound process in the postoperative period, we took measurements of all the patients' wound areas on the 1st and 5th days, monitored the gross impression daily. In the patients who received PDT, we observed an earlier wound cleansing and remitting of the inflammatory process, acceleration of the edge epithelization, and earlier appearance of the granulation tissue by an average of 2 days. In the group of patients who were treated with PDT in the postoperative period, on the 5th day, the wound defect decreased by an average of 1 cm^2, which amounted to 22.4%, in the control group – by 18%. The analysis of cytological and morphological patterns also revealed an accelerated switch from the inflammatory stage of the wound process to the reparative one - the reparative processes in the PDT group began earlier by 2 days. The microbiological analysis of wound exudate showed a downregulation of microflora after a PDT session - only in 6 cases pathogens were identified in the repeated seeding, which amounted to 12.24% of the group compared to 38% of the control group. After a session of photodynamic therapy, patients noted a significant reduction in pain, including during dressings. The pain syndrome immediately after the session decreased by 2–3 points. By the 5th day it became moderate – 4–5 points. In the control group, this indicator on the 2nd day was 8 points, decreasing by the 5th day to 6 points. For all analyzed indicators, the groups had statistically significant differences ($p < 0.001$).

Acceleration of postoperative wounds healing enabled to shorten the inpatient stay by 6 days and bring a vast improvement to the treatment quality for this group of patients, which allows considering photodynamic therapy as a high potential method for postoperative treatment of purulent finger and hand diseases.

Keywords: photodynamic therapy, purulent diseases of the hand, panaritium, phlegmon, wound process, photosensitiser, necroectomy.

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ВЛИЯНИЕ ФОТОДИНАМИЧЕСКОЙ ТЕРАПИИ НА ДИНАМИКУ РАНЕВОГО ПРОЦЕССА У ПАЦИЕНТОВ С ГНОЙНЫМИ ЗАБОЛЕВАНИЯМИ ПАЛЬЦЕВ И КИСТИ

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Резюме

Несмотря на высокий уровень развития современной хирургии, в клинической практике современных хирургов продолжает расти количество пациентов с гнойными заболеваниями пальцев и кисти. В последние годы отмечается тенденция к развитию более тяжелых форм панарициев и флегмон у всё более молодого контингента больных. Все чаще врачи отказываются от использования классического метода ведения послеоперационной раны кисти с установкой дренажных трубок. Это явление полиэтиологично и требует особого внимания в связи с нередкими печальными последствиями неудачного лечениях. Высокая актуальность данной проблемы в ГБУЗ ГКБ № 4 г. Москвы стала основанием для проведения исследования влияния фотодинамической терапии (ФДТ) на течение раневого процесса у пациентов с данной патологией.

Целью данной работы является разработка методики с использованием ФДТ для улучшения результатов лечения пациентов с гнойными заболеваниями кисти при открытом ведении послеоперационных ран.

В данном исследовании проведен сравнительный анализ динамики раневого процесса у 99 больных, из них 49 (49,5%) пациентам в послеоперационном периоде выполняли курс ФДТ, 50 (50,5%) послеоперационную рану вели открытым способом. ФДТ выполняли на 2–е сутки после операции. Для ФДТ использовали гелевую форму фотодитазина (фотосенсибилизатор хлоринового ряда) в виде аппликации из расчета 1 мл геля на 3–5 $см^2$ раневой поверхности. Сеанс облучения проводили с использованием лазерного аппарата «Аткус–2» (длина волны 661 нм). Плотность мощности составляла 0,1–1 $Вт/см^2$, время воздействия на рану варьировали от 30 до 400 сек в зависимости от площади раны.

Для оценки динамики раневого процесса в послеоперационном периоде всем пациентам выполняли измерение площади раневого дефекта на 1–е и 5–е сутки, ежедневный контроль макроскопической картины. У пациентов после выполнения ФДТ отмечено более раннее очищение раны и купирование воспалительного процесса, ускорение краевой эпителизации и более раннее появление грануляционной ткани в среднем на двое суток в сравнении с контрольной группой. В этой группе на 5–е сутки площадь раневого дефекта уменьшилась в среднем на 22,4% (1 $см^2$), в контрольной группе – на 18%. При анализе цитологической и морфологической картин выявлено ускорение перехода от воспалительной стадии раневого процесса к репаративной: репаративные процессы в группе с ФДТ начинались раньше в среднем на двое суток, в сравнении с контрольной группой. При микробиологическом анализе раневого экссудата отмечалось снижение количества микрофлоры после курса ФДТ, лишь в 6 случаях (12,2%) в повторном посеве выявлены возбудители, в контрольной группе данный показатель составил 38%. После курса ФДТ больные отмечали существенное снижение болевого синдрома, в том числе и при перевязках. Болевой синдром сразу после курса ФДТ снижался на 2–3 балла, к 5–м суткам становился умеренным: 4–5 балла. В контрольной группе данный показатель на 2–е сутки составлял 8 баллов, снижаясь к 5–м суткам до 6 баллов. По всем анализируемым показателям группы имели статистически значимые различия ($p<0,001$).

Ускорение заживления послеоперационных ран позволило сократить сроки стационарного пребывания на 6 суток и существенно улучшить качество лечения данной группы пациентов, что позволяет считать ФДТ высокоперспективным методом послеоперационного лечения гнойных заболеваний пальцев и кисти.

Ключевые слова: фотодинамическая терапия, гнойные заболевания кисти, панариций, флегмона, раневой процесс, фотосенсибилизатор, некрэктомия.

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Introduction

Over the past decades, certain successes have been achieved in developing an integrated approach to the treatment of purulent pathology of the hand [1]. However, many doctors note a tendency to increase the number of deep forms of panaritium and severe forms of phlegmon, rapid progression of inflammation of the superficial forms of panaritium, which is associated with a decrease in the immune system resistance to infections, insufficient level of medical care at the outpatient stage, the neglect of microtrauma by patients, a tendency to self-medication, and the appearance of antibiotic-resistant strains of microorganisms. At the same time, the contingent of patients has become significantly younger [2].

Purulent pathology of the hand requires special attention due to the fact that unsuccessful treatment frequently has sad consequences, and surgery does not prevent disability [2, 3, 4].

Currently, the main method of treating this pathology is surgery. The application of primary sutures after the most radical necroectomy and drainage is the best option for completing the surgical intervention. However, despite the progress in modern pharmacology and the emergence of new drugs with a wide range of antibacterial effects, many surgeons still choose open wound management in many cases. The time of wound healing with this tactic is significantly longer than with the installation of drainage and irrigation systems. In addition, bandages with this method are very painful, and the probability of secondary infection of the postsurgical wound with the development of mixed infections with nosocomial flora increases significantly [5, 6]. Often, the cause of open wound management can also be an extensive tissue defect after necroectomy and the impossibility to simultaneously reduce the edges of the wound without significant tension. Quite often, there are cases of widespread diffuse purulent lesions, in which it is impossible to perform a single-stage radical necroectomy. Pronounced tissue edema, extensive skin damage up to necrosis of all its layers require the use of open wound management. The use of open wound management makes it possible to achieve good results, but requires a balanced and energetic approach of the attending physician.

Doctors increasingly use photodynamic therapy (PDT) in the treatment of purulent diseases [7, 8]. The evolutionary development of this method of treatment, a large selection of photosensitizers (PS) and laser devices [9], the absence of severe adverse reactions makes PDT one of the advanced methods for the treatment of purulent wounds. A good anti-inflammatory effect, bacteriostatic effect, and a positive effect on the course of the wound process [10-12] gives grounds for studying the use of PDT in purulent hand surgery as well.

In the literature sources available for study, there is no data on the use of PDT in the treatment of purulent pathology of the hand, which was the reason for this study.

Materials and methods

From June 2018 to March 2020, the results of treatment of 99 patients aged from 18 to 90 with purulent diseases of fingers and hand were studied at the Purulent Surgery and Clinical Diagnostics Department of the State Medical Institution City Clinical Hospital No. 4 of the Healthcare Department of Moscow.

Depending on the method of wound management, the patients were divided into 2 groups: in the control group (n=50), patients underwent classic surgical treatment followed by open wound management; patients of the study group (n=49) did not have their wound sutured either, but on the 2nd day after surgical treatment, a course of PDT was administered.

All patients were given a detailed written (in the form of patient brochure) and oral explanation about the method used, after which they were offered to sign a voluntary informed consent. The criteria for inclusion in the trial were the patient's written voluntary consent, age over 18, the presence of purulent hand disease, extensive wound defect after surgery or contraindications to suturing the wound (bitten, crushed wounds), questionable viability of the wound tissues. Patients who refused to participate in the study or refused further observation and hospital stay were excluded from the study.

A random sample was taken from the studied population, with randomization based on random numbers method. The control and study groups were equal in number and composition.

According to the results of the study, men of working professions suffer most often from purulent diseases of the hand (27.3%), there is a high incidence among pensioners (30.3%), while elderly women suffer from purulent diseases of the hand more often than men: 16% vs. 14.1%. This may be explained by the tendency of older women to self-medication and more frequent concomitant diseases that significantly aggravate the course of purulent pathology (polyarthritis, diabetes mellitus); in addition, women of this category do more household chores than men. High numbers of cases among the unemployed (36%) are due to domestic injuries.

We analyzed the causes of purulent diseases of the hand, from which it follows that the most common cause of this pathology is microtrauma (24%), as well as wounds of various etiologies (19%); often patients (13.6%) do not remember or deny the fact of injury.

The most frequently detected pathology in the study was deep forms of panaritium (40.9%), whereas surface forms of panaritium were significantly less common. This is due to the fact that in the absence of adequate therapy, the latter very quickly develop into more severe forms, transforming into deep forms within a few days. In addition, about a third (35%) of patients were hospitalized for a hand phlegmon.

The first stage of treatment in all patients was surgical treatment, performed urgently in the first hours after the patient's admission to the hospital. The volume and nature of the surgical intervention were determined taking into account the prevalence and localization of the purulent focus; they differed depending on the nosological form. Purulent foci were accessed with classic incisions described in traditional methods. If possible, efforts we made to avoid cuts on the working surfaces of the fingers and hand. Incisions should be optimal in localization and size to ensure the necessary wound revision and a comprehensive necroectomy. Only the skin was dissected with a scalpel; all the underlying tissues were pushed apart with hooks and clamps to maximize the integrity of important anatomical structures (neurovascular bundles, tendons). In the presence of long-term non-healing purulent wounds, a gentle excision of the turned-in or callous edges of the wound was performed. After the removal of pus, a radical necroectomy was performed, with a careful treatment of tissues that were inflamed but viable. Then local treatment of postoperative wounds was performed: daily dressings with antiseptic solutions, antibacterial therapy with cephalosporin-type drugs and fluoroquinolones (with medication adjustment according to the results of bacteriological research), infusion, and detoxification therapy. Analgesic therapy was administered if necessary (at the request of the patient) with standard non-steroidal anti-inflammatory drugs. In the presence of concomitant pathology, adequate symptomatic therapy was administered after consultation with corresponding specialists. The purpose of all therapeutic measures was to eliminate the purulent focus and create optimal conditions for the fastest possible healing of purulent wounds with good functional and cosmetic results.

The first dressing with full-fledged wound rehabilitation was performed on day 2 after the operation (on the first day only the upper layers of the bandage were removed, the condition and viability of the skin of the surrounding tissues were evaluated, and the underlying layers of the bandage were left intact for fear of bleeding and severe pain). Subsequently, debridement of wounds was performed daily.

The PDT course involved the use of photoditazine e6 of chlorin type as PS (OOO «Veta-Grand») in the form of a gel in applications at the rate of 1 ml of gel per 3-5 cm^2 of the wound surface. The irradiation was provided with Atkus–2 laser device (ZAO «Poluprovodnikovyie Pribory», St. Petersburg), with the wavelength of 661 nm. The exposure of the wound to drug was according to the manufacturer's instructions: 15-20 minutes in lightless conditions. The irradiation time during external light supply with the use of light guides with a polished end or microlens was calculated according to a standard formula depending on the power density.

$$T\ (sec) = D\ (J/cm^2)/Ps(W/cm^2),\ \text{where}$$

T is the irradiation time,
D is the required light dose (energy density),
Ps is the power density.

The energy density applied to the wound should be on average 30-40 J/cm^2. At an energy density of less than 30 J/cm^2, a weak effect was observed, the wound microflora was not destroyed completely, and at an energy density of more than 40 J/cm^2 and necrotization of healthy wound tissues was observed. The power density of the light emitted by the semiconductor laser was selected in the range of 0.1-1 W/cm^2, the time of exposure to the wound varied from 30 seconds to 10 minutes, depending on the area of the wound. The power density was chosen depending on the size of the light spot. For the convenience of conducting PDT courses, the power density values for the most commonly used laser output power values and light spot sizes were presented in the power density table for different spot sizes and laser power [7].

The PDT course was performed in a dressing ward. The distance from the end of the light guide to the wound surface was 1.5-2 cm (Fig. 1).

A macroscopic assessment of the dynamics of the wound process was carried out on a daily basis, with the recording of the condition of the edges, walls and bottom of the wound (color, number of necrosis areas, fibrinous pellicle), the condition of the surrounding tissues (the degree of hyperemia, edema, infiltration), the nature of the wound discharge (purulent, serous, sanioserous), the amount of exudate (abundant, moderate, scanty), its smell and color, the dynamics of granulation tissue development (timing, color, shine, granularity, bleeding), the dynamics of epithelization at various stages of the wound process.

Рис. 1. Сеанс ФДТ
Fig. 1. Session of PDT

The dynamics of the wound process was assessed in the study based on M. I. Kusin's classification (1977) [14]:

1. Inflammation phase (wound cleansing, vascular changes).
2. Regeneration phase (formation and maturation of granulation tissue).
3. Epithelialization phase.

To assess the healing time of purulent wounds, the area of the wound surface was measured immediately after the surgery and on the day 5 with the determination of the healing acceleration rate.

The area of an irregular wound was calculated by the formula (Khotinyan V. F., 1983) [15]:

$$S = 0.25Lk - C, \text{ where}$$

S is the area of the wound;
L is the perimeter of the wound;
k is the regression coefficient (for wounds close to a square in shape: 1.013; for wounds with irregular contours: 0.62);
C is a constant, equal to 1.29 and 84.34, respectively.

Wound healing V (%) was estimated by the formula:

$$V=(S_1-S_2)/(t^* S_1) *100, \text{ where}$$

S_1 is the area of the wound determined during the previous measurement;
S_2 is the area of the wound at the moment;
t is the number of days between measurements.

During the normal course of healing, the daily decrease in the wound area is 4%.

In both groups, intraoperatively and on day 5, microbiological material was taken for culture seeding to determine the wound microflora. The bacteriological material was collected with a cotton swab and placed in a sterile test tube with a medium, after which it was seeded in culture medium in Petri dishes.

To study the effect of the treatment on the course of the wound process, a cytological study was used by the method of taking smears/prints of the wound surface. During each period of the study, two smear prints were taken from one area of the wound surface. Smears/prints were obtained during dressing after preliminary removal of liquid exudate from the wound surface during the surgery, on days 2 and 4 after the start of treatment. The study took into account the dynamics of cellular elements: unchanged neutrophilic leukocytes, altered neutrophils, immature mononuclear elements, macrophages, young and mature fibroblasts and fibrocytes. The cytogram was calculated with oil immersion method. 400 cells in each case were considered in the study.

Morphological examination of tissues from the purulent wound area in all patients was performed at the beginning of treatment, on the days 3, 5 and 7 days after the start of treatment.

To assess the subjective pain syndrome in the postoperative period, the patient was asked to assess the degree of pain syndrome in points according to the standard visual analog scale [16] daily during dressing.

Statistical processing of the obtained data of our own observations was carried out with Microsoft Office 2017 applications package (Word, Excel). The calculations were performed in MedCalc Statistical Software version 17.0.4 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2017). The work uses the methods of descriptive statistics. The parameters are specified with the use of the median of the standard square deviation. The medians of the minimum and maximum values were used for the nonparametric distribution of the studied indicators.

When comparing the data, an analysis of variance (the Kruskal-Wallis test) was applied, and for repeated changes, an analysis of variance of repeated measurements was used.

The average values were compared with the determination of the measurement error and the reliability of the parameter differences between the studied groups. The significance level (p) is assumed to be less than 0.05.

Results

During irradiation, patients did not notice discomfort or pain; some patients (n = 21) noticed paresthesia of «light tingling» type in the area of exposure to the beam. No hyperthermia and no local inflammatory reaction were observed during the PDT course and after it.

Due to the abundant innervation of the hand area, all patients experienced a high level of pain syndrome at admission, with the average evaluation of 9 (8-10) points. Initially, there were no significant differences in the severity of pain syndrome between the groups (p=0.23). In the traditional treatment group, the severity of the pain syndrome averaged 8 (7-10) points. On day 5, the patients noted that a high level of pain syndrome remained, with average severity level of 6 (4-8) points. On day 9, the severity of the pain syndrome averaged 5 (3-9) points.

In the PDT group, patients also noted severe pain on the day after surgery, but after performing the PDT course, there was a significant decrease in pain syndrome to 6 (4-8) points, including during wound dressing. The pain syndrome became moderate by day 5, at an average of 4.5 (3-7) points, and then decreased to an acceptable level. On day 9, the severity of the pain syndrome was 4 (2-6) points (Fig. 2).

In the group of patients who underwent PDT, the dynamics of the wound process was significantly better: perifocal inflammatory phenomena subsided faster, and the wound defect was cleared of purulent detritus, the appearance of granulation tissue and marginal epithelization began earlier (Table 1).

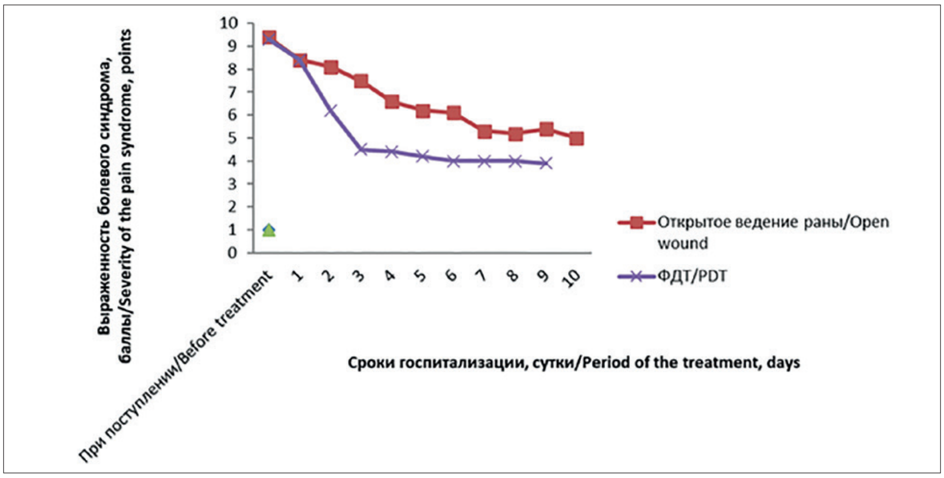

Рис. 2. Динамика выраженности болевого синдрома в группах
Fig. 2. Dynamic of the pain syndrome in groups

Таблица 1
Динамика клинической картины раневого процесса в основной и контрольной группах
Table 1
Dynamic of clinical pattern of the wound process in the control and experimental groups

Группы Group	Стихание перифокального воспаления, сутки Reduction of perifocal inflammation, day	Очищение ран, сутки Purification of wounds, day	Появление грануляций, сутки Appearance of granulations, day	Начало эпителизации, сутки Beginning of epithelialization, day
Без ФДТ Without PDT (n=50)	7 (5–11)	8 (5–12)	8 (4–10)	8 (6–10)
ФДТ PDT (n=49)	5 (2–10)	5 (2–8)	5 (3–12)	6 (3–13)
p	<0,0001	<0,0001	<0,0001	<0,0001

Таблица 2
Изменение размеров ран в основной и контрольной группах
Table 2
Changing the size in groups of the open wounds in the control and experimental groups

Группы Group	Площадь раны интраоперационно, см^2 Wound area after surgery, cm^2	Площадь раны, 5-е сутки, см^2 Wound area 5th day, cm^2	Изменение размера, 5-е сутки, % Changing of the sizes 5th day, %
Без ФДТ Without PDT (n=50)	4,3 (0,9–146,2)	3,4 (0,9–126,9)	18,8 (10,7–22,9)
ФДТ Without PDT (n=49)	4,4 (0,6–134)	3,4 (0,5–105,1)	22,4 (15,0–41,7)
p	0,99	0,77	<0,001

The area of wounds in patients immediately after the surgical stage of treatment ranged from 0.63 cm^2 to 146 cm^2, depending on the nosological form of the disease. In the group with the traditional method of postoperative wound management, the surface area of the wound defect decreased by an average of 0.9 cm^2 over 5 days, amounting to 18.8% of the initial size of the postoperative wound, which indicates a sluggish wound process. In the group of patients who underwent PDT in the postoperative period, on the day 5, the wound defect decreased by an average of 1 cm^2 (22.4%) (Table 2).

The analysis of the results of cytological examination of wound prints allows us to evaluate the nature of the wound process and the effectiveness of the treatment.

The cytological picture in the materials obtained from surgical wounds on the day of surgery was characterized by a pronounced inflammatory reaction standard for the purulent process, the presence of purulent-necrotic exudate in the area of the wound bottom, a large number of dystrophically altered neutrophil leukocytes are found in smears/prints from the wound surface, and the free-lying microflora is observed (Table 3).

In traditional surgical treatment with open management of purulent wounds, the cytological picture is that of a delayed course of the wound process with a prolonged period of purification of wounds from pathogenic microorganisms and foreign particles, with a longer phagocytosis process, the prolonged presence of dystro-

Таблица 3
Динамика цитологических показателей гнойных ран в основной и контрольной группах, %
Table 3
Dynamic of the cytological indicators of purulent wounds in the control and experimental groups, %

Элементы цитограммы Cytogram elements	Интраоперационно во всех группах Upon the surgery in all groups	Группа / Group Без ФДТ / Without PDT		Группа / Group ФДТ / With PDT	
		На 2-е сутки 2nd day	На 4-е сутки 4th day	На 2-е сутки 2nd day	На 4-е сутки 4th day
Нейтрофилы, из них: **Neutrophils incluing:**	97,9±2,1	96,5±2,3	93,2±2,3	93,8±2,2	76,7±2,3
Неизмененные Unchanged	12,5±1,1	20,3±2,3	40,1±4,8	65,3±2,2	56,5±1,1
Дистрофически измененные Dystrophic altered	85,4 ±3,5	76,2±3,	53,1±3,6	28.5±2,2	20,2±2,2
Мононуклеарные фагоциты, из них: **Mononuclear phagocytes including:**	1,9±0,3	2,8±0,3	5,6±0,4	4,5±0,2	13,5±0,4
Моноцитарные Monocytic	1,7±0,1	1,9±0,1	4,0±0,4	3,3±0,2	8,4±0,4
Зрелые макрофаги Mature macrophages	0,2±0,1	0,9±0,1	1,6±0,3	1,2±0,1	4,1±0,2
Фибробласты: Fibroblasts:	Отс. abs.	0,7±0,1	1,2±0,1	1,6±0,1	5,9±0,1
Юные Young	Отс. abs.	0,7±0,1	1,2±0,1	1,3±0,1	4,8±0,1
Зрелые Mature	Отс. abs.	Отс. abs.	Отс. abs.	0,3±0,1	1,1±0,1
Фиброциты Fibrocytes	Отс. abs.	Отс. abs.	Отс. abs.	Отс. abs.	2,0±0,1
Эпителий Epithelium	Отс. abs.	Отс. abs.	Отс. abs.	Отс. abs.	1,9±0,1
Детрит / Detritus*	+++	+++	++	+/-	Отс. abs.
Фибрин Fibrin*	+++	+++	+++	+	+/-
Микрофлора Microflora*	+++	+++	++	+/-	Отс. abs.
Незавершенный фагоцитоз Incomplete phagocytosis*	+++	+++	++	+/-	Отс. abs.

* Степень выраженности гисто-химической реакции
* The rate of the histochemical reaction

phically altered neutrophils, as well as with the preservation of a pronounced amount of both microflora, fibrin and necrotic detritus. A low content of macrophages indicates a sluggish inflammatory process in the exudation stage. The delayed appearance of a small number of cellular elements of the fibroblastic series indicates a delayed onset of the proliferative stage of inflammation.

Changes in the studied cytological patterns after PDT showed an accelerated transition from the inflammatory type cytogram: previously, there was a progressive decrease in the number of neutrophil leukocytes, an increase in the number of monocytic and mature macrophage elements. Fibrous structures of connective tissue appeared earlier, and the number of fibroblasts increased, mainly due to juvenile forms. There were no phenomena of incomplete phagocytosis or free-lying microflora; the amount of fibrin progressively decreased. The cells of the squamous epithelium also appeared faster.

The obtained results of the analysis of the cytological picture indicate a positive effect of PDT on the healing process of postoperative wounds due to the acceleration of the processes of cellular differentiation of the fibroblastic series, and the early onset of epithelialization.

In terms of microbiological picture, monoculture was detected in 79 cases (79.8%) in cultures seeded intraoperatively, whereas associations of pathogens were found in 20 patients (20.2%). Among all pathogenic organisms, Staphylococcus aureus was most

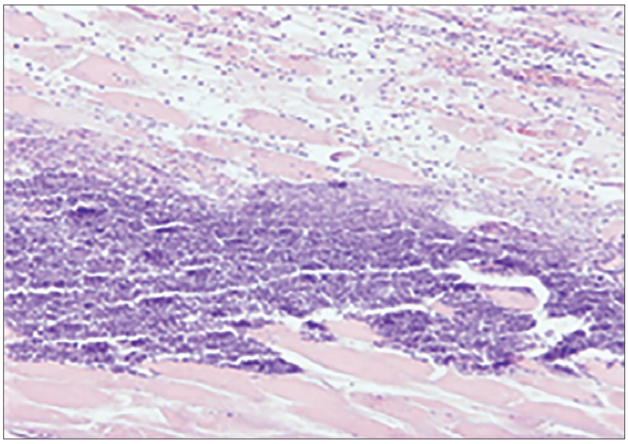

Рис. 3. Гистологическое исследование биоптата из тканей раны во время операции. Нейтрофильная инфильтрация, отек тканей. Некроз мышечных волокон. Окраска гематоксилином и эозином. Увеличение х120.
Fig. 3. Biopsy from wound tissue during surgery. Neutrophilic infiltration, tissue edema. Muscle fiber necrosis. Hematoxylin and eosin staining. Magnification x120.

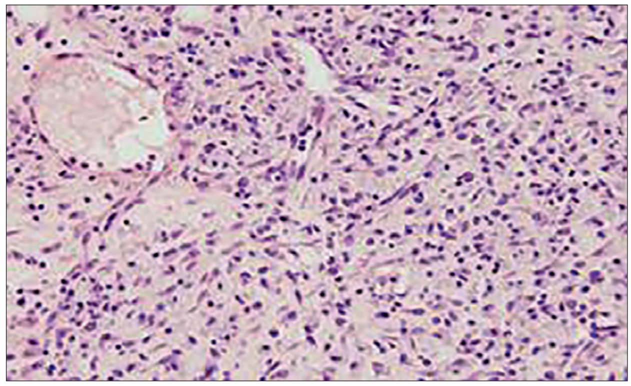

Рис. 4. Гистологическое исследование биоптата из тканей раны пациента контрольной группы на 3-и сутки после операции. Лейкоцитарная инфильтрация (определяются нейтрофилы), полнокровные сосуды, со стазами и плазматическим пропитыванием стенок. Окраска гематоксилином и эозином. Увеличение х200.
Fig. 4. Biopsy from the tissue of the wound of control group on the 3rd day after surgery. Leukocyte infiltration (neutrophils are present), congested vessels, with stasis and plasma impregnation of the walls. Hematoxylin and eosin staining. Magnification x200.

often found in cultures, in 35 cases (35.6%), Escherichia coli was detected in 11 patients (11.1%), epidermal staphylococcus in 7 (7.1%), pyogenic streptococcus in 5 (5.05%) and group B streptococcus (Str. agalacticae) in 6 (6.06%). Much less often (1-2%), Pasteurella, Actinomycetes, Klebsiella, Enterobacter, as well as Aspergillus mold fungi were found in the culture. In 25 cases (25.8%), sterile cultures were obtained, and the analysis of the data revealed that all patients with this result were operated on an outpatient basis or received antibacterial therapy.

In associations, the most common microorganisms were E. coli (2%), *Streptococcus agalactiae* (2%) and *Candida fungus* (2%). The less common ones were Enterobacter (1.52%), *Klebsiella aerogenes* (0.51%), *Streptococcus viridans* (0.51%), and *Proteus* (1%). It is noteworthy that *Klebsiella*, *Proteus* and *Candida* fungi were not detected as a monoculture, forming an association with *Staphylococcus aureus* in all cases.

In the study group, immediately after the PDT course on day 2, new cultures were seeded. In all cases, the results were sterile.

Then, cultures were seeded from patients of both groups on day 5 of inpatient treatment. The results of the analysis of microbiological findings differed significantly depending on the treatment method. In the control group, repeated microbiological examination revealed initial microflora in 19 cases (38%), despite daily dressings with antiseptics and antibacterial therapy received. In the group that underwent PDT, pathogens were detected in 6 cases in repeated cultures, which was 12.24%. Thus, the results of the microbiological study demonstrate a positive effect of PDT on the contamination of the wound surface.

All patients underwent intraoperative histological examination, with the condition similar in both groups: during surgical treatment, pronounced alterative/exudative changes were detected. Necrotically altered tissues with massive infiltration by leukocytes with a polymorphic nuclear structure were found in the bottom of the wound and its walls. The necrosis zone was separated from intact tissues by a torus demarcationis of leukocytes with full-blooded vessels, the permeability of the walls of which was increased; the impregnation with plasma proteins and blood corpuscles was determined, with pronounced stasis and multiple focal perivascular hemorrhages against the background of fibrinoid necrosis of the vascular wall of the microcirculatory bed, which indicated a significant disorder of microcirculation (Fig. 3).

In the group with traditional treatment, on day 3 after surgery, the bottom and edges of the wound are covered with a scab consisting of necrotic tissues and fibrinous exudate. Deeper than this layer is a layer of fibrin fibers with leukocyte infiltration and focal hemorrhages; the neutrophils of this layer are dystrophically altered. The vessels in this zone are characterized by pronounced fullness with microthrombs of various nature, and the phenomena of lymphostasis are determined (Fig. 4).

On day 5, a fibrino-purulent plaque with signs of fragmentation was detected on the wound surface. In various areas of the wound surface, the initial signs of granulation formation appear: chaotically located capillaries with multiple macrophages and rare undirected fibroblasts are formed at various stages of development. Well-expressed perivascular and focal neutrophil infiltrates persist. In the deep layers of the wound edge, the content of neutrophilic leukocytes decreases, and the number of undirected fibroblasts increases, but macrophages prevail in quantity.

On day 7 (Fig. 5) of the postoperative period, there is a decrease in the number of fibrinous overlays and necrotic tissues, the degree of neutrophil infiltration in the surface zone decreases, microthrombs and sludge syndrome phenomena in the vascular lumen are less often detected, the phenomena of tissue edema and perivascular diapedesis hemorrhages disappear. The number of microcirculation disorders decreases. In the perivascular areas, when stained with toluidine blue, rare granule cell with degranulation phenomena are detected. In the area bordering on intact tissues, small foci of granulation tissue appear with small vessels forming, macrophage cells with signs of proliferation, fibroblasts and numerous polymorphonuclear leukocytes.

In patients who underwent PDT, on day 3 after the session (day 5 of the postoperative period), the wound surface is covered with a narrow necrotic scab with fibrinous inclusions, but the thickness of fibrinous deposits is significantly less, there is an intensive cleansing of the surface from necrotic scab elements. Foci of granulation tissue begin to form under the scab, with newly formed capillaries and cellular elements of the macrophage and fibroblastic series. At the border with the underlying healthy tissues, a zone of hemo– and microcirculation disorders is determined, with moderate intravascular fullness and stasis, and an insignificant number of circulatory diapedetic hemorrhages is detected. Edema and neutrophil infiltration are significantly less pronounced, but the number of macrophages and undirected fibroblasts is much higher (Fig. 6).

The functional activity of the macrophage component increases significantly after a course of PDT, which is confirmed by the PAS-positive foamy cytoplasm in cells. The Brachet reaction determines the pyroninophilia of the cytoplasm and nucleoli of fibroblasts and young endotheliocytes, which indicates a pronounced RNA activity (Fig. 7).

By day 5 after PDT (day 7 of the postoperative period), the wound surface is completely cleared of necrotic elements and fibrin, granulation tissue is actively formed in all areas with newly formed vertically oriented capillaries and fibroblasts between them, and pronounced fibrillogenesis (Fig. 8).

The morphological studies conducted indicate that the use of PDT in the treatment of purulent wounds significantly accelerates the wound process and improves the purification of wounds from fibrinous/purulent exudate and scab elements, which is associated with both reparative processes stimulation due to activation the transport of oxygen and nutrients in the forming granulation tissue, and the creation of conditions conducive to earlier and active formation of granulation tissue and faster healing of a purulent wound.

During irradiation, patients did not notice discomfort or pain; some patients (n=21) noticed a slight tingling in the area of exposure to the beam.

The use of PDT made it possible to stop the inflammatory process faster, accelerate the development of the regenerative phase, which was noticed in the shorter duration of treatment. The patients of the control group were in the hospital for an average of 14 (7-29) days, after which they received outpatient medical care for 7 (4-10) days. Wound healing in the group was slowed down by an average of 22 (13-36) days. In the group where patients received PDT, the terms of inpatient treatment averaged 8 (4-21) days, with the outpatient stage averaging 7 (5-9) days; wounds healed completely by day 14 (10-27) ($p<0.0001$).

In the traditional open management of postoperative wounds, repeated amputation in the early postoperative period was performed in 21 patients (42.0%), and amputation was required in 2 patients with pandactylite of the first and second fingers during repeated surgery, 1 patient with anaerobic phlegmon of the hand and forearm had surgery five times. Patients who underwent a course of PDT required repeated surgical treatment in 4 cases (8.2%), while 1 patient with anaerobic phlegmon of the hand and forearm underwent repeated necroectomy 4 times.

There were no complications or allergic reactions during PDT. No hyperthermia and no local inflammatory reaction were observed during the PDT course and after it.

None of the patients who participated in the study applied again with the development of recurrent purulent inflammation. It should be noted that patients from the group with classical treatment more often noted a restriction of the function of the finger or hand due to the development of a dense scar fused with the underlying tissues. Patients whose wounds were exposed to laser radiation, on the contrary, noted the formation of soft scars that did not fuse to the surrounding tissues, did not limit the function and visually seemed neat.

We provide a clinical example illustrating the successful use of PDT.

Patient B., 58 years old, was undergoing inpatient treatment in the department of purulent surgery for pandactylite of the first finger of the left hand.

4 days before admission to the hospital, he was operated on an outpatient basis for subcutaneous panaritium. He receives conservative therapy for type 2 diabetes. Upon admission, the patient's condition was of moderate severity. On examination, a transverse postsurgical wound of a linear shape with abundant purulent discharge was noted. The edges of the wound are severely edematous, with areas of necrosis. A distinct

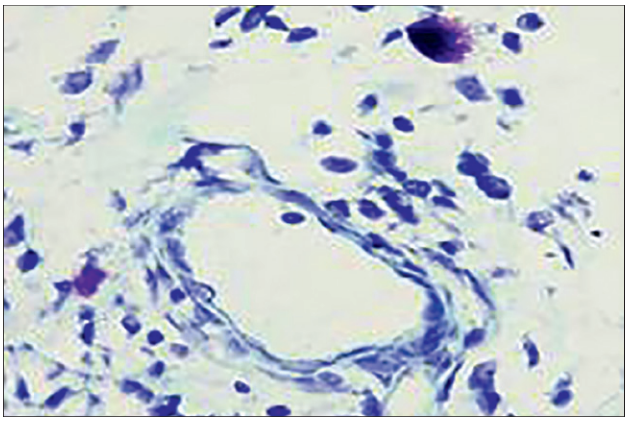

Рис. 5. Гистологическое исследование биоптата из тканей раны пациента контрольной группы на 7–е сутки после операции. Метахроматичные тучные клетки в периваскулярной области. Окраска толуидиновым синим. Увеличение x900.
Fig. 5. Biopsy from the tissue of the wound of control group on 7th day. Metachromatic mast cells in the perivascular region. Toluidine blue staining. Magnification x900.

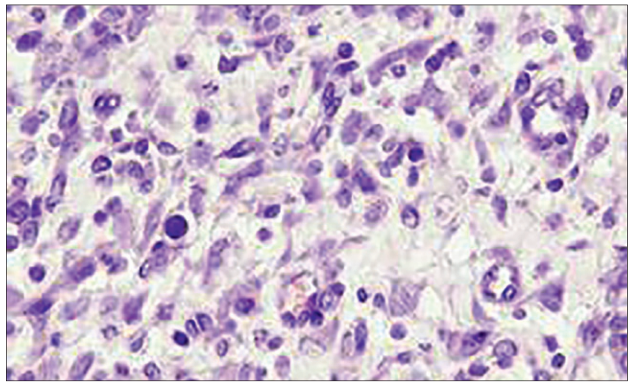

Рис. 6. Гистологическое исследование биоптата из тканей раны пациента исследуемой группы на 5-е сутки послеоперационного периода. Сосудистые элементы с макрофагальными клетками и отдельные неориентированные фибробласты. Окраска гематоксилином и эозином. Увеличение x900.
Fig. 6. Biopsy from the tissue of the wound of experimental group on 5th day of postoperative period. Vascular elements with macrophage cells and some non-oriented fibroblasts. Hematoxylin and eosin staining. Magnification x900.

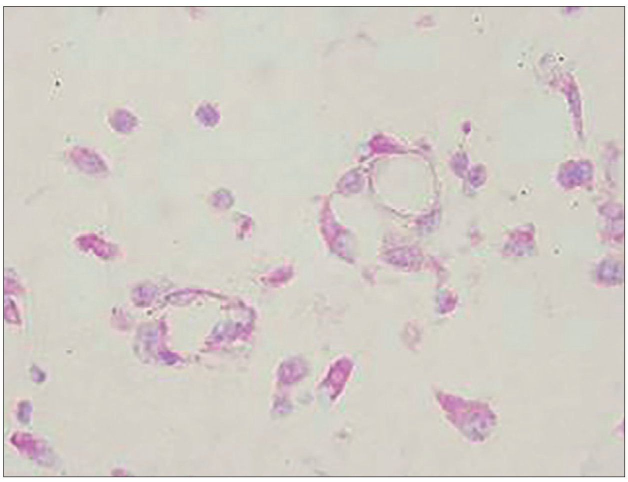

Рис. 7. Гистологическое исследование биоптата из тканей раны после ФДТ на 5-е сутки послеоперационного периода. Пиронинофилия цитоплазмы и ядрышек эндотелиоцитов и фибробластов. Окраска на РНК по Браше. Увеличение x900.
Fig. 7. Biopsy from the tissue of the wound of experimental group on 5th day of postoperative period. Pyroninophilia of the cytoplasm and nucleoli of endotheliocytes and fibroblasts. RNA staining according to Brachet. Magnification x900.

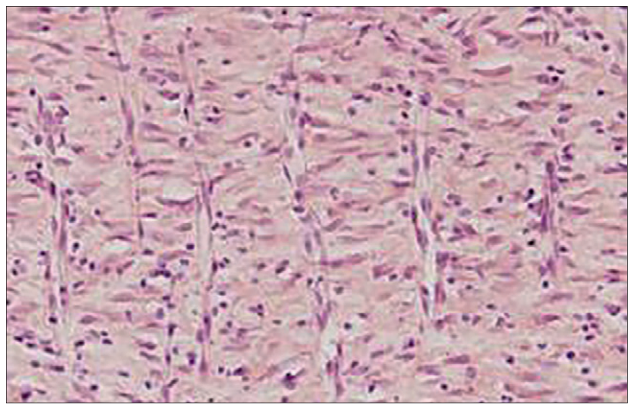

Рис. 8. Гистологическое исследование биоптата из тканей раны на 7–е сутки лечения после операции. Созревание грануляционной ткани с вертикальными сосудами, горизонтально ориентированными фибробластами и выраженным фибриллогенезом. Окраска гематоксилином и эозином. Увеличение x400.
Fig. 8. Biopsy from the tissue of the wound of experimental group on 7th day of treatment after surgery. Maturation of granulation tissue with vertical vessels, horizontally oriented fibroblasts and pronounced fibrillogenesis. Hematoxylin and eosin staining. Magnification x400.

crepitation was detected in the interphalangeal joint. The X-ray showed destruction in the area of the interphalangeal joint. The glucose level at admission was 15.8 mmol/l. Surgical treatment, necrosequestrectomy was performed urgently; due to the pronounced swelling of the wound edges, it was not possible to suture the wound defect. On day 2, examination (Fig. 9) found tissue swelling, pronounced perifocal inflammation, cloudy discharge, and persisting multiple necrosis. A course of PDT with photoditazine was performed (application of 3.5 ml of gel, exposure of 10 minutes, power density of 1 W/cm^2).

In intraoperative culture, *St. pyogenes* was isolated, sensitive to vancomycin, amoxiclav, ciprofloxacin, levofloxacin, erythromycin. On day 2 after the course, a dry black scab was noted in the wound (Fig. 10a), perifocal inflammatory phenomena decreased, the amount of discharge decreased.

Further, the inflammatory phenomena gradually subsided (Fig. 10 b, c). When the new culture was seeded, microflora from the wound was not detected, after

Рис. 9. Пандактилит первого пальца левой кисти на 2–е сутки после операции, экспозиция фотосенсибилизатора.
Fig. 9. Pandactylitis of the 1st finger of the left hand on the 2nd day after surgery, exposure of the photosensitizer.

a b c

Рис. 10. Пандактилит первого пальца левой кисти:
a – на 2–е сутки;
b – на 4–е сутки;
c – на 5–е сутки после ФДТ.
Fig. 10. Pandactylitis of the 1st finger of the left hand:
a –2nd day;
b – 4th day;
c – 5th day after PDT

which secondary stitches were applied to the wound. The patient was discharged on day 9 after surgery, with sutures removed on day 6 after the third surgery. The wound completely healed on day 14. During the control examination: an irregular scar on the lateral surface of the finger without signs of inflammation, soft, not fused to the underlying tissues, pinkish in color. The finger is somewhat shortened after joint resection (Fig. 11), its function is partially limited.

Discussion

This study is part of a previously conducted scientific work [17, 18] aimed at studying the effect of laser radiation on the results of treatment of patients with purulent pathology of the hand.

Currently, there is a wide range of different methods to improve treatment outcomes for this pathology. Foreign scientists widely use open management of postoperative wounds with various auxiliary techniques (plasma

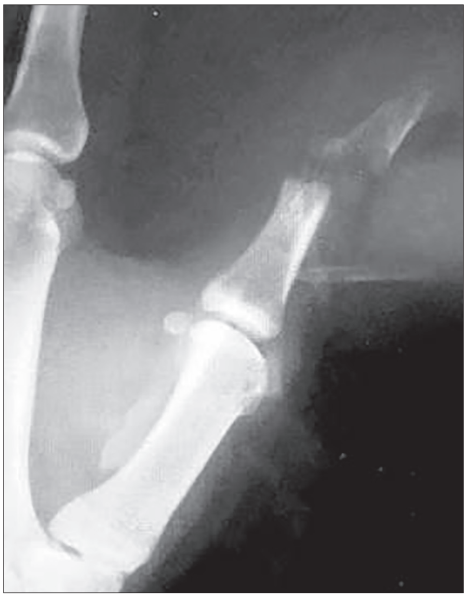

Рис. 11. Рентгенограмма сустава после резекции.
Fig. 11. X-ray of the joint after resection.

therapy, wound management in a liquid environment, cryotherapy, vacuum therapy, etc.). Active use of physical factors in practice, such as ultrasound irradiation, hyperbaric oxygenation, direct current, contributed to improving the effectiveness of treatment of patients with purulent pathology.

Russian and foreign doctors increasingly use plasma flows in the treatment of deep forms of panaritium; the effect of argon plasma intensifies the development of granulation tissue. In recent years, the physical and biostimulating activity in relation to the wounds of the NO-containing plasmodynamic gas flow has been actively studied. One of the new methods used in the treatment of both acute and chronic wounds is the local use of vacuum dressings: the Vacuum–assisted closure (VAC-therapy) method, the principle of ToFig.al negative pressure (TNP). However, the high cost of consumables and the anatomical features of the hand currently create limitations that do not allow for the wide use of this technique in hand surgery.

To date, in the treatment of purulent wounds, an arsenal of technical means is used that have a particular physical effect on the wound, for example, the treatment of wounds with a pulse jet. The technique of using low- and medium-frequency ultrasound in the treatment of purulent wounds has proven itself well. Due to the fact that ultrasound propagates differently in living and devitalized tissues and is reflected at the interface between them, it accelerates the processes of necrotic tissues rejection. At the same time, many authors point to the damaging effect of ultrasound on healthy tissues, which limits its use in hand surgery due to the concentration of important functional structures in a small volume of tissues. Lasers have also achieved great popularity, having become widely used in surgery. The convenience of high-energy lasers, diode laser scalpels in surgical treatment, as well as their high efficiency, allows us to consider their use as a promising technique. Due to the flexibility and elasticity of the light guide and the contact method of diode laser scalpel use, surgery can be performed in hard-to-reach parts of the operated area, which is its significant advantage over other laser systems. In the application of a carbon dioxide laser, a significant factor is its non-contact use and the fact that no consumables are required.

Among the conservative methods in recent years, enzymatic debriming, methods of influencing the wound process with gas flows in the NO therapy mode and low-intensity laser radiation have been widely used to prepare wounds for plastic surgery. However, the use of high-energy lasers and plasma streams in hand surgery requires the development of a technique to exclude damage to delicate anatomical structures that determine the functioning of the entire hand.

All of the above methods require a balanced approach due to the impact on the surrounding tissues, which is critical due to the small volume of tissues in the hand area, and due to the fact that the equipment and consumables used for the treatment are expensive, and the repairs of the equipment are also difficult.

In addition to having a positive effect, the use of PDT is also a relatively safe method. PDT produces a pronounced anti-inflammatory effect, stimulates phagocytosis and accelerates granulation formation due to the absorption of laser energy by tissues; it has a positive effect on microcirculation, which activates the repair process. PDT is characterized by selectivity of exposure, which is due to the absorption of PS by tissues with high proliferative activity, enhanced metabolism and bacterial agents. The specifics of the application of this technique is the absence of pronounced destructive lesions of the wound tissues, the relative painlessness of the procedure, and the possibility of treating deep tissues. The bactericidal effect is limited to the zone of laser irradiation of sensitized tissues, which helps to avoid the side effect observed when using antibiotics and antiseptics for the treatment of surgical infection with local PDT. After a course of PDT, there is a decrease in bleeding during wound dressing.

PDT has a fairly narrow range of contraindications (the presence of severe, non-correctable pathology in patients: hypersensitivity to the drug; severe renal or hepatic failure; decompensated phase cardiovascular diseases; pregnancy and lactation; childhood; the threat of bleeding due to blood clotting disorders), so the treatment can be administered to the absolute majority of patients.

The introduction of PDT into a wide clinical practice will not only improve the results of treatment, but also make it possible to abandon the use of systemic antibacterial drugs and create opportunities for a rapid tranfer of inpatients to outpatient treatment [19, 20].

Conclusions

According to the findings of the study, it can be concluded that PDT has a beneficial effect on the course of the wound process, contributing to the normalization of microcirculation, early cleansing of wounds from detritus, the appearance of granulation tissue and marginal epithelization. In the absence of the possibility of wound suturing, PDT significantly accelerates healing, and, therefore, reduces the duration of hospital stay, which allows us to consider this method highly promising and justified for use in clinical practice.

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ORIGINAL ARTICLES

DIAGNOSTIC CAPABILITIES OF DIFFERENT METHODS OF LASER DOPPLER FLOWMETRY SPECTRAL INDEXES ASSESSMENT IN PATIENTS WITH DIABETIC MICROANGIOPATHY

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Abstract

The article contains the results of a study of two different methods for calculating the spectral parameters of laser Doppler flowmetry in patients with diabetic microangiopathy caused by type 2 diabetes mellitus (main group) and those with excluded diabetes mellitus (control group). Spectral indices were calculated using either average or maximum amplitudes of the frequency ranges. When comparing the contribution of respiratory and pulse fluxmotions using average amplitudes, there were significant ($p < 0.05$) differences between the main and control groups. On the contrary, when using the maximum amplitudes, no significant differences were noted ($p > 0.05$). Also, significant correlations were found between the contributions of respiratory and pulse fluxmotions and the estimated glomerular filtration rate in the main group, using both calculation methods. These studies indicate the feasibility of using a technique based on the analysis of average amplitudes to increase the specificity of laser Doppler flowmetry as a method for diagnosing diabetic microangiopathy.

Key words: laser Doppler flowmetry, diabetes mellitus type 2, diabetic microangiopathy, spectral analysis, assessment of spectral indices.

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ДИАГНОСТИЧЕСКИЕ ВОЗМОЖНОСТИ РАЗЛИЧНЫХ МЕТОДИК ОЦЕНКИ СПЕКТРАЛЬНЫХ ПОКАЗАТЕЛЕЙ ЛАЗЕРНОЙ ДОППЛЕРОВСКОЙ ФЛОУМЕТРИИ У ПАЦИЕНТОВ С ДИАБЕТИЧЕСКОЙ МИКРОАНГИОПАТИЕЙ

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Резюме

В работе представлены результаты исследования двух различных методик расчёта спектральных показателей лазерной допплеровской флоуметрии у пациентов с диабетической микроангиопатией на фоне сахарного диабета 2 типа (основная группа) и лиц с достоверно исключённым сахарным диабетом (контрольная группа). Расчёт спектральных показателей выполнялся с использованием либо средних, либо максимальных амплитуд частотных диапазонов. При сравнении вклада дыхательных и пульсовых флаксмоций с использованием средних амплитуд были получены значимые ($p < 0{,}05$) различия между основной и контрольной группами, тогда как при использовании максимальных амплитуд значимых различий не отмечалось ($p > 0{,}05$). При проведении корреляционного анализа вклада дыхательных и пульсовых флаксмоций и расчётной скорости клубочковой фильтрации в основной группе были выявлены значимые корреляции при использовании обеих расчётных методик. Данные исследования свидетельствуют о целесообразности использования методики, основанной на анализе средних амплитуд, для повышения специфичности лазерной допплеровской флоуметрии как метода диагностики диабетической микроангиопатии.

Ключевые слова: лазерная допплеровская флоуметрия, сахарный диабет 2 типа, диабетическая микроангиопатия, спектральный анализ, расчёт спектральных показателей.

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Introduction

The relevance of the development of diagnostic methods for microvascular complications of diabetes mellitus is beyond any doubt. In 2019, according to the International Diabetes Federation, there were 463 million patients with diabetes in the world aged 20-79 years (9.3% of this age group), and by 2045 the projected incidence will be 700 million [1]. The total number of patients with diabetes in Russia as of 01.01.2019 was 4,584,575 people (3.12% of the population), of which patients with type 2 diabetes accounted for 92.4% (4.24 million). According to NATION national epidemiological study, the proportion of undetected cases of type 2 diabetes in Russia is on average 54% [2]. Cardiovascular diseases, resulting from or aggravated by vascular complications of diabetes, are the main cause of mortality in this group of patients [3, 4].

For the diagnosis of diabetic microangiopathy, intensive research is done into the possibilities of laser Doppler flowmetry (LDF) [5-8]. The wavelet analysis of the amplitude-frequency spectrum of the LDF signal allows us to identify groups of oscillations (harmonics) in certain frequency ranges, the amplitudes of which provide information about the function of local and systemic mechanisms of microcirculation modulation. In particular, with the help of LDF, it was shown that insulin therapy can affect the state of microcirculation even in the short term [9].

The large-scale application of LDF in the diagnosis of microcirculatory disorders is complicated not only by the relatively high cost of equipment, but also by the lack of a unified algorithmic approach to the interpretation of the data obtained. In this regard, there is an urgent need to develop new methods and numerical indices that characterize changes in the parameters of LDF in patients at different stages of diabetic microangiopathy.

The purpose of this work was a comparative analysis of the use of two different methods for calculating the spectral parameters of LDF in patients with diabetic microangiopathy.

Materials and methods

The study was conducted on the basis of St. Petersburg State Medical Institution «City Hospital of St. George the Great Martyr» (St. Petersburg, Russia) among patients receiving treatment on the basis of the Surgical Departments 2 and 4 (the main group) and Cardiology Department 1 (the control group). All patients signed a voluntary informed consent to participate in the study.

The main group included 40 patients. The criteria for inclusion in the main group were: the established diagnosis of type 2 diabetes mellitus, the presence of manifest signs of diabetic microangiopathy (diabetic foot syndrome) and age over 50 years. The following criteria were chosen as non–inclusion criteria: the presence of primary renal diseases in the anamnesis, oncological diseases, systemic connective tissue diseases, administration of nephrotoxic or immunosuppressive drugs, morbid obesity, the presence of varicose vein disease of the lower extremities and post–thrombophlebitic disease, the consequences of acute cerebral circulatory disorders in the form of spastic lower para- and tetraparesis.

For the control group, 30 patients over 50 years of age with reliably excluded diabetes mellitus were selected, in compliance with the above non-inclusion criteria. The summary characteristics of the groups of the surveyed patients are given in the table.

Laser Doppler flowmetry was performed with the diagnostic system «BIOPAC LDF 100C» (Biopac, USA) with a probing radiation wavelength of 830 nm. In each patient, a 10-minute registration of the LDF-gram was performed, in the supine position. The sensor was placed on the skin of the back of the foot in the distal part of the first metatarsal space.

To process the obtained LDF signals, a spectral wavelet analysis based on the Morlet wavelet was used. The following were taken as the boundaries of the corresponding frequency ranges: slow-wave flaxmotion (LF): 0.05–0.2 Hz, respiratory flaxmotion (HF): 0.2–0.4 Hz, pulse flaxmotion (CF): 0.8-1.6 Hz. The calculation of the indicators was based on the methodology proposed by V. I. Kozlov et al. [10].

The contribution of the corresponding frequency range (v: vLF, vHF, vCF) was determined as the percentage ratio of the square of the amplitude of this range (A) to the total power of the spectrum (M), which is the sum of the squares of the amplitudes over 3 ranges.

$$M = A^2LF + A^2HF + A^2CF$$
$$v = A^2 / M \times 100\%$$

Таблица
Характеристика обследованных пациентов
Table
Characteristics of investigated patients

Параметр Parameter	Основная группа Main group	Контрольная группа Control group
Количество обследованных Number of examined	n=40 (20 мужчин, 20 женщин) n=40 (20 male, 20 female)	n=30 (20 мужчин, 10 женщин) n=30 (20 male, 10 female)
Возраст, лет (среднее ± ст. отклонение) Age, years (mean ± st. deviation)	67,6 ± 6,7	65,2 ± 6,3
Продолжительность СД, лет Duration of diabetes, years	> 5	не применимо not applicable
Сахароснижающая терапия Hypoglycemic therapy		
Пероральные препараты Oral medications	n=37	не применимо not applicable
Инсулинотерапия Insulin therapy	n=5	
Форма синдрома диабетической стопы Diabetic foot syndrome form		
Нейропатическая Neuropathic	n=14	не применимо not applicable
Нейроишемическая Neuroischemic	n=26	
Гипертоническая болезнь Essential arterial hypertension	n=40	n=30

The calculation of these spectral indicators was performed with two different methods. In one case, the average amplitudes of the corresponding frequency ranges were used, in the other, the maximum ones.

One of the manifestations of diabetic microangiopathy is diabetic nephropathy, which leads to the development of chronic kidney disease with a progressive decrease in the filtration function of the kidneys. In this regard, it was of interest to evaluate the correlations of the spectral parameters of LDF with the calculated glomerular filtration rate (eGFR). To determine eGFR, the formula CKD–EPI was used, based on the concentration of creatinine in the blood serum.

Statistical processing was performed with the GraphPad Prism 8 software package. The intergroup differences were evaluated with the Mann-Whitney criterion. The Spearman correlation coefficient was used for the correlation analysis. The differences were considered statistically significant at $p < 0.05$.

Results and discussion

In both groups, the spectral parameters of the contribution of slow-wave (vLF), respiratory flaxmotions (vHF) and the contribution of pulse flaxmotions (vCF) were analyzed, obtained with the average or maximum amplitudes of the corresponding harmonic components.

When comparing the contribution of slow-wave flaxmotions in patients of the control and main groups, calculated with average and maximum amplitudes, no significant differences were found in either case ($p>0.05$). There were also no intergroup differences in the flaxmotion index ($p>0.05$), with both calculation methods.

The comparison of the contribution of respiratory flaxmotions with the use of average amplitudes showed that the indicator was significantly higher in the main group ($p<0.05$) (Fig. 1), with no significant differences observed with the use of maximum amplitudes ($p>0.05$) (Fig. 2).

On the contrary, the contribution of pulse flaxmotions with the use of average amplitudes was significantly higher in the control group ($p<0.05$) (Fig. 3), while no differences were detected with the use of maximum amplitudes ($p>0.05$) (Fig. 4).

The correlation analysis revealed significant correlations of the contribution of respiratory and pulse flaxmotions with the estimated glomerular filtration rate in the main group with both calculation methods ($p<0.05$). A negative correlation was observed for the contribution of respiratory flaxmotions (Fig. 5), while a positive correlation was observed for the contribution of pulse flaxmotions (Fig. 6). In the control group, there was no significant correlation between the LDF indicators and the calculated glomerular filtration rate ($p> 0.05$).

The obtained data can be interpreted as follows. According to our previous studies [11], a decrease in the contribution of slow-wave flaxmotion and the flaxmo-

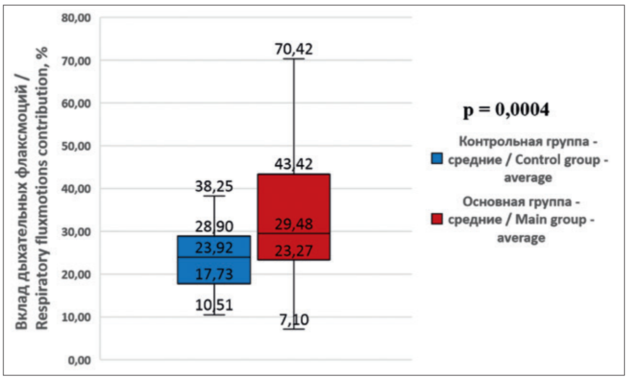

Рис. 1. Вклад дыхательных флаксмоций в основной и контрольной группах при использовании средних амплитуд
Fig. 1. The contribution of respiratory fluxmotions in the main and control groups when using average amplitudes

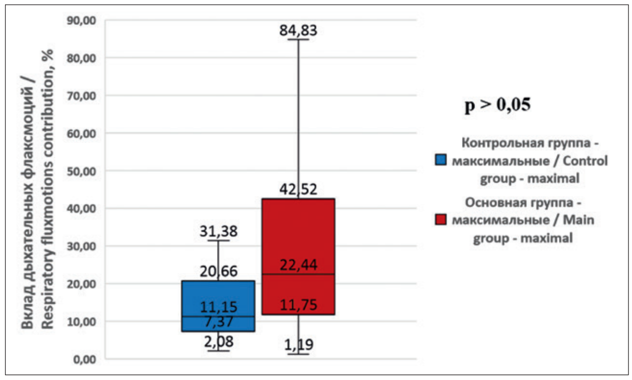

Рис. 2. Вклад дыхательных флаксмоций в основной и контрольной группах при использовании максимальных амплитуд
Fig. 2. The contribution of respiratory fluxmotions in the main and control groups when using maximum amplitudes

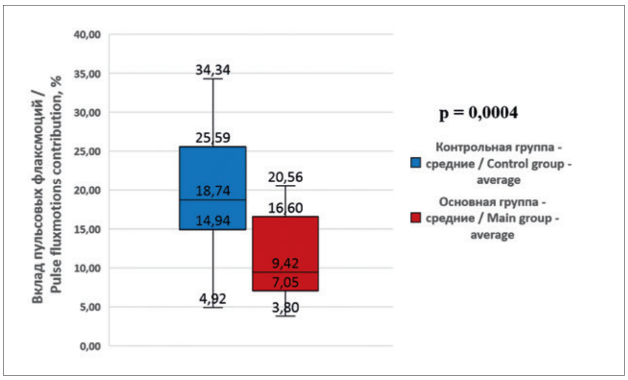

Рис. 3. Вклад пульсовых флаксмоций в основной и контрольной группах при использовании средних амплитуд
Fig. 3. The contribution of pulse fluxmotions in the main and control groups when using average amplitudes

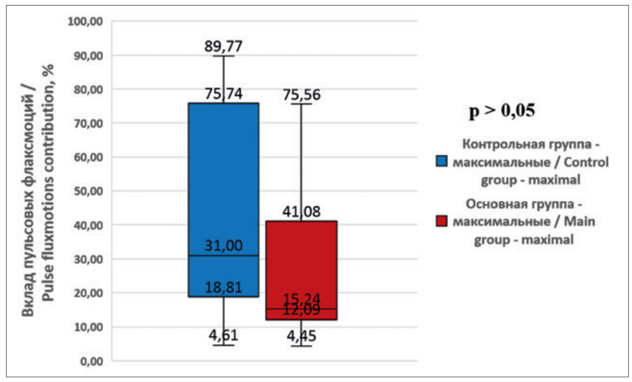

Рис. 4. Вклад пульсовых флаксмоций в основной и контрольной группах при использовании максимальных амплитуд
Fig. 4. The contribution of pulse fluxmotions in the main and control groups when using the maximum amplitudes

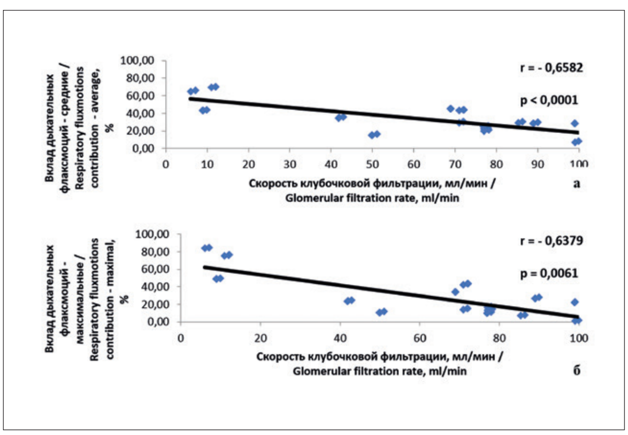

Рис. 5. Корреляции вклада дыхательных флаксмоций с расчётной скоростью клубочковой фильтрации при использовании средних (а) и максимальных (б) амплитуд
Fig. 5. Correlations of the contribution of respiratory fluxmotions with the estimated glomerular filtration rate using the mean (a) and maximum (b) amplitudes

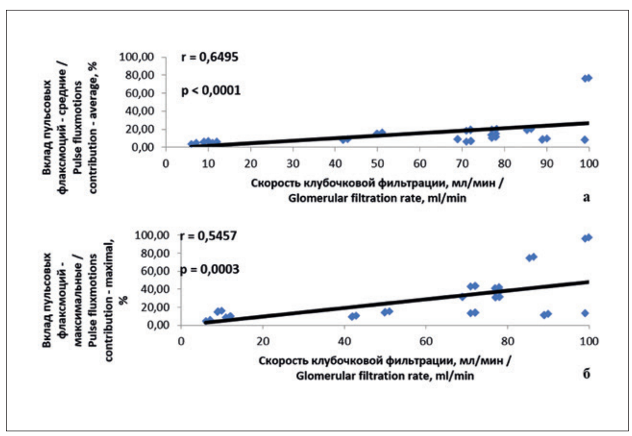

Рис. 6. Корреляции вклада пульсовых флаксмоций с расчётной скоростью клубочковой фильтрации при использовании средних (а) и максимальных (б) амплитуд
Fig. 6. Correlations of the contribution of pulse fluxmotions with the calculated glomerular filtration rate using the mean (a) and maximum (b) amplitudes

tion index was observed in various pathologies of microcirculation, including those not associated with diabetes mellitus. The reason for this lies in the integration of the circulatory, lymphatic and interstitial compartments of the internal environment of the body at the level of the microcirculatory bed. Changes in the microcirculatory blood flow can be caused by pathological processes both at the level of the microvessels themselves and by disorders of arterial inflow or venous outflow [13]. The balance of factors of modulation of microcirculatory blood flow in the case of a decrease in the role of local factors is shifted towards systemic factors, such as the suction effect of the chest and the propulsive activity of the left ventricle.

As it was shown earlier [11], it is the nature of the relationship between the systemic factors of microcirculation modulation, which is displayed by the indicators of the contribution of respiratory and pulse flaxmotions, that is the criterion for differentiating pathogenetic variants of microcirculatory dysfunction.

In the present study, the calculation using the maximum amplitudes of harmonics did not reveal significant differences in microcirculatory dysfunction in patients of the main and control groups, despite the fundamentally different mechanisms of its development. It was possible to differentiate between microcirculation insufficiency caused by diabetic microangiopathy and other causes only when using a calculation algorithm based on average amplitudes.

At the same time, the contribution of respiratory flaxmotions was significantly higher in patients of the main group (with diabetic microangiopathy), whereas in the control group the contribution of pulse flaxmotions was higher. This can be explained by the formation of persistent disorders of venous outflow with stasis phenomena [10, 12]. The reverse situation in patients with essential arterial hypertension and excluded diabetes mellitus may be due to remodeling of the arteriole wall with a decrease in the number of myocytes in the middle layer of the vessel wall (tunica media), a decrease in the compliance of the vascular wall and an increase in its rigidity.

It is worth paying attention to the fact that the patients of the main group also had essential arterial hypertension, and, therefore, the wall of the arterioles in them was also highly likely to undergo remodeling. Moreover, according to available data [13], changes in arterioles caused by diabetic microangiopathy are similar in their morphomechanical properties to those caused by primary arterial hypertension: in both cases, the phenomena of hyalinosis and sclerosis are noted, which often complicates morphological identification of the particular type of damage. Type 2 diabetes mellitus itself is a risk factor for the development of primary arterial hypertension, so in patients of the main group, remodeling of the walls of arterioles may have a dual pathogenesis (due to diabetic microangiopathy and essential arterial hypertension) [4]. From our point of view, the lower values of the contribution of pulse flaxmotions in the main group are due to the fact that the lesion of arterioles becomes somehow less important in connection with the lesion of the venous link, which causes an increase in the contribution of respiratory flaxmotions.

An additional factor in reducing the contribution of pulse flaxmotions in patients with type 2 diabetes is the atherosclerosis-type lesions of large arterial vessels. At the same time, the transmission of the pulse wave to the periphery becomes difficult or completely impossible. This explains the decrease in the contribution of pulse flaxmotions to the total power of the amplitude-frequency spectrum of the LDF signal in these patients when registering LDF in the basin of the above-mentioned arteries.

The revealed correlations in the main group can be explained in the framework of the pathogenesis of diabetic microangiopathy. As it was shown earlier [7, 11], in diabetic microangiopathy, there is a progressive dysfunction of local microcirculation mechanisms caused by a decrease in the number of pacemaker myocytes in the t. media of arterioles, the phenomena of neuropathy and endothelial dysfunction. At the same time, the role of systemic factors of microcirculatory blood flow modulation, such as the pulse wave and the suction effect of the chest, increases in the compensatory manner.

An increase in the contribution of respiratory flaxmotions is associated with an increase in the phenomena of venous stagnation [5]. Venous stasis with subsequent formation of microcirculatory stasis is a natural component of the progression of diabetic microangiopathy [13]. The increase in the contribution of cardiac flaxmotions, apparently, is a consequence of remodeling of the wall of blood vessels, primarily arterioles. It is known that in diabetic microangiopathy, there is a decrease in the number of smooth myocytes in the t. media of arterioles, sclerosis and hyalinosis [13, 14]. As a result, the rigidity of the arteriole wall increases, which makes it difficult to transmit the pulse wave to the periphery and reduces the amplitude of fluctuations in the microcirculatory blood flow under the influence of the pulse wave. Diabetic nephropathy is a particular variant of diabetic microangiopathy and at the same time a factor in its progression due to increased activation of local renin-angiotensin-aldosterone systems [14]. In this regard, it can be assumed that the severity of the above processes: remodeling of the arteriole wall and the progression of venous congestion, will correlate with the estimated glomerular filtration rate as an indicator of the progression of diabetic nephropathy, which is confirmed by the data obtained.

Conclusion

In connection with the obtained results, in our opinion, for the differential diagnosis of diabetic microangiopathy, it is advisable to use a technique that allows to obtain higher values of the contribution of respiratory flaxmotions and lower values of pulse flaxmotions. This requirement is largely met by the method using the average amplitudes of harmonic components. This feature can increase the specificity of laser Doppler flowmetry as a method for diagnosing diabetic microangiopathy.

The revealed correlations are: the negative contribution of respiratory flaxmotions and the positive contribution of pulse flaxmotions, indicate that as chronic kidney disease progress, the changes in the nature of microcirculation modulation described above are increasingly observed. Therefore, the data of laser Doppler flowmetry are consistent with the ideas about the pathogenesis of diabetic microangiopathy. Thus, laser Doppler flowmetry allows us to identify the progressive nature of diabetic microangiopathy.

Based on the above, it can be concluded that laser Doppler flowmetry can be considered as a potential method of dynamic monitoring of microcirculation, in particular, in patients with diabetic microangiopathy. By improving the methods of spectral analysis results processing, it is possible to significantly expand the diagnostic potential of the method and its value for practical medicine.

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

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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 cm³, 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 cm³, 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.

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

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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 см³, 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 см³, 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.

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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);
π/6 = 0.52 is a constant value;
V is the volume of the tumor (in cm³).

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

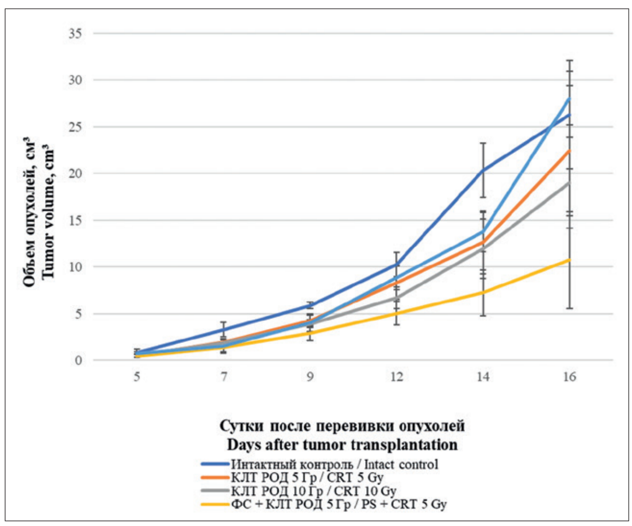

Рис. 1. Динамика роста перевивных опухолей модели ЛСП при исследуемых схемах терапевтического воздействия
Fig. 1. Dynamics of growth of transplanted tumors of the PLS model under the studied therapeutic regimens

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	К 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

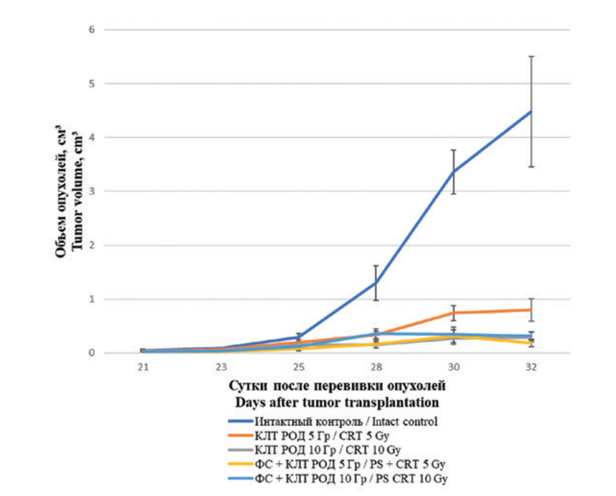

Рис. 2. Динамика роста перевивных опухолей модели РС1 при исследуемых схемах терапевтического воздействия
Fig. 2. Dynamics of growth of transplanted tumors of the PC1 model under the studied therapeutic regimens

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.

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ORIGINAL ARTICLES

PHOTOBIOMODULATION OF ACUTE PAIN SYNDROME AFTER SEPTOPLASTY

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Abstract

The paper evaluates the effectiveness of the use of therapeutic laser exposure (photobiomodulation therapy – PBMT) to minimize acute pain in the early postoperative period in patients after septoplasty. The study included two groups of patients. Patients of the first group (31 patients) underwent septoplasty with standard management in the postoperative period. Patients of the second group (31 patients) also underwent septoplasty, and then added PBMT to the standard measures of the postoperative period at 3, 6 and 24 h after septoplasty (λ = 0.890 µm, P = 10 W, 2 min) and then intranasally 48 h after septoplasty (λ = 0.630 µm, P = 8 W, 2 min). In patients of both groups, heart rate variability and pain were assessed using a visual analog scale within 48 hours after septoplasty. In patients of the second group, after the use of PBMT, the indicators of heart rate variability had a significantly lower total power, compared with patients of the first group. So, after PBMT, the ultra-low-frequency component of the spectral analysis of heart rate variability in the first group was 18580 ± 2067 ms^2, which is significantly higher than in the second group (8086 ± 3003 ms^2) (p <0.001). The low-frequency component of heart rate variability was also significantly higher in the first group (1871 ± 405 ms^2) compared to the second (1095 ± 190 ms^2) (p <0.005), which indicates an increase in the tension of the sympathetic part of the autonomic nervous system in the group without the use of PBMT. In the first 3 hours after surgery, the severity of pain between the groups did not differ significantly (p = 0.07). In the period from 6 to 24 hours after surgery, patients who did not undergo PBMT experienced significantly higher pain than patients with PBMT (p <0.001). Thus, in our study, the group of patients with PBMT showed better results in pain and heart rate variability compared to the classical rehabilitation of patients after septoplasty.

Key words: septoplasty, pain, photobiomodulation, heart rate variability.

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ФОТОБИОМОДУЛЯЦИЯ ОСТРОГО БОЛЕВОГО СИНДРОМА ПОСЛЕ СЕПТОПЛАСТИКИ

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Резюме

В работе оценена эффективность фотобиомодуляционной терапии (ФБМТ) для минимизации острого болевого синдрома в раннем постоперационном периоде у пациентов после проведения септопластики. В исследование были включены две группы наблюдения в количестве 31 пациент каждая. В первой группе была проведена септоплатика со стандартным ведением в послеоперационном периоде. Во второй группе к стандартным мероприятиям послеоперационного периода добавляли ФБМТ через 3, 6 и 24 ч после септопластики (λ=0,890 мкм, P=10 Вт, 2 мин) и далее интраназально через 48 ч после операции (λ=0,630 мкм, P=8 Вт, 2 мин). В обеих группах оценивали вариабельность сердечного ритма (ВСР) и болевой синдром при помощи визуально-аналоговой шкалы в течение 48 ч после септопластики. У пациентов второй группы на фоне применения ФБМТ показатели ВСР имели значимо меньшую общую мощность по сравнению с пациентами первой группы. После проведения ФБМТ ультранизкочастотный компонент спектрального анализа ВСР в первой группе составил 18580±2067 мс^2, во второй группе – 8086±3003 мс^2 (p<0,001). Низкочастотный компонент ВСР также был значимо выше в первой группе: 1871±405 мс^2 и 1095±190 мс^2 соответственно (p<0,005), что свидетельствует о повышении напряжения симпатического отдела вегетативной нервной системы в группе без применения ФБМТ. В первые 3 ч после септопластики интенсивность боли между группами не имела достоверных различий (p=0,07). В период от 6 до 24 ч после хирургического вмешательства пациенты, которым не проводилась ФБМТ, испытывали более интенсивную боль, чем пациенты второй группы (p<0,001). Таким образом, в нашем исследовании группа пациентов с ФБМТ показала лучшие результаты по выраженности болевого синдрома и ВСР по сравнению с классической реабилитацией пациентов после септопластики.

Ключевые слова: септопластика, боль, фотобиомодуляция, вариабельность сердечного ритма.

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Introduction

Nasal septoplasty is one of the most commonly performed procedures in rhinosurgery. Frequent complications after the intervention are nasal bleeding, septal hematoma, acute rhinosinusitis, and pain syndrome [1, 2].

Septoplasty consists in the separation of the muco-supraperichondrial and/or muco-periosteal leaves and the removal of curved areas of the cartilaginous and/or bony parts of the nasal septum. As a rule, smooth sections of the extracted part of the nasal septum are placed back between the two leaves of the perichondrium. The nasal cavity is tamponed after surgery to avoid complications [3].

A special position is occupied by the issue of rehabilitation of patients after septoplasty, including high-quality anesthetic aid, analgesic therapy, and the use of topical medicines. We previously demonstrated that septoplasty as such [4], as well as with poor-quality anesthetic aid, provokes the development of the distress syndrome: an imbalance of the autonomic nervous system (ANS), a pronounced pain syndrome and a deterioration of the quality of life in the early postoperative period, which is confirmed by changes in the ANS balance and changes in HRV [5].

In order to reduce the manifestation of side effects after septoplasty, such as pain, tissue edema, inflammation, ecchymosis, photobiostimulation has recently been increasingly used [6], which improves and accelerates tissue repair, and, consequently, the healing of the surgical wound. These effects of photobiostimulation are based on improving intracellular calcium metabolism and accelerating the synthesis of ATP in mitochondria [7, 8]. Photobiomodulation therapy (PBMT) is a form of light therapy. In PBMT, light sources such as lasers or light-emitting diodes (LEDs) with a wavelength of 0.6–1 microns and a power of less than 500 MW per diode are used [9] to cause a photochemical reaction that leads to an increase in ATP synthesis in mitochondria, signal transmission in biological membranes and cells, DNA synthesis, cell proliferation, differentiation and modulation of pro-and anti-inflammatory mediators that reduce pain and inflammation [10, 11, 12]. PBMT is widely used for the treatment of various diseases: diabetic ulcers, blood diseases, musculoskeletal complications, coronary heart disease, as well as for wound healing, reducing pain and inflammation, restoring and regenerating tissues [13, 14].

It is known that after septoplasty, PBMT is used intranasally after the removal of tampons, or immediately in the case of splints [15]. At the same time, there are practically no data where the effectiveness of PBMT was evaluated with the exposure during tamponade in the first two days after septoplasty.

Taking into account the above, this study was conducted to evaluate the effectiveness of photobiomodulation (PBM) to minimize acute pain syndrome in the early postoperative period in patients after septoplasty.

Materials and methods

Rhinosurgery

Septoplasty under general anesthesia was performed in 62 patients, including 40 men and 22 women aged 18 to 44 years. The patients were randomly divided into 2 groups of 31 patients each, with an equal number of men and women. Women underwent septoplasty during the periovulatory period, since it is known that it is during this phase of the menstrual cycle that the risk of nosebleeds after rhinosurgery is minimal [16]. Immediately after the operation, all patients had an anterior nasal packing with gauze swabs in glove rubber for two days. All patients underwent septoplasty using local infiltration anesthesia with 1% procaine solution (250 mg) with 0.1% epinephrine solution (10 mg) and general anesthesia, for which Fentanyl (30 mcg/ml), Propofol (150 mg), cisatracurium besilate (nimbex) (6 mg), tranexamic acid (Tranexam) (1000 mg), atropine (0.5 mg) and metoclopramide (Cerucal) (10 mg) were used. In order to prevent the development of acute bacterial inflammation of the paranasal sinuses, oral antibacterial therapy with azithromycin was prescribed according to the scheme: 500 mg once in the morning for three days with the first administration in the morning on the day of surgery.

Photobiomodulation therapy

After 3 hours, 6 hours, and 24 hours after septoplasty, laser therapy was performed in patients of the second group. The emitter heads generated infrared pulsed laser radiation with a wavelength of 890 nm and an installed power of 10 W (LASMIK-01 device, Russia). The emitter heads were installed in the projection of the lateral cartilage and the large cartilage of the ala of nose on both sides for 2 minutes.

48 hours after the operation, patients had nasal tampons removed in both groups. In the second group, an intranasal PBMT with a nozzle was performed in a continuous, modulated mode of operation in the red optical range, with a wavelength of 630 nm and a radiation power of 8 MW. The heads were installed in both nostrils for 2 minutes (LAZMIK-01 device, Russia).

Analysis of heart rate variability and pain syndrome

To assess HRV, a daily Holter ECG monitoring was performed with MT-200 devices (Schiller, Swiss). The ECG monitoring system was put on patients 30 minutes before the septoplasty and removed 24 hours after it. HRV parameters were studied in the frequency range: low frequencies (LF, ms^2), ultra-low frequencies (ULF, ms^2), high frequencies (HF, ms^2) and total power (ms^2).

Pain syndrome was assessed with a visual analog scale (Fig. 1) in 1, 3, 6, 12, 24 and 48 hours after septoplasty, and in the second group, immediately after laser therapy sessions. Patients were asked to put a vertical line or a dot in the place on the scale that, in their opinion, corresponded to the pain they were experiencing. The scale length was 100 mm. The pain intensity was measured in mm [5].

Statistic analysis

All statistical data processing was performed with the JASP software package, version 0.14.0 (University of Amsterdam, the Netherlands) for Windows®. Continuous variables (pain value, LF, ULF, HF, Total power) were presented as the mean ± error of the mean (M±SE) and analyzed using the t–test of independent samples after checking normality with the Shapiro-Wilk test. Normally distributed data were evaluated with Student's t-test of independent samples, and abnormally distributed data were evaluated with Mann-Whitney U-test. Values of $p<0.05$ were considered statistically significant.

Results

Heart rhythm variability

After PBMT sessions, the ultra-low-frequency component of HRV spectral analysis was significantly lower in the second group (8086±3003 ms^2) than in the first (18580±2067 ms^2) ($p<0.001$) (Fig. 2a). The low-frequency HRV component was significantly higher in the first group (1871±405 ms^2) than in the second (1095±190 ms^2) ($p<0.005$), which indicates increased tension of the sympathetic part of the ANS in the group without PBMT (Fig. 2b). Based on the analysis of the high-frequency component of HRV, a decrease in the activity of the parasympathetic nervous system during the perioperative day was also recorded in the second group as a whole: 1157±220 ms^2 versus 1630±263 ms^2 in the first group ($p<0.01$) (Fig. 2c). In the second group, the total HRV power (13498±3226 ms^2) was significantly lower ($p<0.001$) than in the first (26808±2371 ms^2) (Fig. 2d).

Рис. 1. Визуально–аналоговая шкала оценки интенсивности острого болевого синдрома
Fig. 1. Visual analog scale for assessing the intensity of acute pain syndrome

The pain syndrome

In the first 3 hours after the surgical intervention, the pain intensity did not differ between the groups ($p=0.07$). In the first group, the intensity of pain increased after 6 hours compared to 3 hours after surgery, but no significant difference was found ($p=0.01$). After 6 hours, in the second group, the intensity of the pain syndrome began to decrease compared to the previous period ($p<0.05$) (Fig. 3). Further, the intensity of the pain syndrome continued to decrease in both groups, and 48 hours after the septoplasty, the patients either did not feel pain or it was very low and did not cause noticeable discomfort. At the same time, in the period from 6 to 24 hours after surgery, patients who did not undergo PBMT experienced pain that was significantly higher than in patients who underwent PBMT ($p<0.001$) (Fig. 3, table).

Discussion

It is known that the removal of tampons is advisable two days after surgery, when there is a decline in inflammatory processes and the restoration of the mucous membrane, normalization of blood supply to cartilage and bone tissues begin [1, 2], so we considered it important to use PBMT during the first two days. In the available literature, we have not found any works where PBMT was performed in patients after septoplasty with intra-nasal tampons and with a high frequency of therapy sessions on the first day after the rhinosurgical intervention.

The generally accepted theory on the mechanism of the biological effect of PBM is the absorption of light by chromophores [17]. PBMT leads to the following effects: reduction of edema and inflammation, reduction of pain, collagen synthesis, increased elasticity, increased tissue perfusion and increased tissue vascularization, increased cell proliferation, especially of fibroblasts, which generally contributes to the restoration of damaged tissues [6]. Recent studies have shown that PBMT is effective

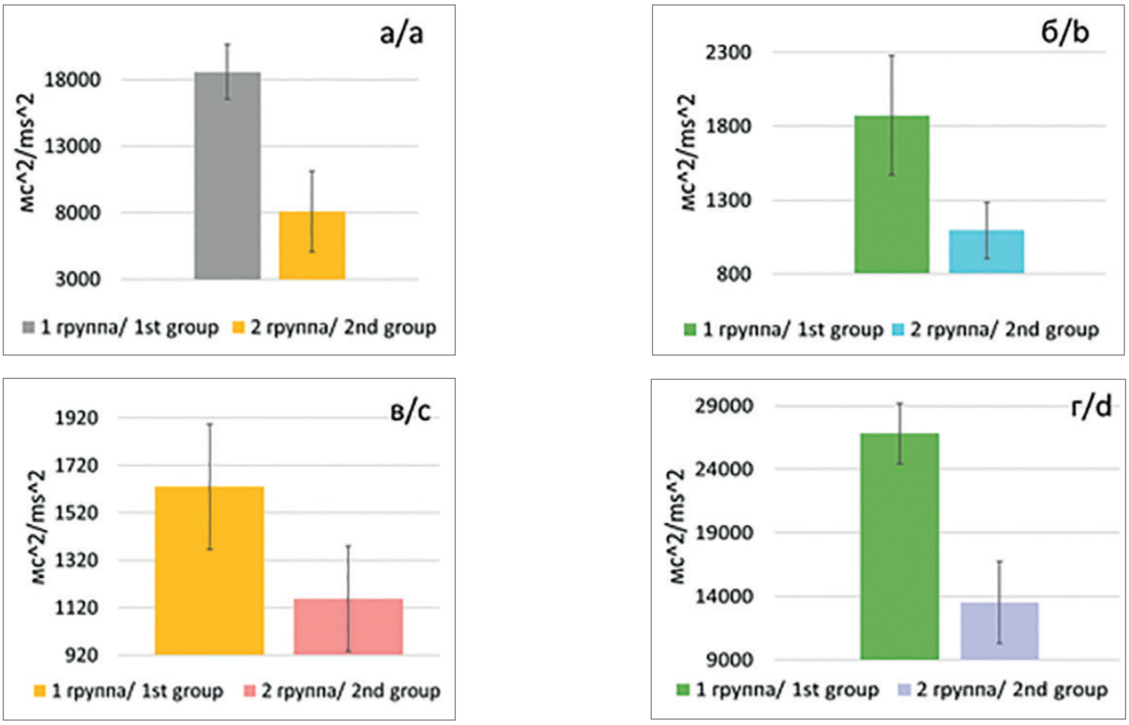

Рис. 2. Изменения показателей частотной области вариабельности сердечного ритма с применением ФБМТ после септопластики и без нее: a – ULF, b – LF, c – HF, d – общая мощность
Fig. 2. Changes in the indicators of the frequency domain of heart rate variability with the use of PBMT after septoplasty and without it: a – ULF (ultralow-frequency), b – LF (low-frequency), c – HF (high-frequency), d – Total power

Таблица
Интенсивность острого болевого синдрома после септопластики
Table
Intensity of acute pain after septoplasty

Группа Groups	Динамика интенсивности острого болевого синдрома после операции, мм Dynamics of the intensity of acute pain syndrome after surgery, mm					
	1 ч 1 h	3 ч 2 h	6 ч 6 h	12 ч 12h	24 ч 24 h	48 ч 48 h
1 группа 1st group	17,15±2,46	21,82±2,83	25±2,02	21,64±2,36	16,68±1,01	3,68±1,01
2 группа 2nd group	14,16±2,31	18,88±2,45	16,43±2,08	12,83±2,38	10,84±1,15	3,84±1,15

for various conditions: from diabetic foot to androgenic alopecia and mucositis after chemotherapy, as well as for wound healing and inflammation [7, 8, 17, 18, 19]. PBMT can play a role in reducing the number of new hemorrhages after surgical interventions in the maxillofacial region. At the same time, PBMT is positioned as a new alternative to other interventions, since it is an easy-to-use and minimally invasive method [6].

Hersant et al. evaluated the effect of a low-intensity laser on the results of graft survival in facial plastic surgery. The authors have shown that PBMT contributes to a higher survival rate of the graft and accelerates wound healing [20]. Enwemeka et al. found that PBMT is effective in promoting the restoration of damaged tissues during all three phases and reduces pain syndrome [21].

The effects of PBMT described above, especially the restoration of damaged tissue and neovascularization, provide a reduction in edema and inflammatory reactions, a decrease in the likelihood of hemorrhage [6] and, therefore, of pain in the tissue after septoplasty. With the intranasal use of laser therapy, systemic effects are also achieved through cells and blood components [22], which can probably contribute to a positive neurotherapeutic effect [23]. The tissues around the nasal

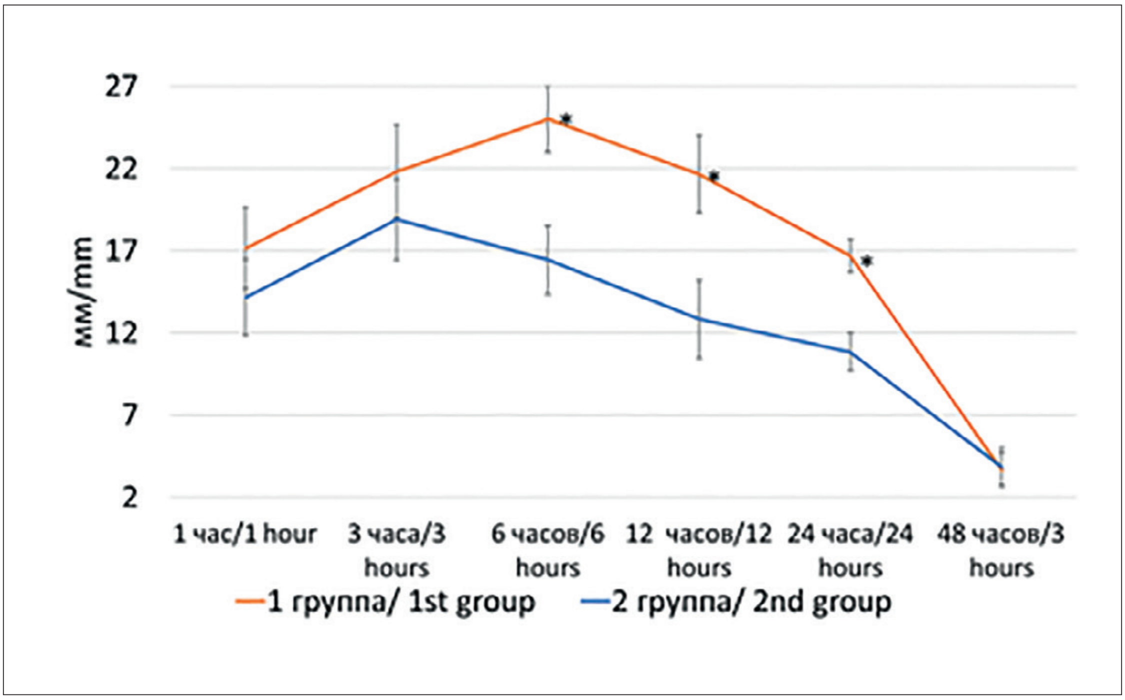

Рис. 3. Интенсивность болевого синдрома после септопластики
* – достоверные различия между группами, p=0,001
Fig. 3. Intensity of pain after septoplasty
* – significant differences between groups, p=0.001

cavity have an abundant blood supply with a relatively slow blood flow. It was shown that PBMT improves blood rheology [24], reduces its viscosity [25] and improves the blood clotting status [26] in various pathological conditions. A significantly lower intensity of pain syndrome was observed in the second group compared to patients of the first group, indicates relatively low inflammatory reactions from the blood system in the damaged area after the use of PBMT [27].

In patients who had PBMT, HRV indicators had significantly lower overall power compared to patients who did not receive laser therapy. Thus, the ultra-low frequency component, which is often associated with circadian rhythms [28], was lower in the second group. An increase in the ULF power indicates a failure of circulatory rhythms as a result of surgical traumatization against the background of inflammatory phenomena in the group which did noy have PBMT. The high-frequency (HF) component of HRV shows the tone of the parasympathetic nervous system, while the low-frequency (LF), according to a number of authors, can reflect both sympathetic (mainly) and parasympathetic tone [29]. The decrease in LF and HF after septoplasty with the use of PBMT reflects a decrease in sympathetic and parasympathetic tone after correction of nasal septum deviation. The shift of the balance of the ANS towards its sympathetic component is physiologically justified and corresponds to the degree of severity of the stress factors impact. An increase in the tone of the parasympathetic nervous system under stress may indicate the body's inadequate response [30], which may reflect the degree of surgical damage in the maxillofacial region [31]. It has been shown that after septoplasty LF HRV can sharply decrease [29]. In our study, in a group of patients with the classical variant of postoperative rehabilitation, the activity of both the sympathetic and parasympathetic parts of the ANS was increased. Studies have shown a relationship between blood rheology, cognitive functions [27] and mood improvement [32]. It was suggested that the systemic effects of PBMT after blood irradiation may also ultimately have a neuroprotective effect [23, 33, 34]. It is known that intranasal blood irradiation has the same neurological consequences as intravenous or intravascular PBMT [35]. These facts may facilitate the understanding of a lower level of pain syndrome, smaller changes in the balance of the ANS in response to surgical damage after septoplasty in patients with the use of PBM in the early postoperative period.

Conclusion

In our study, a group of patients who had PBMT showed better results when the indicators of pain syndrome and HRV were evaluated compared to the classical rehabilitation of patients after septoplasty. In our opinion, it is necessary to further develop protocols for the rehabilitation of patients after septoplasty with various types of nasal cavity tamponade.

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PHOTODYNAMIC THERAPY FOR FACIAL SKIN CANCER DEVELOPED IN THE ZONE OF PREVIOUS RADIOTHERAPY (CLINICAL CASE)

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Abstract

The results of a 13-year clinical observation of a patient after treatment for basal cell carcinoma of the skin of the right cheek Ist cT1N0M0 are presented. The history of the course of the disease is associated with the fact that the patient underwent radiation therapy in early childhood for hemangioma of the lower eyelid of the right eye and right cheek. In 2008, against the background of post-radiation changes in the area of the right cheek, basal cell carcinoma was diagnosed at the Moscow Oncological Research Institute. P.A. Herzen. At the Center for Laser and Photodynamic Diagnostics and Tumor Therapy, the patient underwent organ-preserving PDT treatment. A course of photodynamic therapy (PDT) with 5-aminolevulic acid was carried out. Subsequently, the patient was followed up until 2021 without relapse in the PDT area. In 2016, the patient was diagnosed with a relapse of the disease in the form of a new focus of basal cell carcinoma of the upper eyelid skin on the right Iast cT1N0M0. The patient underwent a course of PDT with a chlorin e6-based photosensitizer. Complete regression of the tumor was achieved, the period of relapse-free follow-up was 5 years.

Keywords: basal cell skin cancer, photodynamic therapy, radiation therapy, photosensitizer, chlorin e6, 5-aminolevulinic acid, induced cancer.

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ФОТОДИНАМИЧЕСКАЯ ТЕРАПИЯ ПРИ РАКЕ КОЖИ ЛИЦА, РАЗВИВШЕГОСЯ В ЗОНЕ ПРЕДШЕСТВУЮЩЕЙ ЛУЧЕВОЙ ТЕРАПИИ (КЛИНИЧЕСКОЕ НАБЛЮДЕНИЕ)

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Резюме

Приведены результаты 13-летнего клинического наблюдения пациентки после лечения базальноклеточного рака кожи правой щеки I ст cT2N0M0. Анамнез заболевания связан с тем, что пациентке в раннем детстве по поводу гемангиомы нижнего века правого глаза и правой щеки выполнена лучевая терапия. В 2008 г., на фоне постлучевых изменений в области правой щеки, диагностирован базальноклеточный рак в МНИОИ им. П.А. Герцена. В Центре лазерной и фотодинамической диагностики и терапии опухолей пациентке проведено органосохраняющее лечение методом ФДТ. Проведен курс фотодинамической терапии (ФДТ) с 5-аминолевульновой кислотой. В последующем больная наблюдалась 13 лет без рецидива в зоне ФДТ. В 2016 г у пациентки диагностирован рецидив заболевания в виде нового очага базальноклеточного рака кожи верхнего века справа IA ст cT1N0M0. Пациентке проведен курс ФДТ с фотосенсибилизатором на основе хлорина е6. Достигнута полная регрессия опухоли, срок безрецидивного наблюдения – 5 лет.

Ключевые слова: базальноклеточный рак кожи, фотодинамическая терапия, лучевая терапия, фотосенсибилизатор, хлорин е6, 5-аминолевулиновая кислота, индуцированный рак.

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Introduction

Photodynamic therapy (PDT) is a method of antitumor therapy successfully used in clinical practice. In several decades of its use in Russia, the method has proven to be effective and safe for the treatment of patients with cancer of various localizations. PDT is used for malignant neoplasms of the skin, genitourinary system organs, gastrointestinal tract, brain, bronchi, and other nosologies [1-3]. In recent years, the range of indications for the use of the method has been constantly expanding, and new effective photosensitizers and PDT methods have appeared [4-6].

Clinical example

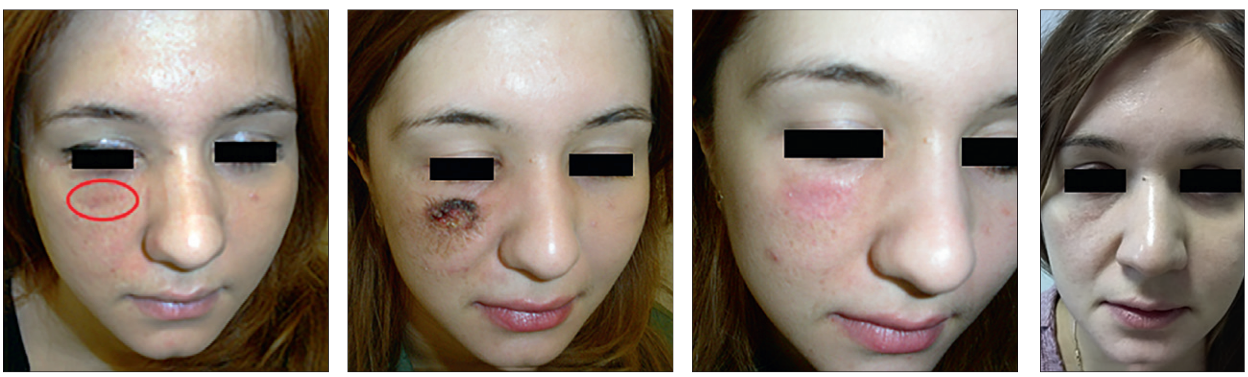
We present a clinical observation of the treatment of a patient with a diagnosis of primary multiple metachronous cancer: 1) basal cell skin cancer (BCSC) of the right cheek, I degree, cT2N0M0, the condition after PDT in 2008; 2) BCSC of the upper eyelid of the right eye IA deg. cT1N0M0, the condition after PDT in 2016.

In 1991, patient Sh., DOB: 1986, aged 5, underwent radiation therapy in connection with a hemangioma of the right cheek spreading to the lower eyelid of the right eye, at Helmholtz Moscow Research Institute of Eye Diseases.

In 2008, the patient noted a lesion on the skin of the right cheek in the area of previous treatment, and applied independently to P. A. Hertsen Moscow Oncology Research Center. When examined, the patient was found to have, against the background of post-radiation skin changes, an area of superficial tumor infiltration of the skin with fuzzy borders, with a maximum size of 2.3 cm (Fig. 1a). A cytological study of the lesion was performed, and BCSC was diagnosed. The patient was discussed at an extended medical board, and PDT was recommended.

In May 2008, the patient underwent a course of PDT with a drug based on 5-aminolevulinic acid. The patient tolerated treatment satisfactorily, without complications. Complete regression of the tumor was achieved after one course of PDT (Fig. 1c). Subsequently, the patient was observed without tumor recurrence in the treatment area with periodic confirmation of the achieved effect by control cytological studies from the PDT zone (Fig. 1d; 2c, d).

In September 2015, the patient noted the appearance of a lesion on the skin of the upper eyelid of the right eye; a biopsy of the tumor mass was performed in the ophthalmological clinic; BCSC was diagnosed according to the histological test. In December 2015, the patient independently applied to P. A. Hertsen Moscow Oncology Research Center. During examination, a trace from the tumor biopsy and a tumor infiltration of the skin of the upper eyelid is visualized in the upper eyelid area. The data of the revision of histology slides No. 51232/15 showed the presence of BCSC (Fig. 2a). The patient was discussed at a medical board, and PDT of the skin tumor on the upper eyelid of the right eye was recommended.



a b c d

Рис. 1. Лечение БКРК правой щеки:
a – опухоль правой щеки до лечения;
b – некроз опухоли через неделю после ФДТ;
c – полная регрессия опухоли через месяц после ФДТ;
d – состояние без рецидива после лечения через 6 лет после ФДТ (2014 г.)

Fig. 1. Treatment of basal cell carcinoma of the skin of the right cheek:
a – tumor of the right cheek before treatment;
b – tumor necrosis a week after PDT;
c – complete tumor regression one month after PDT;
d – condition without relapse after treatment 6 years after PDT (2014)

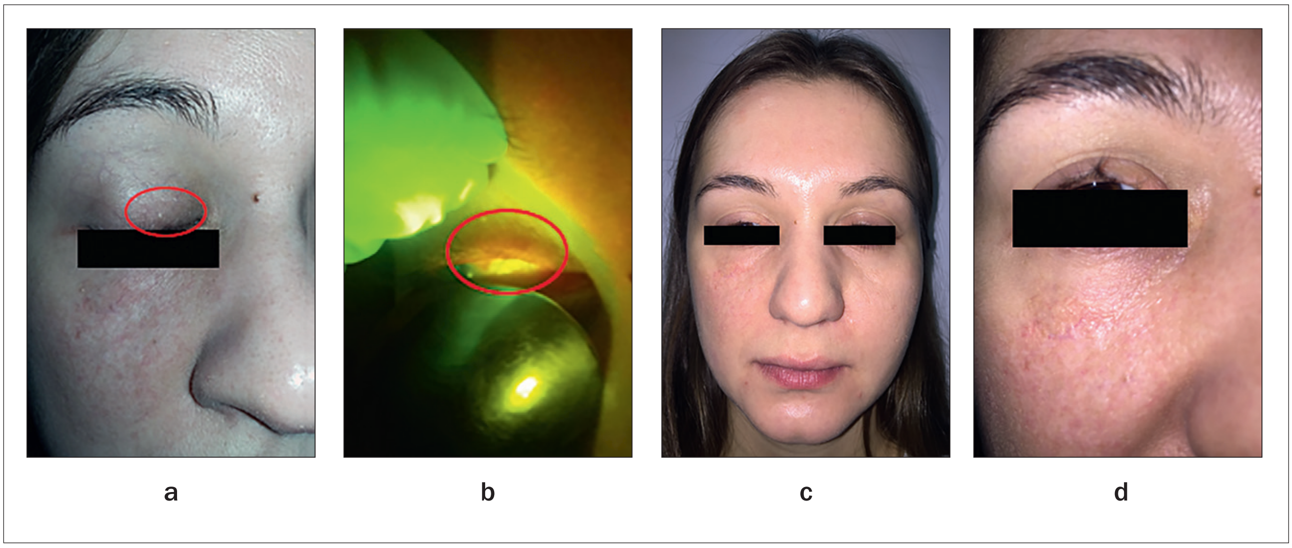


Рис. 2. Лечение БКРК верхнего века правого глаза:
а – опухоль до ФДТ (после биопсии);
б – флуоресценция опухоли при проведении ФД (определение границ опухоли);
в, г – полная регрессия опухоли верхнего века справа через 1 год после ФДТ, состояние без рецидива опухоли правой щеки через 9 лет после ФДТ (2017 г.)

Fig. 2. Treatment of basal cell skin cancer of the upper eyelid of the right eye:
a – tumor before PDT (after biopsy);
b – tumor fluorescence during PD (definition of tumor boundaries);
c, d – complete regression of the tumor of the upper eyelid on the right 1 year after PDT, condition without tumor recurrence in the right cheek 9 years after PDT (2017)

On 26.01.2016, a course of PDT with chlorin e6 as photosensitizer was performed on the skin tumor of the upper eyelid of the right eye. Before the laser irradiation session, a fluorescence diagnostics (FD) session was performed. The boundaries of the upper eyelid tumor were evaluated for planning radiation fields (Fig. 2b), and other skin areas were examined, including the area of scarring after PDT of the tumor on the right cheek. No additional areas of increased fluorescence were detected. A laser irradiation session was performed with due account for the boundaries of the tumor lesion determined by the results of FD. The patient tolerated treatment well, without complications. Complete regression of the tumor was achieved (Fig. 2c, d). The patient has had follow-up monitoring and has been found relapse-free after PDT in the area of tumor treatment on the upper eyelid on the right eye for 5 years, and on the right cheek, for 13 years.

Discussion

The patient was diagnosed with two foci of skin cancer in areas that were located either directly in the radiation exposure zones or along the edge of the irradiation zone 22 years and 29 years after radiation therapy for a benign skin pathology. Is it possible to see the development of these foci of skin cancer as a consequence of previous radiation therapy?

One of the most significant effects of radiation therapy (RT) on normal tissues is mutagenesis, which is the basis for the development of radiation-induced malignant neoplasms. Radiation-induced malignant neoplasms are late complications that occur after RT, the frequency of which increases among survivors, including both children and adults [7].

There are three main criteria by which malignant neoplasms are classified as RT-induced: the occurrence at the site of previous irradiation, a latent period of at least 2 years after the start of RT, and a histology different from the primary tumor (if present) [8-10].

Friedman D. L. et al. (2010) conducted a retrospective study to evaluate the frequency of the development of second primary multiple neoplasms in survivors of childhood cancer [11]. Of the 14,359 patients with a 5-year overall survival, 1,402 subsequently developed 2703 neoplasms. Cumulative incidence at 30 years after the childhood cancer diagnosis was 20.5% for all subsequent neoplasms, including 7.9% for second malignant neoplasms (excluding nonmelanoma skin cancer), 9.1% for nonmelanoma skin cancer, and 3.1% for meningioma. The association of RT with an increased risk of developing second neoplasms was proved by the authors by use of multivariable Poisson regression. Cumulative incidence at 30 years after childhood cancer diagnosis was 20.5% for

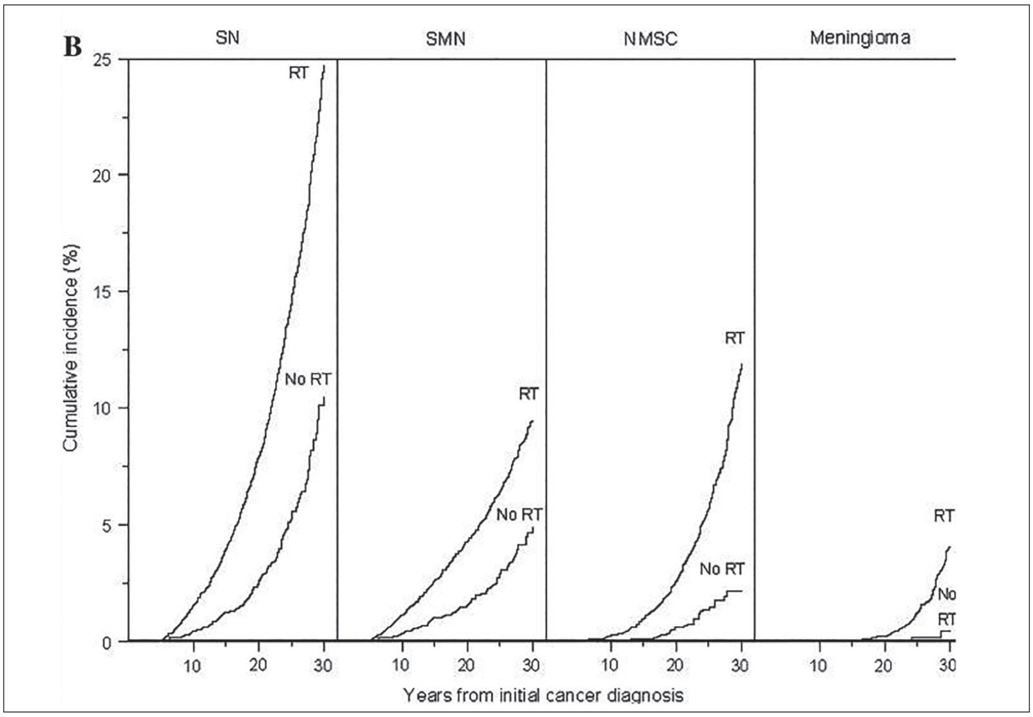


Рис. 3. Кумулятивная частота вторых новообразований через 30 лет после первого рака у пациентов с ЛТ и без ЛТ (Friedman D.L. и соавт., 2010 [11]); RT – ЛТ; No RT – без ЛТ; SN – второе новообразование (доброкачественное или злокачественное); SMN – второе злокачественное новообразование; NMSC – немеланомный рак кожи

Fig. 3. Cumulative incidence of second neoplasms (SNs) at 30 years after initial cancer diagnosis, stratified by radiation therapy (RT) treatment or no RT (Friedman D.L. et al., 2010 [11]); SN – second neoplasm; SNM – second malignant neoplasm; NMSC – nonmelanoma skin cancer

all first of the subsequent neoplasms and was higher for patients treated with radiation therapy for their primary cancer than for those not receiving radiation therapy (Fig. 3). The results of the study showed that RT increased the risk of any subsequent neoplasm by 2.7 times. The analysis confirmed that the effect of RT was associated with an increased risk of second tumors of the central nervous system, soft tissue and bone sarcomas, as well as thyroid cancer [11].

After the brain is included in the irradiation zone, the most common RT-induced second tumors are neoplasms of the central nervous system. For instance, in 1991 Neglia J. P. et al. [12] conducted a retrospective study including 9720 children who had previously been diagnosed with acute lymphoblastic leukemia and who were treated in accordance with the therapeutic protocols of the Children's Cancer Research Group using radiation of the cranial and craniospinal zones. The average follow-up time was 4.7 years (from 2 months to 16 years). The authors found that 43 second neoplasms occurred in the children included in the study, including 24 (55.8%) had neoplasms of the central nervous system (14 patients with high-grade astrocytoma and glioblastoma multiforme, 3 with primitive neuroectodermal tumor, 2 with meningioma, 2 with astrocytoma or low-grade glioma, 1 with medulloblastoma, 1 with brain stem glioma, 1 with ependymoma), 10 (23.3%) new leukemias and lymphomas (6 patients had non-Hodgkin's lymphoma, 2 – acute non-lymphoblastic leukemia, 1 – immunoblastic sarcoma, 1 – Hodgkin's disease) and 9 (20.9%) had other neoplasms (3 cases of thyroid cancer, 2 cases of mucoepidermoid carcinoma of the parotid gland, 1 of dysgerminoma, 1 of melanoma, 1 of ganglioneuroblastoma, 1 of leiomyosarcoma of the ileum). The authors point out that these figures represent a 7-fold excess of all cancers and a 22-fold excess of neoplasms of the central nervous system compared to the general population of this age. All neoplasms of the central nervous system occurred in children who had previously undergone radiation therapy. There was no association with the effects of cyclophosphamide or anthracyclines. This proves the inducing effect of RT on the development of tumors of the central nervous system [12].

In their study, Armstrong G. T. et al. (2011) analyzed the incidence of primary multiple metachronous malignancies in patients who had treatment for childhood cancer [13]. Of the 14,358 childhood cancer survivors, 1382 (9.6%) patients were diagnosed with one metachronous multiple primary tumor. Of these, 386 (27.9%) patients, after treatment of their tumors, subsequently developed other metachronous primary multiple tumors. At the same time, among patients with a subsequent repeated metachronous tumor, 153 (39.6%) were diagnosed with more than two metachronous primary multiple tumors. The cumulative incidence of repeated primary multiple metachronous tumors 20 years after the diagnosis of the first primary multiple metachronous tumor was 38.8%. At the same time, the cumulative frequency of repeated primary multiple metachronous tumors in the group of patients who survived RT of the first cancer was 41.3% after 15 years, compared with 25.7% for patients who did not receive RT [13].

The study by Travis L. B. et al. (2003) presents the results on the association of RT with the development of second tumors in patients with Hodgkin's disease [14]. Hodgkin's disease usually affects cervical and mediastinal lymph nodes, and classical RT in Hodgkin's disease targets the areas of the lymph nodes, which leads to irradiation of the breast and lung tissues. The authors showed that the risk of breast cancer after RT+CT in Hodgkin's disease depends on the radiation dose, while a dose of 4 Gy or more is associated with a 3.2-fold increase in risk compared to patients receiving lower doses, and the risk increases up to eight times at doses of more than 40 Gy. The authors conclude that the risk of breast cancer after CT+RT seems to be primarily associated with RT, since treatment with alkylating agents alone led to a decrease in the risk of developing breast cancer. The probability of developing breast cancer decreased with an increase in the number of cycles of alkylating agents and a reduction in the use of RT in these patients [14].

A review by Braunstein S. et al. (2013) presents data on the frequency of second tumor development after RT of primary neoplasms of various localization. The authors point to an increased risk of developing RT-induced tumors after irradiation of the pelvic organs and abdominal cavity. Thus, patients after RT of testicular cancer are at an increased risk of developing RT-induced tumors of the intestinal and genitourinary tracts, and patients after RT of cervical and endometrial cancer are at an increased risk of a second cancer of the colon and rectum, bladder and genitals. People who have survived prostate cancer are also at risk of developing radiation-induced tumors, which is especially important, given that these patients usually receive treatment at a much older age than patients with testicular or cervical cancer. A study of men with prostate cancer treated in the period from 1988 to 2003 showed that the relative risk of developing a second bladder cancer is 1.88 for patients who received remote RT, compared with prostatectomy. Patients after RT of head and neck cancer are at an increased risk of developing RT-induced tumors in the head and neck, esophagus or lungs, with 15% probability of occurrence of an RT-induced tumor within 5 years [7].

The most common type of induced skin cancer in patients after RT is BCSC [15]. At the same time, RT-induced BCSC usually occurs with the use of low and moderate doses of radiation, e. g., when RT is used for the treatment of pathology other than malignant neoplasms: shingles, hypertrophic tonsillitis, common acne, atopic dermatitis, and hyperthyroidism. There is evidence indicating that squamous cell skin cancer (SCSC) develops more often after higher doses of radiation [10, 16-18].

There are both radiation-dependent and independent risk factors for developing RT-induced skin cancer. Radiation-dependent risk factors include a higher total radiation dose, the RT technique (two-dimensional conformal RT > RT with intensity modulation > 3-dimensional conformal RT > proton therapy), increased sensibility to ultraviolet light / lighter skin type and a younger age during radiation exposure. Risk factors that do not depend on radiation include genetic predisposition to malignant neoplasms, lifestyle aspects (alcohol, tobacco, and medications) and exposure to other carcinogens [8].

The development of RT-induced malignant neoplasms is characterized by a number of features. Thus, carcinogenesis in this case is induced by fairly low levels of radiation doses, and the risk increases with the dose. At higher radiation doses (as well as when exposed to sunlight on previously irradiated areas), the duration of the latency period is significantly lower [7, 10]. The second feature is the fact that young age during exposure to RT is a risk factor for carcinogenesis [7, 19, 20]. There are also indications that the incubation period between exposure to ionizing radiation and the appearance of BCSC symptoms is shorter in young patients [10]. Another feature is that the development of RT-induced tumors is characterized by a long latency period, which is usually several years, but can be decades [7]. The literature describes cases of induced skin cancer 2 to 65 years after radiation therapy. Most often, according to literary data, this period is 20-45 years [10]. Finally, although BCSC is usually characterized by slow growth, minimal invasiveness into the underlying tissues and high cure rates, RT-induced BCSC tends to be more aggressive and more prone to relapses [19, 21, 22].

There are few studies describing the molecular mechanism underlying the pathogenesis of aggressive radiation-induced BCSC [19]. A few years ago, Boaventura P. et al. found that the frequency of the D-Loop D310 mitochondrial mutation was associated with a higher radiation dose, although the role of this mutation in the development of BCSC in children has yet to be shown [23].

Previously, indications for the clinical use of radiation therapy included various benign conditions, for example, rheumatological, dermatological, and infectious diseases. This is an important context in which late radiation effects can be identified, because, unlike malignant diseases, the long survival of these patients allows us to track radiation-induced malignant neoplasms with a long latent period [7].

Before the advent of antifungal drugs in the 1950s, X-ray irradiation was widely used for the treatment of shingles. It is estimated that about 200,000 children worldwide have received X-ray treatment for this disease [19]. The first study of the long-term effects of RT in dermatomycosis on the head was reported by Albert R. E. et al. in 1968. Among 2,043 children treated at the New York University Hospital, 14 cases of malignant tumors were detected, 7 of which were cases of BCSC [19, 24]. A subsequent study involving 2,215 patients, the results of which were published in 1976, confirmed that RT in children with shingles infection on the head was associated with an increased risk of skin cancer (including BCSC), as well as malignant neoplasms of the brain, parotid gland, bones, and thyroid gland. In all subsequent studies, BCSC was the main type of skin cancer affected by therapeutic radiation, while the frequency of SCSC and melanoma did not change significantly. The treated patients had a high prevalence of multiple forms of BCSC, most of which were of the nodular type [19, 25].

In the study of Shore R. E. et al., 2224 children who received RT for dermatomycosis on the head (ringworm of the scalp) were observed for 50 years to determine the incidence of cancer. The control group consisted of 1380 patients with shingles of the scalp who received only topical medications. The study assessed the relative risk of developing BCSC during irradiation of the scalp as the ratio of the probability of developing BCSC in the group exposed to RT to the probability of its development in the unexposed group. BCSC developed in 124 patients in the group that had RT, and in 21 patients in the group without RT. Thus, with scalp irradiation at a total dose of 4.8 Gy, the relative risk of developing BCSC was 3.6. Cases of the development of melanoma of scalp and neck were not observed, isolated cases of SCSC were registered. Among patients with BCSC, about 40% had multiple forms. The study also showed that the level of risk for developing BCSC is approximately constant over time from the moment of exposure, which suggests that the risk is likely to persist throughout life [20].

In a multicenter retrospective study by Ron E. et al., it was shown that CT of the scalp in children with dermatomycosis led to a four-fold increase in the incidence of skin cancer, primarily BCSC, and to a three-fold increase in the incidence of benign skin tumors. However, as in previous studies, the risk of developing malignant melanoma in such patients was not increased [26].

Maalej M. et al. reported on 98 patients who developed RT-induced cancer of the scalp after irradiation in childhood for shingles, including 81 (82%) patients who had only one RT session. In 98 patients, 150 foci of malignant neoplasms were registered, 125 of which were BCSC, 16 SCSC, 2 malignant non-Hodgkin's lymphomas, 4 foci of melanoma, and 3 other tumors. The period from RT to the development of skin cancer averaged 36±14 years [15].

The study by Mseddi M. et al. describes 33 patients with BCSC induced by previous RT of shingles foci. The latency period was 21-51 years [27].

Currently, a significant group of patients with RT-induced skin malignancies are patients who have undergone radiation for oncological diseases in childhood. Thus, the study of Watt T. C. et al. showed the connection between eadiation therapy and an increased risk of developing BCSC. The study included 199 childhood cancer survivors, who subsequently developed BCSC. The comparison group consisted of 597 childhood cancer survivors without BCSC. This study revealed a dose-response relationship showing an increase in the incidence risk ratio with a coefficient of 1.09 per 1 Gy. Thus, in patients who received a dose of 35 Gy, the risk of developing BCSC was 39.8 times higher than in survivors who did not receive radiation therapy [28].

Over 40 years of the use of hematopoietic cell transplantation, another large cohort of patients who have undergone RT and have high risks of developing induced malignant neoplasms has appeared. In these patients, an increased frequency of malignant neoplasms was detected, the most frequent being BCSC [19]. Many of the patients undergo preliminary total irradiation of the entire body as a preparation for hematopoietic cell transplantation. Leisenring W. et al. reported that the use of a regime with total body irradiation was a significant risk factor for the development of BCSC, but not for SCSC, in a study involving 4,810 survivors with allogeneic hematopoietic cell transplantation who received treatment between 1969 and 2003. A single or fractional dose of 14 Gy significantly increased the frequency of BCSC: more than 1.8 times compared to regimens without total irradiation [29]. Schwartz J. L. et al. present the results of a study in which the risks of developing BCSC were assessed in 6306 patients treated with hematopoietic cell transplantation with or without total body irradiation, and reported that the overall relative risk of developing BCSC was 1.76 in patients with total irradiation who were exposed to prescribed radiation doses from 7.5 to 18.4 Gy. The risk of developing BCSC was highest in patients exposed at the age of under 10 years, and decreased by 10.9% per year for patients older than 10 years. There was no increased risk of developing BCSC associated with total whole-body irradiation for patients over the age of 40 years during hematopoietic cell transplantation [30].

The authors of all the described studies indicate the need for careful monitoring of patients with a history of RT. Unfortunately, as already noted, RT-induced BCSC tends to be more aggressive, more difficult to treat, and more prone to relapses than sporadic lesions. Patients with a history of RT are recommended to undergo regular lifelong examination of the irradiated areas. Moreover, it is very important to inform patients that they should contact their doctor in case of any suspicious lesions.

Conclusion

This clinical observation demonstrates the importance of follow-up monitoring of cured cancer patients even after the completion of a 5-year relapse-free period. The patient developed a skin tumor 8 years after radical PDT of another skin tumor. It is only regular observation by an oncologist that makes it possible to diagnose the second tumor at an early stage, when photodynamic therapy, an organ-preserving method with a high cosmetic effect, can be applied.

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