MORPHOLOGICAL EVALUATION OF THE EFFECTIVENESS OF TREATING INFECTED WOUNDS WITH HIGH-INTENSITY PULSED BROADBAND IRRADIATION

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Abstract

The objective of this study was to investigate the effectiveness of high-intensity pulsed broadband irradiation in treating infected wounds. A morphological study was conducted on wound specimens from 105 Wistar rats, in which infected wounds were experimentally induced (three groups). The first group was treated with high-intensity pulsed broadband irradiation, the second group received traditional ultraviolet irradiation, and the third group was treated only with antiseptics. Monitoring was performed before treatment, on the 7th, 14th, and 21st days of treatment. Non-parametric statistical methods were used for data analysis. Prior to treatment, the wounds exhibited signs of the acute inflammation phase. By the 7th day, the first group's wounds were in the proliferation phase. In the second and third groups, edema and infiltration persisted. By the 14th day, the first group's wounds showed signs of granulation tissue formation and transition to the regeneration stage. In the second group, there was a reduction in infiltration, the appearance of new capillaries, and an increase in fibroblasts. In the third group, inflammatory symptoms persisted. By the 21st day, the first group showed remodeling of connective tissue with signs of delicate scar formation. In the second group, signs of connective tissue remodeling were observed, while in the third group, there was reduced infiltration with slow formation of new vessels. Thus, the use of high-intensity pulsed broadband irradiation in the early stages effectively mitigates inflammation, activates local immune response, and accelerates reparative processes.

Keywords: infected wounds, wound healing process, high-intensity pulsed broadband irradiation, ultraviolet irradiation, infiltration, connective tissue remodeling.

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МОРФОЛОГИЧЕСКАЯ ОЦЕНКА ЭФФЕКТИВНОСТИ ЛЕЧЕНИЯ ИНФИЦИРОВАННЫХ РАН ВЫСОКОИНТЕНСИВНЫМ ИМПУЛЬСНЫМ ШИРОКОПОЛОСНЫМ ОБЛУЧЕНИЕМ

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Резюме

Целью исследования явилось изучение эффективности высокоинтенсивного импульсного широкополосного облучения инфицированных ран. Проведено морфологическое исследование препаратов ран 105 крыс линии Wistar, которым в эксперименте моделировали инфицированные раны (три группы животных). В 1-й группе для лечения ран использовали высокоинтенсивное импульсное широкополосное облучение, во 2-й группе – традиционное ультрафиолетовое облучение, и в 3-й применяли только антисептик. Оценку эффективности производили до лечения, на 7-е, 14-е и 21-е сутки лечения. До начала лечения картина ран соответствовала фазе острого воспаления. На 7-й день в 1-о группе морфологическая картина соответствовала фазе пролиферации. Во 2-й и 3-й группах отек и инфильтрация к этому сроки сохранялись. К 14-му дню в 1-й группе наблюдали признаки формирования грануляционной ткани и переход ран в стадию регенерации. Во 2-й группе уменьшалась инфильтрация, появлялись новые капилляры, увеличивалось количество фибробластов. В 3-й группе, воспалительные явления сохранялись. К 21-му дню в первой группе наблюдалось ремоделирование соединительной ткани с признаками образования нежного рубца. Во 2-й группе животных наблюдались явления ремоделирования соединительной ткани. В препаратах ран 3-й группы инфильтрация уменьшена, новые сосуды образуются с замедлением. Таким образом, использование высокоинтенсивного импульсного широкополосного облучения инфицированных ран в более ранние сроки купирует воспаление, активизирует местную иммунную реакцию и ускоряет репаративные процессы.

Ключевые слова: инфицированные раны, раневой процесс, высокоинтенсивное импульсное широкополосное облучение, ультрафиолетовое облучение, инфильтрация, ремоделирование соединительной ткани.

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Instroduction

Nowadays, doctors of many specialties use various physical methods of influence in the treatment of a large number of diseases, including inflammatory ones. Thus, in surgery, phototherapy methods are widely used, which include laser and ultraviolet irradiation of tissues.

Due to the high drug resistance of microorganisms, some authors suggest looking for alternative ways to treat infected wounds using physical methods of influence [1, 2]. The sensitivity of many microorganisms to ultraviolet radiation is well known and thoroughly characterized. Over the past decade, antimicrobial therapy based on the use of optical radiation has achieved significant success against antibiotic resistance among various strains of microorganisms. Such kind of treatment includes methods using the antimicrobial properties of blue light, antimicrobial photodynamic therapy and bactericidal ultraviolet irradiation. Phototherapy has an advantage over traditional antibiotics, since it quickly destroys microbial cells and the likelihood of the development of photoresistance in microbes is low. As many authors claim, antimicrobial approaches based on optical radiation have great potential for the treatment of antibiotic-resistant infections and related diseases [3, 4, 5].

According to experimental and clinical studies, the use of technologies based on high-intensity continuous-spectrum ultraviolet radiation allows for the shortest possible time to reduce the contamination of infected wounds, which makes it possible to recommend the use of such phototherapeutic devices for the treatment of severe infectious diseases occurring against the background of severe immunodeficiency and allergic phenomena [6]. The effect of pulsed high-intensity optical irradiation is a highly effective method with a powerful biocidal and immunostimulating effect [7].

Currently, there are many works devoted to the clinical, bactericidal and immunological effectiveness of various phototherapy methods, while studies are rarely found that describe in detail the morphological basis for the effectiveness of their use, including ultraviolet irradiation.

Our work is devoted to studying the effectiveness of high-intensity pulsed broadband irradiation in the treatment of infected wounds using morphological research methods.

Materials and Methods

A morphological study of wound specimens from 105 animals was conducted, which were experimentally modeled with infected wounds. The study was approved by the Interuniversity Ethics Committee (extract from protocol No. 06-23 dated 15.06.23) and was conducted in the vivarium of the Russian University of Medicine of

the Ministry of Health of the Russian Federation. Mature male Wistar rats weighing 200-250 g were used for the experiment. Infected wounds were modeled under aseptic conditions after anesthesia with a 2% solution of xylazine and zoletil 100. The skin in the withers area was cut with a diameter of 20 mm. Hemostasis was performed, after which a trigger was introduced into the wound in the form of a gauze ball moistened with a mixture of cultures from control strains of Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Candida albicans in equal volumes and dilutions, containing 10^9 microbial bodies in 1 ml. The wound with the trigger was sutured. On the next day, the sutures and the trigger were removed, and the animals were randomly divided into three groups. Every day, all animals of all groups without exception underwent wound toilet with 0.1% chlorhexidine solution.

Animals of the 1st (main) group (n=30) underwent high-intensity pulsed broadband irradiation during the treatment. This method was performed using a device based on a pulsed xenon lamp of the "pulsed for pumping lasers straight tube 5/60" type, operating in a pulse-periodic mode with a pulse frequency of 5 Hz and an average electric power of 100 W. The average radiation power of the lamp in the UV-C range of the spectrum (200-280 nm) was 3 W, the pulse power of UV-C radiation was 24 kW.

The software of the used device included the following therapy modes: 1st mode – 50 pulses with an irradiation cycle duration of 10 s; 2nd mode – 100 pulses for 20 s; 3rd mode – 200 pulses with a duration of 40 s. Taking into account the contamination and vastness of the modeled wounds, to combat infection and stop inflammation, wound irradiation in the first five days of treatment was carried out using mode 3 at a distance of 5 cm from the wound. Starting from the sixth day of treatment and for the next five days, mode 2 was used at a distance of 10 cm from the wounds.

On the wounds of animals of the 2nd group, daily, for 10 days, a 3-minute traditional ultraviolet irradiation with the "Solnyshko" ultraviolet quartz irradiator, based on a UV mercury bactericidal lamp of the arc compact bactericidal ultraviolet lamp of type 7, with an electric power of 7 W. The power of UV-C radiation (254 nm) of the lamp was 1.2 W.

And in the 3rd control group of animals, wound treatment was carried out only with the help of an antiseptic by daily toilet and applying a bandage with a 0.1% chlorhexidine solution to the wound.

All manipulations were performed in compliance with the requirements of the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, 1986), the World Medical Association Declaration on the Humane Treatment of Animals (Helsinki, 2000), and in accordance with the requirements of Order No. 267 of the Ministry of Health of the Russian Federation dated 19/06/2003 "Rules for the handling, maintenance, anesthesia and euthanasia of experimental animals".

To control the morphological picture before the start of treatment, 5 animals were withdrawn from the experiment in each group. On the 7th, 14th and 21st days, 10 animals were withdrawn from the experiment in each group. Animals were withdrawn using an overdose of intramuscular anesthesia (2% xylazine and zoletil 100). After euthanasia, soft tissues in the wound area were excised and fixed in 10% formalin solution, followed by specimens of paraffin blocks and histological specimens using standard methods. Morphological examination was performed on specimens stained with hematoxylin and eosin, with a section thickness of 5 μm.

Electron microscopic examination was performed on a JEM 100 CX electron microscope (JEOL, Japan) in transmission mode at an accelerating voltage of 80 kV. For this purpose, biological material was fixed in a 2.5% glutaraldehyde solution, then in a 1% osmium oxide solution and embedded in a mixture of araldite resins. Semi-thin sections (1-1.5 μm) were stained with toluidine blue. Ultra-thin sections were contrasted with uranyl acetate and lead citrate.

For qualitative and quantitative study, histological specimens were pre-scanned on a digital scanner PANNORAMIC 250 Flash (3DHISTECH Ltd. Hungary) and then studied using the program Pannoramic Viewier 1.15.4 (3DHISTECH Ltd. Hungary).

Statistical processing of the obtained results was carried out using the programs Microsoft Office Excel and Statistica 10.0.1011 (StatSoft, Tibco, USA). The analysis was carried out using nonparametric statistical methods, since the preliminary study showed unequal dispersion of the studied features. Descriptive statistics data are presented as a median and interquartile range of 25% and 75%. Comparison of three groups was performed by the Kruskal-Wallis test, where at $p < 0.05$ the sign was considered statistically significantly different in the three groups. Then, paired comparison between the groups was performed using the Mann-Whitney criterion and Bonferroni correction. The sign was considered statistically significantly different between the two groups at $p<0.0167$. Preliminary comparison of the groups over time was performed using the Friedman test for related traits with calculation of the Kendall concordance coefficient (KC). The sign was considered statistically significantly different at $p<0.05$. Then, sequential pairwise comparison of the sign within the groups was performed using the Wilcoxon test with the Bonferroni correction. The sign was considered statistically significantly different between the two groups at $p<0.0167$.

Results

Neutrophilic and lymphocytic infiltration with hemorrhagic areas were observed in the wounds of animals of all groups before treatment (Fig. 1a). The vessels and capillaries found in the specimens were paralytically dilated or filled with erythrocytes. The vascular endothelium was swollen. Collagen fibers present in the wounds were damaged and swollen. In the area of the wound bottom, among the fatty tissue, a large number of mast cells in varying degrees of degranulation were noted (Fig. 1b, c). Foci of hemorrhage were visible in the muscle tissue. The tissue was edematous and

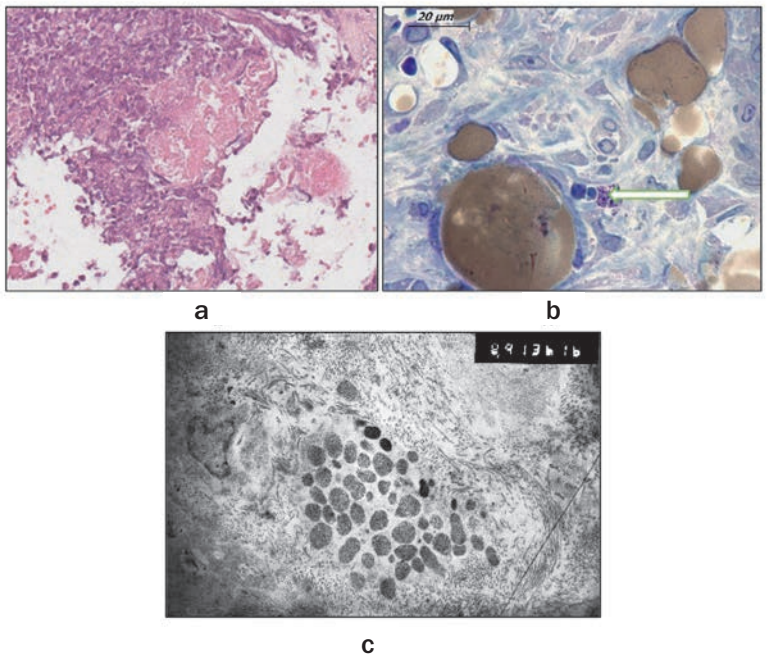

Рис. 1. Препараты ран животных до лечения: a – раневой дефект, некроз ткани, участки кровоизлияния; b – тучная клетка – стрелка; c – тучная клетка в состоянии дегрануляции; Увеличение: a – 200; b – 1000; c – электронограмма x8900. Окраска: a – гематоксилин-эозин; b – толуидиновый синий; c – уранил ацетат и цитрат свинца.
Fig. 1. Specimens of animal wounds before treatment: a – wound defect, tissue necrosis, areas of hemorrhage; b – mast cell – indicated by an arrow; c – mast cell in a state of degranulation. Magnification: a – 200x; b – 1000x; c – electron micrograph x8900. Staining: a – hematoxylin and eosin; b – toluidine blue; c – uranyl acetate and lead citrate.

Таблица 1
Количественный состав клеток в гистологических препаратах ран до начала лечения
Table 1
Quantitative composition of cells in histological wound specimens before treatment

Клеточный состав Cell composition	1-я группа Group 1	2-я группа Group 2	3-я группа Group 3	p – между исследуемыми группами p – between groups
Нейтрофилы Neutrophils	316 (274;346)	303 (276;345)	314 (261;364)	p=0,88
Лимфоциты Lymphocytes	62 (59;68)	59 (57;66)	65 (60;68)	p=0,051
Макрофаги Macrophages	0	0	0	
Дегранулирующие базофилы Degranulating basophils	11 (10;12)	12 (11;13)	11 (10;13)	p=0,049
Базофилы Basophils	0	0	0	
Плазмоциты Plasmacells	1 (1;1)	1 (1;1)	1 (1;1)	p=0,47
Фибробласты Fibroblasts	0	0	0	
Сравнение клеточного состава внутри групп в динамике Comparison within groups over time	p(Н/N)<0.0001, КК=1; p(Л/L)<0.0001, КК=1; p(М/M)<0.0001, КК=1; p(ДБ/DB)<0.0001, КК=0,98; p(Б/B)<0.0001, КК=1; p(П/P)<0.0001, КК=0,58; p(Ф/F)<0.0001, КК=0,98	p(Н/N)<0.0001, КК=1; p(Л/L)<0.0001, КК=0,9; p(М/M)<0.0001, КК=0,78; p(ДБ/DB)<0.0001, КК=1; p(Б/B)<0.0001, КК=0,92; p(П/P)<0.0001, КК=0,92; p(Ф/F)<0.0001, КК=1	p(Н/N)<0.0001, КК=1; p(Л/L)<0.0001, КК=0,93; p(М/M)<0.0001, КК=0,93; p(ДБ/DB)<0.0001, КК=0,98; p(Б/B)<0.0001, КК=0,93; p(П/P)<0.0001, КК=0,81; p(Ф/F)<0.0001, КК=1	

contained damaged vessels with swollen endothelium. The edges of the wound were bordered by thickened epidermis, with signs of edema.

Statistical analysis of the quantitative composition of cells before the treatment revealed no significant difference between the groups (Table 1).

By the 7th day of treatment, edema persisted in the specimens of animals of all groups. A tendency toward a decrease and transformation of the infiltrate was noted. In the 1st group, the infiltration areas acquired the appearance of a layered structure, and hemosiderin granules appeared. The pigment granules were located freely in the intercellular space and macrophages, staining the latter brown (Fig. 2a, b). In other groups, the decrease in infiltrate was less significant. Thus, in the 2nd group of animals, infiltration in the form of rosettes around small vessels was observed. In the wound specimens of animals of the 3rd group, thrombosed blood vessels were noted, both small and larger. Neutrophils in the wound had signs of NETosis. In all groups, degranulation of mast cells was noted (Fig. 2c). In the specimens of the 1st and 2nd groups of animals, young fibroblasts appeared, which in the 1st group of animals were lined up in strands oriented parallel to the wound surface (Fig. 2d). In the 2nd group of animals such orientation of cells was not determined (Fig. 2e).

In all groups, by the 7th day of treatment, the number of neutrophils was significantly reduced compared to the previous day of control (Tables 1, 2). Differences in the number of neutrophils in wound specimens between the groups (Table 2) were statistically significant. Thus, in the 1st group their number was significantly less compared to the 2nd and 3rd groups ($p < 0.0001$ for both groups), and in the 2nd group the number of neutrophils was significantly less compared to the 3rd group ($p < 0.0001$).

Also, in the specimens of animals of the 1st group, the number of lymphocytes was reduced compared to the previous day of control and compared to other groups (Tables 1, 2). In animals of all groups, macrophages appeared in the wounds, which were significantly more in the wound specimens of animals of the 1st group ($p<0.0001$ compared to the 2nd and 3rd groups) and the

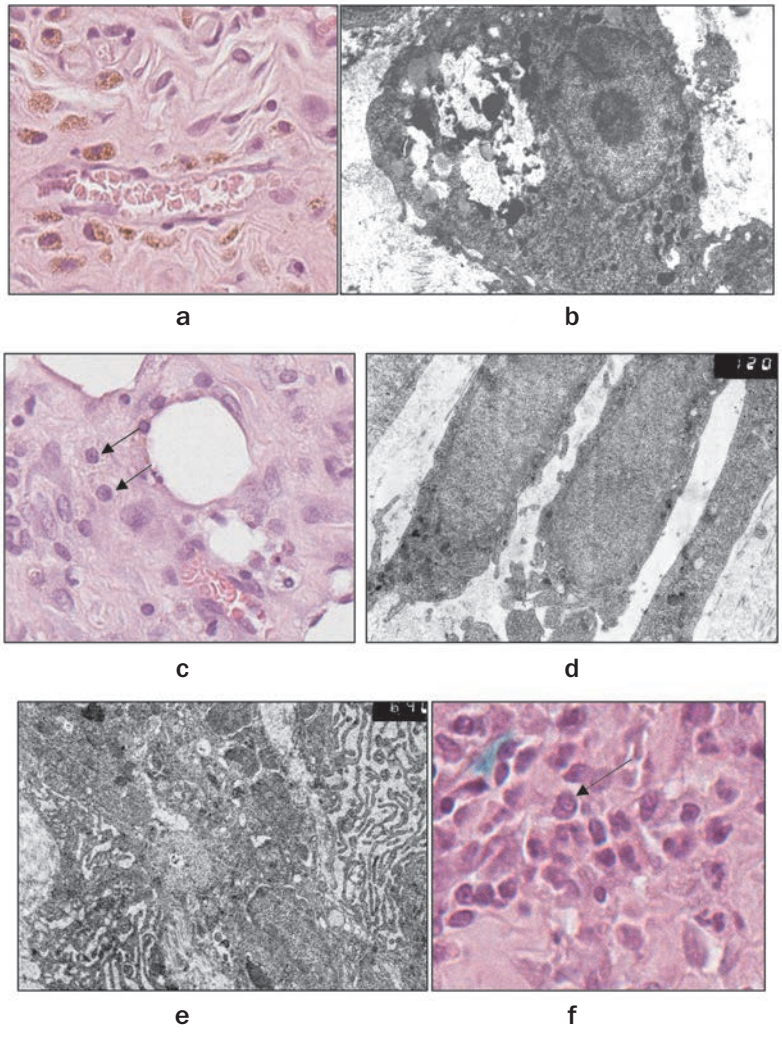

Рис. 2. Препараты ран животных на 7-й день лечения (a-d – 1-а группа; e-f – 2-я группа): a – в соединительной ткани гранулы гемосидерина коричневого цвета; b – функционально активный макрофаг, в центре клетки крупная фагосома с клеточным детритом; c – тучные клетки в состоянии полной дегрануляции; d – фибробласты ориентированные параллельно поверхности раны; e – функционально активные фибробласты без выраженной пространственной ориентации; f – в поле зрения плазмоцит.
a, c, f увеличение: – 400, окраска – гематоксилин-эозин; b, d, e – электронограммы, увеличение – 12000, окраска – уранил ацетат и цитрат свинца.
Fig. 2. Specimens of animal wounds on the 7th day of treatment (a-d: Group 1; e-f: Group 2): a – brown hemosiderin granules in connective tissue; b – functionally active macrophage. Large phagosome with cellular debris in the center; c – mast cells in a state of complete degranulation; d – fibroblasts aligned parallel to the wound surface; e – functionally active fibroblasts without distinct spatial orientation; f – plasma cell in the field of view.
Magnification: a, c, f – staining – hematoxylin and eosin, 400x; b, d, e – electron micrographs, staining – uranyl acetate and lead citrate, magnification – 12000x.

Таблица 2
Количественный состав клеток в гистологических препаратах ран на 7 день лечения
Table 2
Quantitative composition of cells in histological wound specimens on the 7th day of treatment

Клеточный состав Cell composition	1-я группа Group 1	2-я группа Group 2	3-я группа Group 3	p – между исследуемыми группами p – between groups
Нейтрофилы Neutrophils	64 (59;67)	82 (77;86)	176,5 (167;188)	p<0,0001 p(1-2) <0,0001 p(1-3) <0,0001 p(2-3) <0,0001
Лимфоциты Lymphocytes	13 (12;14)	60 (53;67)	73 (72;74)	p<0,0001 p(1-2) <0,0001 p(1-3) <0,0001 p(2-3) <0,0001
Макрофаги Macrophages	34 (30;36)	17 (16;18)	4 (2;6)	p<0,0001 p(1-2) <0,0001 p(1-3) <0,0001 p(2-3) <0,0001
Дегранулирующие базофилы Degranulating basophils	1 (1;1)	4 (3;5)	7 (6;8)	p<0,0001 p(1-2) <0,0001 p(1-3) <0,0001 p(2-3) <0,0001
Базофилы Basophils	6 (5;8)	1 (1;1)	0	p<0,0001 p(1-2) <0,0001 p(1-3) <0,0001 p(2-3) <0,0001
Плазмоциты Plasma cells	3,5 (2;6)	2 (2;2)	1 (1;1)	p<0,0001 p(1-2) <0,0001 p(1-3) <0,0001 p(2-3) <0,0001
Фибробласты Fibroblasts	13,5 (12;17)	2,5 (2;4)	0	p<0,0001 p(1-2) <0,0001 p(1-3) <0,0001 p(2-3) <0,0001
p – внутри каждой исследуемой группы между 7-ым днем контроля и днем до начала лечения p – within each group between Day 7 and pre-treatment	p(Н/N)<0.0001 p(Л/L)<0.0001 p(М/M)<0.0001 p(ДБ/DB)<0.0001 p(Б/B)<0.0001 p(П/P)<0.0001 p(Ф/F)<0.0001	p(Н/N)<0.0001 p(Л/L)=0,62 p(М/M)<0.0001 p(ДБ/DB)<0.0001 p(Б/B)<0.0001 p(П/P)<0.0001 p(Ф/F)<0.0001	p(Н/N)<0.0001 p(Л/L)<0.0001 p(М/M)<0.0001 p(ДБ/DB)<0.0001 p(П/P)=0,69	

2nd group (p<0.0001 compared to the 3rd group). On the 7th day of wound treatment, in response to the therapy, the number of plasma cells in the wound specimens of animals of the 1st and 2nd groups increased (Tables 1, 2; Fig. 2f).

Morphological examination of wound specimens on the 14th day of treatment showed that in specimens of the 1st group of animals, inflammatory tissue infiltration was minimal. Fibroblasts oriented parallel to the wound surface were in a state of high functional activity (as confirmed by ultrastructural analysis), synthesized proteins, including collagen (Fig. 3a). New vessels grew toward the wound center and perpendicular to the wound surface (Fig. 3b). Fibroblasts were grouped along the vessels (Fig. 3c). Mast cells were single, in a state of partial degranulation. (Fig. 3d,e). In the 2nd group, infiltration decreased, in some specimens, formation of new capillaries was noticeable. In the 3rd group, a scab still remained, infiltrate and edema under the scab, neutrophils and macrophages were visible (Fig. 3f). In some areas, infiltration extended to muscle and adipose tissue. There were no histological signs of connective tissue remodeling.

Quantitative analysis showed that the number of neutrophils and lymphocytes on the 14th day of treatment became significantly lower than on the 7th day (Tables 2, 3). The dynamics of the number of macrophages in each group was different. Thus, in the 1st group, their number compared to the 7th day of treatment and compared to other groups was statistically significantly lower ($p < 0.0001$ for all the above indicators). In the 2nd group of animals, the number of macrophages remained at the same level compared to the previous day of control ($p = 0.89$), but was significantly lower than in the 3rd group ($p < 0.0001$). In the wound specimens of the 3rd group, the macrophage indicators increased compared to the

7th day of treatment, which indicated a later response of the body to the treatment. At the same time, a significant decrease in the number of degranulating basophils was detected in all groups ($p<0.0001$ for all groups), while their content was increased in groups 1 and 2 ($p<0.0001$). The mast cell population of groups 1 and 2 restored their

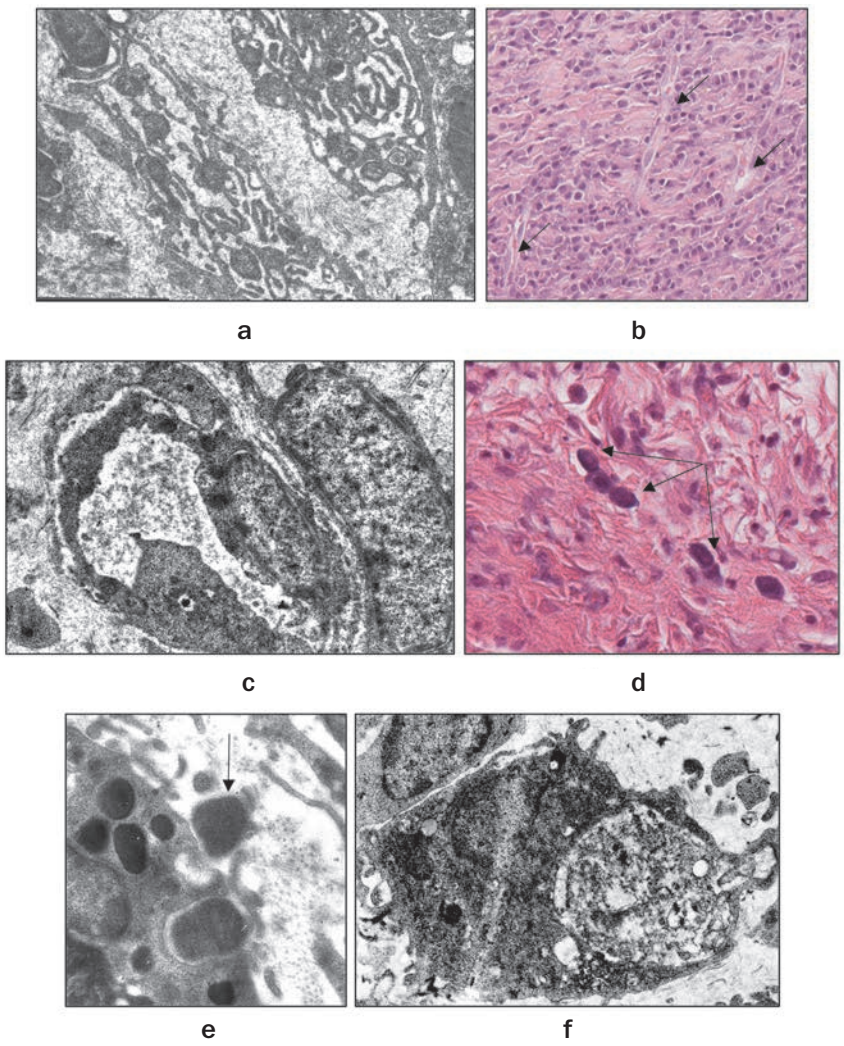

Рис. 3. Препараты ран животных на 14-й день лечения (a-e – 1-я группа; f –3-я группа):
a – функционально активные синтезирующие коллаген фибробласты;
b – к центру раны растут новые кровеносные сосуды – стрелки;
c – фибробласт находится рядом с новообразованным капилляром;
d – тучные клетки – стрелки;
e – дегрануляция тучной клетки, гранула (стрелка) находится среди коллагеновых фибрилл;
f – в цитоплазме макрофага крупная фагосома с клеточным детритом.
Увеличение: b – 200; d – 400; a, c, e, f – электронограммы; a, c, f – 12000; e – 30000
Окраска: b, d – гематоксилин-эозин; a, c, e, f – уранил ацетат и цитрат свинца.
Fig. 3. Specimens of animal wounds on the 14th day of treatment (a-e: Group 1; f: Group 3):
a – functionally active fibroblasts synthesizing collagen;
b – new blood vessels growing towards the wound center – indicated by arrows;
c – fibroblast adjacent to a newly formed capillary;
d – mast cells – indicated by arrows;
e – degranulating mast cell. Granule (arrow) among collagen fibrils;
f – large phagosome with cellular debris in macrophage cytoplasm.
Magnification: b – 200x; d – 400x; a, c, e, f – electron micrographs; a, c, f – 12000x; e – 30000x.
Staining: b, d – hematoxylin and eosin; a, c, e, f – uranyl acetate and lead citrate.

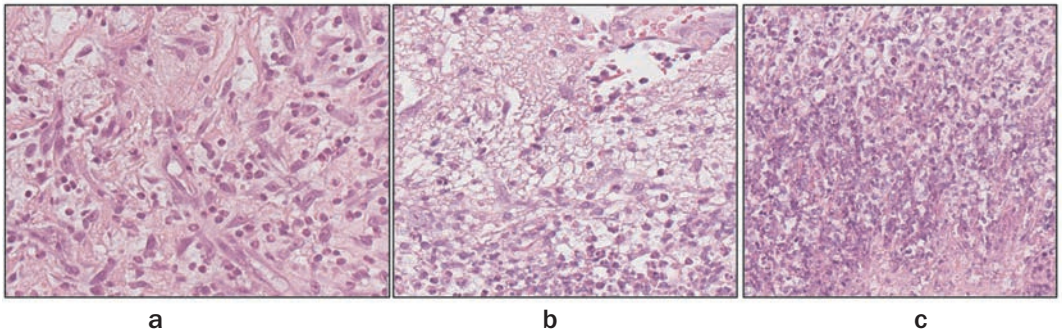

Рис. 4. Препараты раны животных на 1-й день лечения (a – 1-я группа; b – 2-я группа; c – 3-я группа): a – ремоделирование соединительной ткани, множество молодых фибробластов, в том числе в состоянии митоза; b – ремоделирование ткани замедлено, единичные новообразованные сосуды, макрофаги, сохраняется инфильтрация и отек соединительной ткани; c – инфильтрация и отек соединительной ткани; a, b, c – увеличение – 200, окраска – гематоксилин-эозин.
Fig. 4. Specimens of animal wounds on the 21st day of treatment (a: Group 1; b: Group 2; c: Group 3): a – connective tissue remodeling with numerous young fibroblasts, some in mitosis; b – slowed tissue remodeling, few newly formed vessels, macrophages, persistent infiltration, and connective tissue edema; c – persistent infiltration and connective tissue edema.
Magnification: a, b, c – 200x, staining – hematoxylin and eosin.

Таблица 3
Количественный состав клеток в гистологических препаратах ран на 14-й день лечения
Table 3
Quantitative composition of cells in histological wound specimens on the 14th day of treatment

Клеточный состав Cell composition	1-я группа Group 1	2-я группа Group 2	3-я группа Group 3	p – между исследуемыми группами p – between groups
Нейтрофилы Neutrophils	10,5 (7;14)	27 (25;34)	56 (49;61)	p<0,0001 p(1-2)<0,0001 p(1-3)<0,0001 p(2-3)<0,0001
Лимфоциты Lymphocytes	2 (2;2)	27 (25;29)	55 (51;60)	p<0,0001 p(1-2)<0,0001 p(1-3)<0,0001 p(2-3)<0,0001
Макрофаги Macrophages	6 (5;7)	17 (14;21)	33 (25;42)	p<0,0001 p(1-2)<0,0001 p(1-3)<0,0001 p(2-3)<0,0001
Дегранулирующие базофилы Degranulating basophils	0	1 (1;1)	2 (2;2)	p<0,0001 p(1-2)<0,0001 p(1-3)<0,0001 p(2-3)<0,0001
Базофилы Basophils	13 (12;15)	6 (5;8)	1 (1;1)	p<0,0001 p(1-2)<0,0001 p(1-3)<0,0001 p(2-3)<0,0001
Плазмоциты Plasmacells	2 (2;2)	1 (1;1)	1 (1;1)	p<0,0001 p(1-2)<0,0001 p(1-3)<0,0001 p(2-3)=0,7
Фибробласты Fibroblasts	45 (33;52)	17 (15;19)	5 (4;6)	p<0,0001 p(1-2) <0,0001 p(1-3) <0,0001 p(2-3) <0,0001
p – внутри каждой исследуемой группы между 14-ым и 7-ым днями p – within each group between Day 14 and Day 7	p(Н/N)<0.0001 p(Л/L)<0.0001 p(М/M)<0.0001 p(ДБ/DB)<0.0001 p(Б/B)<0.0001 p(П/P)<0.0001 p(Ф/F)<0.0001	p(Н/N)<0.0001 p(Л/L)<0.0001 p(М/M)=0,89 p(ДБ/DB)<0.0001 p(Б/B)<0.0001 p(П/P)<0.0001 p(Ф/F)<0.0001	p(Н/N)<0.0001 p(Л/L)<0.0001 p(М/M)<0.0001 p(ДБ/DB)<0.0001 p(Б/B)<0.0001 p(П/P)=0,53 p(Ф/F)<0.0001	

functional activity. On the part of the connective tissue component, an increase in the content of fibroblasts was noted in groups 1 and 2 (Tables 2, 3). Single basophils and fibroblasts appeared in the wound specimens of group 3.

By the 21st day of treatment, there was a significant dynamics of morphological signs. Thus, when examining wound specimens of animals in group 1, in which high-intensity pulsed broadband irradiation was used in the treatment, connective tissue remodeling was observed in favor of complete restoration of the structure. In group 2 animals, in which traditional ultraviolet irradiation was used in the treatment of wounds, the macrophage reaction persisted. In the connective tissue under the scab, capillaries were present, although in smaller quantities than in group 1. In wound specimens of animals in groups 1 and 2, there is a large number of inactive mast cells in the connective tissue (Table 4). In group 3, an admixture of neutrophils (Table 4) and thrombosis of small vessels are preserved. Edema persists, which is expressed in connective and adipose tissue.

Discussion

Wound healing is a complex biological process with successively overlapping physiological and morphological phases. To restore the barrier function of damaged skin, coordination of cellular and molecular processes is necessary. At the beginning of the wound healing process, in the first phase of inflammation, cellular elements such as neutrophils and macrophages migrate to the wound, mobilizing local and systemic defense. In the next phase, proliferation, connective tissue cells, fibroblasts and keratinocytes, begin to

Таблица 4
Количественный состав клеток в гистологических препаратах ран на 21-й день лечения
Table 4
Quantitative composition of cells in histological wound specimens on the 21st day of treatment

Клеточный состав Cell composition	1-я группа Group 1	2-я группа Group 2	3-я группа Group 3	p – между исследуемыми группами p – between groups
Нейтрофилы Neutrophils	0	6,5 (5;9)	23 (19;26)	p<0,0001 p(1-2) <0,0001 p(1-3) <0,0001 p(2-3) <0,0001
Лимфоциты Lymphocytes	0	3 (2;4)	14 (12;18)	p<0,0001 p(1-2) <0,0001 p(1-3) <0,0001 p(2-3) <0,0001
Макрофаги Macrophages	2 (2;2)	10 (8;13)	25 (23;27)	p<0,0001 p(1-2) <0,0001 p(1-3) <0,0001 p(2-3) <0,0001
Дегранулирующие базофилы Degranulating basophils	0	0	0	p<0,0001 p(1-2) <0,0001 p(1-3) <0,0001 p(2-3) <0,0001
Базофилы Basophils	19 (18;20)	9 (7;11)	2 (2;2)	p<0,0001 p(1-2) <0,0001 p(1-3) <0,0001 p(2-3) <0,0001
Плазмоциты Plasmacells	2 (2;2)	0	0	p<0,0001 p(1-2) <0,0001 p(1-3) <0,0001
Фибробласты Fibroblasts	66 (59;77)	34 (27;40)	14 (12;16)	p<0,0001 p(1-2) <0,0001 p(1-3) <0,0001 p(2-3) <0,0001
p – внутри каждой группы между 21-ым и 14-ым днем p – within each group between Day 21 and Day 14	p(Н/N)<0.0001; p(Л/L)<0.0001; p(М/M)<0.0001; p(Б/B)<0.0001; p(П/P)=1 p(Ф/F)<0.0001	p(Н/N)<0.0001 p(Л/L)<0.0001 p(М/M)<0.0001 p(ДБ/DB)<0.0001 p(Б/B)<0.0002 p(П/P)<0.0001 p(Ф/F)<0.0001	p(Н/N)<0.0001 p(Л/L)<0.0001 p(М/M)<0.0001 p(ДБ/DB)<0.0001 p(Б/B)<0.0001 p(П/P)<0.0001 p(Ф/F)<0.0001	

actively proliferate, triggering the process of connective tissue remodeling. The third phase of the wound healing process is the phase of regeneration or epithelialization, when fibroblasts begin to synthesize collagen, and the organization of a new matrix begins [8].

Various methods are used to analyze and evaluate wound healing, including planimetry and morphological examination. Planimetry allows clinical evaluation of the visible characteristics of the wound and, due to the presence of a scab, often does not correlate with the healing indices visualized by morphology, which makes morphological examination the "gold standard" for assessing wound healing [9].

There are data on the effectiveness of phototherapy in the treatment of wounds, including infected ones, however the studies mainly concern laser or photodynamic therapy, and the authors point out the clinical, bactericidal and morphological effectiveness of their use [10, 11, 12]. There are few works devoted to studying the effectiveness of ultraviolet irradiation in the treatment of infected wounds, moreover they show only bactericidal effectiveness [13, 14].

K. Narita *et al.* in an experiment on mice, having modeled a wound infected with St. Aureus (MRSA), irradiated it with two types of lamps with radiation in the range of 222 nm and 254 nm. The authors performed bacteriological, histological and immunohistochemical studies. In the morphological study, the authors found a decrease in the inflammatory response to ultraviolet irradiation of infected wounds in the 222 nm range. In the study, histological analysis was performed only on the 5th and 8th days, and only neutrophilic infiltration was assessed without a statistical assessment of the full morphological picture [15].

V.V. Bagrov *et al.* showed the bactericidal, clinical and morphological effectiveness of antibacterial therapy in combination with high-intensity optical irradiation of infected wounds compared to the traditional use of Levomekol ointment (dioxomethyltetrahydropyrimidine (methyluracil), chloramphenicol). Histological analysis showed an earlier transition of the wound process (day 14) to the granulation phase in animals whose wounds were exposed to high-intensity optical irradiation, compared to the wounds of animals in the control group [16].

Thus, the data on the use of ultraviolet irradiation of infected wounds is limited and is presented mainly by a description of bactericidal effectiveness. In the existing studies, morphological signs of tissue regeneration are presented only at the initial stages of the wound process, at the stages of inflammation and proliferation, and there is no data on the full morphological picture occurring in the wound during treatment with ultraviolet irradiation until the completion of regenerative processes.

In our study, we present a qualitative and quantitative analysis of the morphological picture using high-intensity pulsed broadband irradiation, traditional ultraviolet irradiation and classical local application of an antiseptic.

Our experimental study of the treatment of infected wounds showed that before the treatment, the morphological picture of the wounds corresponded to the acute inflammation phase. On the 7th day in the 1st group, the morphological picture corresponded to the proliferation phase. In the 2nd and 3rd groups, edema and infiltration persisted. By the 14th day, signs of granulation tissue formation and the transition of wounds to the regeneration stage were observed in the 1st group. In the 2nd group, infiltration decreased, new capillaries appeared, and the number of fibroblasts increased.

In the 3rd group, inflammatory phenomena persisted. By the 21st day, there were no signs of inflammation in the wound specimens of the 1st group, connective tissue remodeling with signs of formation of a delicate scar was observed. In the specimens of the 2nd group, signs of infiltration were minimal, connective tissue remodeling phenomena were observed, in which new capillaries were present. In the wound specimens of the 3rd group of animals, infiltration was reduced, new vessels were formed with a slowdown.

Conclusion

The morphological study showed that the use of high-intensity pulsed broadband irradiation of infected wounds, in contrast to traditional ultraviolet irradiation and treatment of wounds with antiseptics at an earlier stage, stops the inflammatory reaction of tissues, activates the local immune response and accelerates the processes of connective tissue remodeling.

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