A PLANIMETRIC STUDY OF EXPERIMENTALLY MODELED INFECTED WOUNDS EXPOSED TO HIGH-INTENSITY PULSED BROADBAND RADIATION

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Abstract

An experimental model of an infected wound was created in 90 Wistar rats using a mixture of cultures of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Candida albicans*. The animals are divided into 3 groups of 30 individuals. The first group consisted of animals treated with pulsed high-intensity broadband irradiation using an experimental apparatus with a pulsed xenon lamp operating in a pulsed periodic mode with an average UV-C (200-280 nm) radiation power of 3 W, and a pulsed UV-C power of 24 kW. In the second group, traditional ultraviolet irradiation of wounds with a mercury bactericidal lamp was used for treatment, with an average UV-C (254 nm) radiation power of 1.2 W. The third group received only a local antiseptic treatment. The computer planimetry was used for monitoring the effectiveness of treatment. Parameters such as wound area, rate, and degree of epithelialization were recorded on days 1, 7, 14, and 21 of treatment. The study showed that in the first group of animals, the rate and degree of epithelialization, as well as the reduction in wound area at each control stage, were statistically significantly greater compared to the use of traditional ultraviolet irradiation and local antiseptic monotherapy. This dynamic is associated with the earlier cleansing of wounds from pathogenic microorganisms and the morphological changes that correspond to an earlier transition from the inflammatory phase to the proliferation and regeneration phases. Therefore, the local treatment of infected wounds with antiseptic agents in combination with pulsed high-intensity wideband radiation promotes the earlier epithelialization of the wounds.

Keywords: purulent wounds, infected wounds, high-intensity pulsed broadband radiation, ultraviolet radiation, regeneration, epithelialization, planimetry

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ПЛАНИМЕТРИЧЕСКОЕ ИССЛЕДОВАНИЕ ЭКСПЕРИМЕНТАЛЬНО МОДЕЛИРОВАННЫХ ИНФИЦИРОВАННЫХ РАН ПРИ ВОЗДЕЙСТВИИ ВЫСОКОИНТЕНСИВНОГО ИМПУЛЬСНОГО ШИРОКОПОЛОСНОГО ОБЛУЧЕНИЯ

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Резюме

Изучено действие широкополосного облучения на инфицированные раны. Экспериментально моделирована инфицированная рана у 90 крыс линии Wistar при помощи смеси культур *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Candida albicans*. Животные были разделены на 3 группы по 30 особей. Первую группу составили животные для лечения, которых использовано импульсное высокоинтенсивное широкополосное облучение экспериментальным аппаратом с импульсной ксеноновой лампой, работающей в импульсно-периодическом режиме со средней мощностью УФ-С (200-280 нм) излучения – 3 Вт, импульсной мощностью УФ-С – 24 кВт. Во второй группе для лечения использовали традиционное ультрафиолетовое облучение ран ртутной бактерицидной лампой, со средней мощностью УФ-С (254 нм) излучения – 1,2 Вт. В третьей группе использовали только местное применение антисептика. Для контроля эффективности проводимого лечения нами использована компьютерная планиметрия. Учтены параметры площади, скорости и степени эпителизации ран в 1-й, 7-й, 14-й и 21-й дни лечения. Исследование показало, что у животных первой группы скорость и степень эпителизации и, соответственно, уменьшение площади ран на каждом этапе контроля статистически значимо больше по сравнению с использованием традиционного ультрафиолетового облучения и местной монотерапии антисептиком. Такая динамика связана с более ранним очищением ран от патогенной микробной флоры и более ранним переходом воспалительной фазы в фазу пролиферации и регенерации. Таким образом, местное лечение инфицированных ран антисептиками с комбинацией импульсного высокоинтенсивного широкополосного облучения способствует более ранней эпителизации ран.

Ключевые слова: гнойные раны, инфицированные раны, высокоинтенсивное импульсное широкополосное облучение, ультрафиолетовое облучение, регенерация, эпителизация, планиметрия.

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Introduction

The factors of ineffective prevention and treatment of purulent complications in surgery are increasing resistance of microorganisms to antibacterial therapy, decreasing reactivity of the organism, increasing number of surgeries, their complexity, including in comorbid and elderly patients. All this determines the need to improve the existing and develop new types of prophylaxis and treatment [1,2,3].

In this regard, the works based on the effect of optical radiation on infected wounds are of considerable interest. In the scientific bases there are data on the successful application of various types of this radiation in wound infection: visible, infrared, ultraviolet radiation, as well as photodynamic therapy. The mentioned types of optical radiation have a destructive effect on *Pseudomonas bacillus* and multidrug-resistant strains (e.g., MRSA) and they also improve regenerative processes in the wound [4,5,6,7].

The biocidal effect of optical radiation is based on the presence of different spectral absorption bands in different microorganisms. The maximum destructive effect will be achieved in microorganisms whose maximum spectral sensitivity will coincide or is maximally close to the spectral line of the radiation source. The biocidal effect of UV radiation has been known for a relatively long time, as well as the fact that different parts of the UV spectrum have different biological activity. The UV-C region (from 200 to 280 nm) has the maximum biocidal effect, causing the death of various types of microorganisms - bacteria, spores and viruses. In this regard, the use of high-intensity pulsed broadband optical irradiation is of great interest. However there are very few works devoted to this method of exposure, which does not allow making reliable conclusions about the feasibility of its application, and requires further study and improvement [8,9].

The aim of the study was to evaluate the rate of wound surface reduction under high-intensity pulsed broadband irradiation of experimentally modeled infected wounds.

Materials and Methods

An experimental study with modeling of infected wound on 90 male Wistar rats was carried out. The approval of the Interuniversity Ethics Committee was obtained (extract from protocol No. 06-23 dated 15.06.23). Under aseptic conditions and general anesthesia with the solution of xylazine and zoletil 100 in the withers area a bordering incision with removal of skin and subcutaneous tissue up to fascia was performed, after which a defect with a diameter of 20 mm was formed. Infection of soft tissues was performed with a trigger moistened in 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 skin defect was sutured and aseptic dressing was applied. After 1 day, the wound was opened by removing the sutures. Daily all animals without exception were treated with 0.1% chlorhexidine solution. The studied animals were randomly divided into three groups of thirty animals each.

In the first, main, group of animals for wound treatment, high-intensity pulsed broadband irradiation was performed using an experimental apparatus developed by the Research Institute of Power Engineering of Bauman Moscow State Technical University. The apparatus is equipped with a pulsed xenon lamp of PPD (pulsed, pumping, direct) 5/60 type, operating in pulse-periodic mode with pulse frequency of 5 Hz and average electric power of 100 W. The average power of the lamp radiation 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 device had three modes with irradiation cycle duration of 10 s (mode 1-50 pulses), 20 s (mode 2-100 pulses) and 40 s (mode 3-200 pulses). We selected the following method of wound treatment: in the first 5 days of treatment we used radiation mode 3 (200 pulses with irradiation cycle duration of 40 s) with irradiation distance of 5 cm from the wound; starting from the 6th day of treatment the next 5 days mode 2 was used (100 pulses with cycle duration of 20 s) at a distance of 10 cm.

In the second group of animals for the treatment of wounds traditional UV irradiation with the device UQI-01 (Ultraviolet Quartz Irradiator) "Solnyshko", UV quartz irradiator with a mercury bactericidal lamp type ACBU-7 (arc, compact, bactericidal, universal) with a power of 7 W, and UV-C with a radiation power 1.2 W - 275-180 nm were used. Irradiation was carried out daily for 10 days for 3 min at a distance of 10 cm from the wound. And in the third, control, group of animals the treatment of wounds was carried out only with the help of antiseptic by daily toileting and applying a dressing with 0.1% chlorhexidine solution to the wound.

One of the criteria of treatment efficiency was the study of wound healing parameters, which include the rate and degree of epithelialization, indicators of area reduction and direct control of wound area in different periods of treatment. All these parameters were analyzed.

To measure the wound area, ImageJ - a computer program for image analysis and editing - was used on the Windows XP platform. For this purpose, at each control stage (day 1, day 7, day 14, day 21 of treatment) we photographed the wound and saved the file in JPEG format. Then each of the JPEG files was opened in ImageJ program and wound area parameters were calculated. The rates and degrees of wound epithelialization were calculated in the periods between days of area control, and therefore time periods or intervals were identified. The first of such periods was the time interval from the beginning of treatment to the 7th day. The second period was from the 7th to the 14th day of treatment, and the third control period was from the 14th to the 21st day of treatment.

The study of wound epithelization rate was performed according to the following formula:

$$ER=S-Sn/t\ (mm^2)$$

where ER is the rate of wound epithelization per day, S is the area of the defect in the previous study, Sn is the area of the defect in the current study and t is the number of days between studies.

Analysis of the dynamics of the epithelization rate was performed using the following formula:

$$Sc=(S-Sn)x100/Sxt\ (\%)$$

where Sc is the degree of ulcer epithelization per day (in %), S is the ulcer area in the previous study, Sn is the ulcer area in this study, t is the number of days between the previous and this study.

Results

The analysis of wound epithelization rate showed that in the first 7 days of treatment against the background of cleansing in the first group of animals, to which pulsed high-intensity broadband irradiation was used in the treatment of wounds, the dynamics of epithelization rate was the highest compared to that in the animals of the second and third groups (Fig. 1). Thus, in the first group the epithelization rate in the first 7 days of treatment was 1.43 (1.29;1.71) mm/day ($p<0.0001$ in relation to the indicators of the second and third groups), while in the second and third groups it was 0.86 (0.57;0.87) mm/day ($p=0.0024$ in relation to the indicators of the third group) and 0.57 (0.57;0.71) mm/day, respectively. In the time interval between the 7th and 14th days the rates of wound epithelization in animals of the first and second groups did not differ significantly ($p=0.61$), and were equal to 0.71 (0.71;0.86) mm/day and 0.71 (0.57;0.86) mm/day, which was statistically significantly higher compared to the same indicators in animals of the third group (0.57(0.43;0.71) mm/day $p=0.004$ and $p=0.016$, respectively).

When comparing the epithelization rate within the groups (Fig. 1) between the first and second time intervals, it was revealed that the rate of wound epithelization decreased after the 7th day of treatment compared to the first 7 days of treatment in the first group of animals ($p<0.0001$). In the interval between

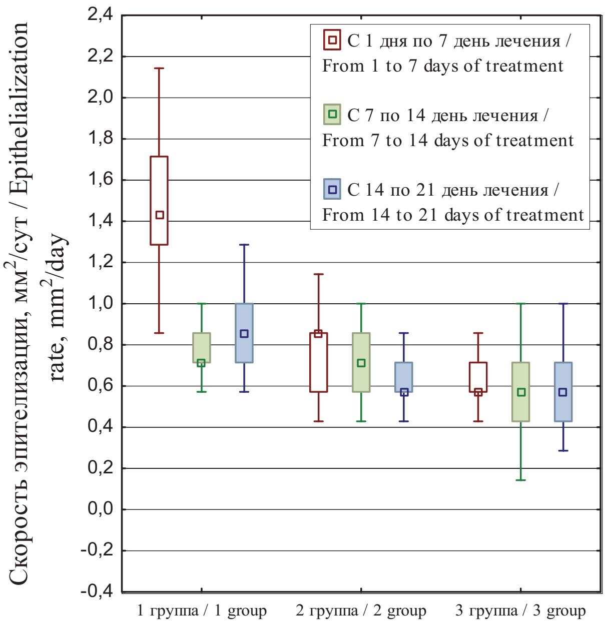

Рис. 1. Показатели скорости эпителизации ран у животных с разными видами лечения (мм/сут)
Fig. 1. Indicators of wound epithelialization rate in animals with different types of treatment (mm/day)

the 14th and 21st days of treatment the rate of wound epithelization in the first group of animals was 0.86 mm/day, which is statistically significantly higher compared to the indicators of the animals of the second and third groups ($p<0.0001$ for both groups). At the same time, in the first group, where pulsed high-intensity broadband irradiation was used during wound treatment, and in the second group, where traditional ultraviolet irradiation was used, in the period from the 14th to the 21st days of treatment the epithelization rate was significantly lower in comparison with the first control period (in the first 7 days of treatment) ($p<0.0001$ for the first group, $p=0.006$ for the second group).

During the first 7 days of treatment in animals of the first group the degree of wound epithelization was 3,97 (3,47;4,4) % per day ($p<0,0001$ in relation to the indicators of the second and third groups), in the second group it was 2,2 (1,59;2,38) % per day ($p<0,0001$ in relation to the indicators of the third group). In animals of the third group, where only antiseptic degree was used in wound treatment, epithelization was equal to 1.59 (1.5;1.88) % per day (Fig. 2).

In the period from the 7th to the 14th day the degree of wound epithelization in animals of the first group decreased in comparison with the previous period to 2.7 (2.38;3.06) % per day ($p<0.0001$). At the same time it was statistically significantly higher compared to the indicators of animals of the second and third groups ($p=0.003$ and $p<0.0001$, respectively). In animals of the second and third groups, the dynamics of the degree of epithelization did not change significantly compared to the previous control period (Fig. 1). In the control period from day 14 to day 21 an increase in the degree of wound epithelization up to 3.99 (3.43;4.55) % per day was observed in animals treated with pulsed high-intensity broadband irradiation, which was significantly higher compared to the indicators in the previous control period ($p<0.0001$) and compared to the indicators of the animals of the second and third groups ($p<0.0001$). In animals of the second and third groups, the indicators of the degree of epithelization were not significantly different from the previous control periods and compared to each other, and were equal to 2.2 (2.04;2.65) % per day and 2.04 (1.71;2.46) % per day (Fig. 2), respectively.

The study showed that during the whole period of observation the degree of wound epithelization in animals of the first group was 2.78 (2.65;2.83) % per day, which was statistically significantly higher compared to the indicators in the first and second groups ($p<0.0001$). In animals of the second group the degree of wound epithelization was equal to 1.85 (1.72;2.06) % per day ($p=0.00012$ compared to the third group), and in animals of the third group 1.58 (1.5;1.85) % per day (Fig. 2).

The area of wounds before treatment in animals of all three groups was not statistically different (Fig. 3). By the 7th day of treatment there was a statistically significant positive dynamics within each group compared to the initial indicators ($p<0.0001$ for each group). It should be noted that when comparing the wound area between the groups there was a significant difference ($p<0,0001$). In the first group of animals, where treatment was carried out by pulsed high-intensity broadband irradiation, the

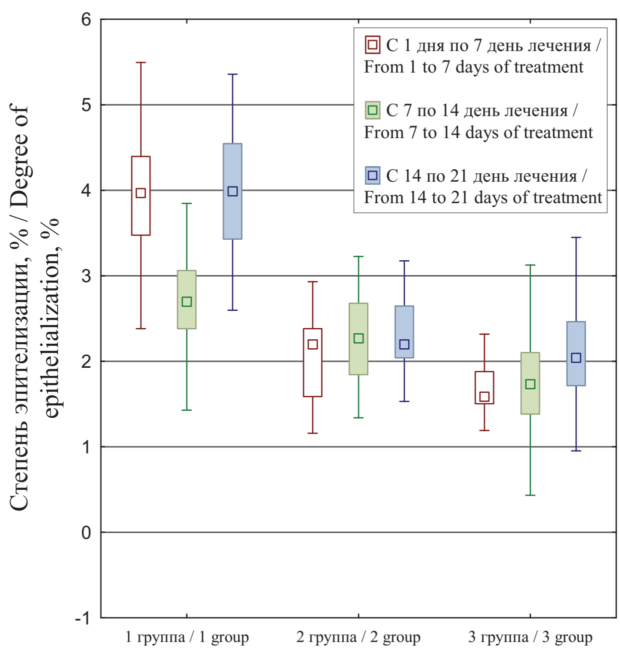

Рис. 2. Показатели степени эпителизации ран у животных с разными видами лечения (%).
Fig. 2. Indicators of the degree of epithelialization of wounds in animals with different types of treatment (%).

wound area decreased by 10 (9;12) mm², and was equal to 27 (26,28) mm² (p<0,0001 in relation to the indicators of the second and third groups). And in the third group, the area was 33 (32;34) mm², decreasing by 4 (4;5) mm².

By day 14 of treatment, the wound area in all groups of animals was statistically significantly smaller compared to day 7 of control (p<0.0001). In the first group of animals the wound area decreased compared to day 7 of the control by 6 (4;6) mm² and was equal to 22 (21;23) mm²; in the second group it decrease by 5 (4;6) mm² and amounted to 27 (26;28) mm²; and in the third group the wound decreased by 4 (4;5) mm², reaching 29 (28;30) mm². At this point in the study, there was a statistically significant difference in wound area between the groups.

The wound area on day 21 of treatment within each group was statistically significantly smaller compared to the previous day's control. In group one, the wound surface area was 15 (15;16) mm² (p<0.0001 compared to the rates in groups one and two), while in group two it was 22 (21;24) mm² (p<0.0001 compared to the rates in group three), and in group three it was 24 (23;26) mm².

Further analysis showed that in the first group of animals, where pulsed high-intensity irradiation was applied to treat infected wounds, complete epithelization of wounds was achieved within 37 (36;38) days (Fig. 4), which was statistically significantly less compared to the group where conventional UV irradiation was performed (p<0.0001) and to the group using only antiseptic (p<0.0001). In the second group of animals, we observed complete epithelialization of wounds by the 48th (47;49) day of treatment, which was significantly less (p<0.0001) compared to the third group, where complete epithelialization was achieved by the 61st (59;61) day.

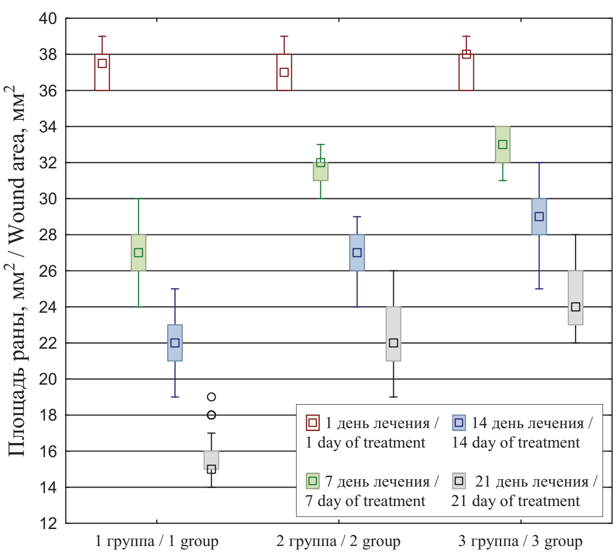

Рис. 3. Динамика площади ран у животных с разными методами лечения.
Fig. 3. Dynamics of wound area in animals with different treatment methods.

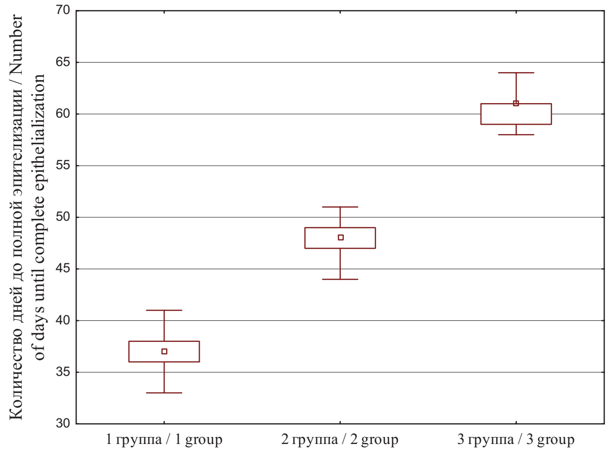

Рис. 4. Сроки полной эпителизации инфицированных ран у животных трех групп с различными методами лечения.
Fig. 4. The timing of complete epithelialization of infected wounds in animals of three groups with different treatment methods.

Discussion

In modern scientific literature there are a sufficient number of studies devoted to the treatment of wounds, including infected ones. According to Ashja Zadeh M.A. *et al*., the area of experimentally infected wounds in rats with diabetes when using the extract of Crocus Pallasii Subsp. Haussknechtii Boiss leaves by the 21st day of treatment is reduced by more than three times compared to classical treatment methods. At the same time, the authors claim bactericidal effectiveness when using this extract [10]. There is also evidence that the combined use of local antiseptic polyhexanide and exposure of the wound surface to erbium laser in chronic purulent wounds contributes to significant cleansing, decontamination and, consequently, earlier epithelization of wounds [11]. Another method of exogenous physical treatment of infected wounds is cryotherapy. Thus, according to some authors, irrigation of the wound surface with liquid nitrogen, both monotherapy and in combination with local application of antiseptics, as well as in combination with antibacterial therapy, leads to a rapid decrease in signs of inflammation, activates the processes of granulation and epithelialization [12].

To assess the effectiveness of wound treatment in modern surgery, numerous methods of control are used, including bacterial, cytologic, morphologic, which includes both light microscopy, immunohistochemical methods and electron microscopy, as well as morphometry and other control methods. One of the most used criteria for analyzing the results of wound treatment, including infected wounds, is planimetry, a method of dynamic control of the wound area. With the development of computer technology in modern practical surgery, doctors use electronic programs and mobile applications for planimetry [13]. There is data on the use of computer planimetry, which allows to analyze different parameters of the wound surface, such as the area, the presence of necrosis in the wound, fibrin plaque, the appearance of granulation and epithelization, which makes it possible to determine the choice of further treatment, compare and evaluate different approaches and methods of local therapy [14].

We modeled an experimentally infected wound using a mixture of cultures of *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Candida albicans*. The animals were divided into three groups depending on the type of treatment. The first group consisted of animals for the treatment of which a combination of pulsed high-intensity broadband irradiation and local application of antiseptic was used. In the second group a combination of traditional ultraviolet irradiation of wounds was used with local application of antiseptic. In the third group only local application of antiseptic was used. To control the effectiveness of treatment computerized planimetry was applied. At each stage of the control photofixation of the wound was carried out, subsequently the photo file in JPEG format was entered into the ImageJ program and counting of the wound area was performed. The planimetric study showed that in animals that were treated with pulsed high-intensity broadband irradiation for the treatment of infected wounds, the rate and degree of epithelialization and, accordingly, the reduction in the area of wounds at each stage of control were statistically significantly greater compared to the use of traditional ultraviolet irradiation and local antiseptic monotherapy. Such dynamics is associated with earlier clearing of wounds from pathogenic microbial flora [15] and morphologic picture [16, 17], i.e. earlier transition of the inflammatory phase to the phase of proliferation and regeneration.

Conclusion

Local treatment of infected wounds with antiseptics and combination of pulsed high-intensity broadband irradiation promotes earlier epithelization of wounds.

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