International Journal of Biomedicine 12(1) (2022) 138-141
http://dx.doi.org/10.21103/Article12(1)_OA15

ORIGINAL ARTICLE

Experimental Medicine

INTERNATIONAL JOURNAL OF BIOMEDICINE

Comparative Evaluation of Isolated and Complex Use of Hexetidine and Photoditazine in Combination with Ultrasound Therapy in the Treatment of Purulent Wounds under Experimental Conditions

Ivan S. Dragovoz, Dmitry S. Zotov, Maria A. Zatolokina*, Arsen Yu. Grigoryan, Ekaterina S. Mishina, Vadim V. Tsymbalyuk

Kursk State Medical University, Kursk, Russia

Abstract

Background: Currently, despite the significant success of the pharmaceutical industry in producing drugs with antibacterial activity, the treatment of soft tissue diseases accompanied by purulent-inflammatory processes within them remains one of the topical problems of general surgery. Not only the widespread introduction of antibacterial agents in practical medicine, but also the steady increase in the resistance of microorganisms to existing drugs leads to the need to search for new combinations aimed at the local treatment of purulent wounds. The purpose of our work was to study the course of the wound process in a purulent wound by using hexetidine and photoditazine in combination with ultrasound treatment.

Methods and Results: This experimental study was carried out on 144 male Wistar rats. The course of the wound process was evaluated on a model of a purulent wound. The animals were divided into 4 equal groups (36 animals in each group), depending on their treatment. Treatment was carried out using daily dressings for 15 days. The dynamics of changes in the area of the wound were assessed by the planimetry method. The percentage of area reduction of the wound was calculated. A histological examination of a skin area (1.0 cm×1.5 cm) taken in the zone of a simulated purulent wound was carried out on Days 5 and 10 of the experiment. Our study confirmed the effectiveness of the use of hexetidine, photoditazine, and a combination of them, along with ultrasound treatment, in the local treatment of a purulent wound. At the same time, the combined effect of an antiseptic and a photosensitizer showed significantly better results in the first and second phases of the course of the wound process than did their isolated use.(**International Journal of Biomedicine. 2022;12(1):138-141.**)

Key Words: purulent wound • hexetidine • photoditazine • ultrasound therapy • methylcellulose

For citation: Dragovoz IS, Zotov DS, Zatolokina MA, Grigoryan AYu, Mishina ES, Tsymbalyuk VV. Comparative Evaluation of Isolated and Complex Use of Hexetidine and Photoditazine in Combination with Ultrasound Therapy in the Treatment of Purulent Wounds under Experimental Conditions. International Journal of Biomedicine. 2022;12(1):138-141. doi:10.21103/Article12(1)_OA15

Abbreviations

UST, ultrasound treatment; **MC**, methylcellulose; **H**, hexetidine; **PD**, photoditazine; **DMTHP**, dioxomethyltetrahydropyrimidine; **CP**, chloramphenicol.

Introduction

At present, the question of treating the purulent-inflammatory process of soft tissues is one of the most urgent. Despite the vast range of treatment options for this process, purulent-inflammatory diseases occupy a leading position. They account for 30%-45% of the conditions and complications in surgical patients, both outpatient and inpatient.[1,2] This problem is associated with the uncontrolled use of antibiotics and the emergence of resistant microorganisms.[3,4] Therefore, at present, interest has increased in antiseptics and photosensitizers, which have proven themselves in the treatment of purulent wounds while not causing resistance in microorganisms.[5] However, despite the variety of different means of treatment, the number

of patients with purulent-inflammatory diseases of soft tissues is not decreasing.[6] In this regard, there is a need to use the latest treatment methods, using physical factors of influence. One of these methods is UST, which has not only a bactericidal and bacteriostatic effect on microorganisms, but also enhances the effect of antiseptics.[7,8] Thus, the search for and development of the most optimal combination of a drug with ultrasound therapy is an urgent and priority task in modern surgery.

The purpose of our work was to study the course of the wound process in a purulent wound by using hexetidine and photoditazine in combination with ultrasound treatment.

Materials and Methods

This experimental study was carried out on 144 male Wistar rats. The course of the wound process was evaluated on a model of a purulent wound.

In vivo experiments were carried out in compliance with the norms of humane treatment, in accordance with the current legislation on working with laboratory animals, and was approved by the Ethics Committee of the Kursk State Medical University (Protocol No. 7 dated November 30, 2018).

The purulent wound (at the withers, with an area about 150 mm^2) was modeled according to P. Tolstykh,[3] in an operating room under isoflurane inhalation anesthesia. Next, a gauze ball soaked in 1 ml of the billionth suspension of *Staphylococcus aureus* was introduced into the wound and sutured. After 24 hours, all animals developed a purulent wound with all the characteristic signs (hyperemia and swelling of the skin around the defect, purulent discharge from the wound). Then the animals were divided into 4 equal groups (36 animals in each group), depending on their treatment: Group 1 (MC[2.0g]+H[0.5g]+UST), Group 2 (MC[2.0g]+PD[1.0g]+UST), Group 3 (MC[2.0 g]+H[0.5g]+PD[1.0g]+UST), and Group 4 (official drug (DMTHP[0.04g]+CP[0.0075g]-ointment)+UST). Treatment was carried out for 15 days. Dressings were performed daily with the study combination. In Groups 2 and 3, daily phototherapy was carried out using the Nevoton apparatus (Nevoton LLC, Russia) (the wavelength of light radiation was 650–670nm). UST was carried out daily, starting from Day 4 after the experiment, using a dual-frequency device (UZT - 1.3.01F - "Med TeKo") in this study, a frequency of 2.64±0.03 MHz was used, the oscillation intensity was 1.0W/cm^2. The duration of each therapeutic exposure was 5 minutes. The dynamics of changes in the area of the wound were assessed by the planimetry method. The percentage of area reduction (PAR) of the wound was calculated according to the formula:

PAR= (W1-Wx)/W1×100%, where W1 is the initial wound area (mm^2), Wx is the area on measurement day (mm^2).

A histological examination of a skin area (1.0 cm×1.5 cm) taken in the zone of a simulated purulent wound was carried out on Days 5 and 10 of the experiment. Light microscopy of the preparations obtained was carried out using a Levenhuk C320 microscope at ×200 and ×400 magnifications, microphotography was performed with a Levenhuk C310 NG digital camera.

Statistical analysis was performed using Microsoft Excel 2010. For descriptive analysis, results are presented as median (Me), first quartile (Q1) and third quartile (Q3). A non-parametric Kruskal-Wallis test was used for comparisons of median values among study groups, followed by post-hoc testing using un-paired Mann-Whitney U tests. A probability value of $P<0.05$ was considered statistically significant.

Results

The study showed that in all observation groups, there was a decrease in the area of the wound surface throughout the entire period of treatment. In Groups 2 and 3, with the use of the photosensitizer the wound was completely healed by day 15. The dynamics of these changes is presented in Table 1.

Table 1.

Dynamics of changes in the area of wounds (PAR, %) (Me (Q_1; Q_3))

Group	Group 1	Group 2	Group 3	Group 4
Day 3 (n=30)	12.2 (8.6; 15.2)*	13.1 (11.9; 14.7)*	35.9 (27.2; 41.6)*^#	8.7 (6.1; 10.6)
Day 5 (n=24)	25.3 (23; 28.1)*	22.8 (21.8; 25.2)*	57.6 (54.9; 63.2)*^#	15.7 (12.8; 17.1)
Day 8 (n=18)	66 (63.7; 66.9)*	48.4 (45; 52.8)	82.7 (80.5; 83.9)*^#	41.7 (38.8; 43.2)
Day 10 (n=12)	80.2 (77.0; 83)*	91.2 (90.4; 91.9)*	97.3 (96.8; 97.3)*^	60.5 (58.7; 64.5)
Day 15 (n=6)	93.7 (93.4; 95.1)*	-	-	81.3 (81.2; 82.1)

*n - Number of animals at the time of the measurement; *– P<0.05 between Group 4 and Groups 1-3; ^– P<0.05 between Group 1 and Groups 2 and 3; # – P<0.05 between Group 2 and Group 3.*

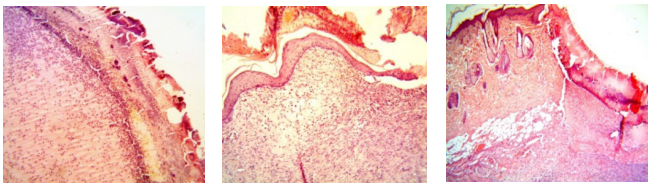
The microscopic examination showed that in **Group 1,** on Day 5 of the experiment (Fig.1A), the outside of the wound defect was covered with a wide scab, the material substrate of which was necrotic masses and fibrin deposits. The tissues surrounding the wound defect showed signs of interstitial edema. Round cell infiltration was visualized far beyond the boundaries of the wound defect. The field of view was dominated by neutrophils and macrophages, which, in turn, are the main structural elements of the leukocyte shaft that delimits the granulation tissue; in the thickness of that shaft, cells of the leukocyte-lymphocytic series were visualized.

On Day 10 (Fig.1B), the wound area was completely covered with stratified squamous keratinized epithelium of heterogeneous thickness throughout the wound defect. The newly formed connective tissue was not mature enough; the border with the intact dermis was well defined. In the field of view, the cellular component predominated over the fibrous component.

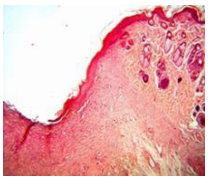
In Group 2, on Day 5 of the experiment (Fig.1C), deposits of necrotic and fibrinoid masses were visualized in the area of the wound defect, under which an extensive zone of hemorrhagic impregnation of tissues was determined, separated, in turn, by a leukocyte shaft from the newly formed

granulation fabrics. The infiltration zone was extensive and extended to the muscle tissue. Histiocytic cells were visualized in the field of view. In the bottom of the wound, we observed local accumulations of macrophages and the initial stages of the formation of multinucleated cells.

On Day 10 (Fig.1D), epithelialization of the wound was observed in the form of marginal creeping of the stratified squamous keratinizing epithelium from the side of the lateral edges of the wound defect of the skin inwards. Complete epithelialization was not observed by this time. Outside of the newly formed epidermis, scab elements (necrotic and fibrin deposits) continued to be visualized. The newly formed epithelium was thin without a clear structural organization of the layers and the complete absence of the stratum corneum. There was a clearly defined border between the granulation tissue and the intact dermal tissue.



A B C



D

Fig. 1. *Micrograph of the skin section in the area of the wound defect. H&E staining, ×100 magnification.*

Group 1: A - on Day 5; B - Day 10.

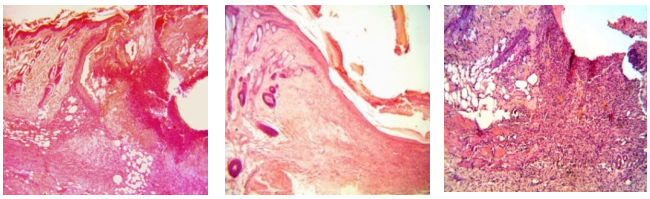
Group 2: C - on Day 5; D - on Day 10.

In Group 3, on Day 5 of the experiment (Fig.2A), an extensive area of the scab was visualized in the area of the skin defect, the material substrate of which was necrotic masses, fibrin deposits, and cellular detritus. In the area of the newly formed granulation tissue, separated by a leukocytic ridge of the scab, vertically oriented small blood vessels and focal accumulations of adipocytes were determined. The field of view was dominated by inflammatory cells. In the deeper layers of the wound, a macrophage-histiocytic reaction was pronounced.

On Day 10 (Fig.2B), complete epithelialization of the wound defect was observed. The newly formed thin epithelial layer covered the entire area of the wound defect and consisted of well-identified layers of stratified squamous keratinized epithelium. Above the "young" epithelium, elements of the scab containing necrotic masses and degeneratively altered lymphohistiocytic elements continued to be preserved in some areas.

In Group 4, on Day 5 of the experiment (Fig.2C), an extensive zone of round cell infiltration continued to be visualized outside the area of the wound defect. In the newly formed granulation tissue, a large number of vertically oriented blood vessels were determined. The field of view was dominated by the cellular component over the fibrous one.

On Day 10 (Fig.2D), epithelialization of the wound defect of the skin was observed. The wound defect was filled with scar tissue; the fibrous component predominated in the field of view. The border between the cicatricial and intact dermis was well defined, mainly due to the fact that the newly formed fibers were not yet combined into bundles, but were located separately.



A B C

D

Fig. 2. *Micrograph of the skin section in the area of the wound defect. H&E staining, ×100 magnification.*

Group 3: A - on Day 5; B - Day 10.

Group 4: C - on Day 5; D - on Day 10.

In conclusion, our study confirmed the effectiveness of the use of hexetidine, photoditazine, and a combination of them, along with UST, in the local treatment of a purulent wound. At the same time, the combined effect of an antiseptic and a photosensitizer showed significantly better results in the first and second phases of the course of the wound process than did their isolated use. Thus, this combination of hexetidine with photoditazine can be recommended for further preclinical studies for local treatment of the soft tissue purulent-inflammatory process.

Competing Interests

The authors declare that they have no competing interests.

References

1. Arkhipov DV, Andreev AA, Atyakshin DA, Glukhov AA, Ostroushko AP. [Jet oxygen sorption treatment in the local treatment of purulent wounds of soft tissues]. Bulletin of Experimental and Clinical Surgery. 2020;13(1):41–45. doi:10.18499/2070-478X-2020-13-1-41-45. [Article in Russian].

2. Katorkin SE, Bystrov SA, Lisin OE, Rozanova AA, Bezborodov AI. [Evaluation of the effectiveness of the use of modern dressings in the complex treatment of purulent wounds]. Stationary-Replacing Technologies: Ambulatory Surgery. 2019;1-2:146–152. doi:10.21518/1995-1477-2019-1-2-146-152. [Article in Russian].

3. Bezhin AI, Pankrusheva TA, Zatolokina MA, Grigoryan AYu, Zhilyaeva LV, Kobzareva EV, Mishina ES.

***Corresponding author**: Prof. Mariya A. Zatolokina, PhD, ScD. Department of Histology, Embryology, and Cytology. Kursk State Medical University. Kursk, Russia. E-mail: marika1212@mail.ru*

[Morphological features of wound surface healing when using new preparations based on carboxymethyl cellulose]. Modern Problems of Science and Education. 2015;3. Available from: https://science-education.ru/ru/article/view?id=20333. [Article in Russian].
4. Hoffmann JP, Friedman JK, Wang Y, McLachlan JB, Sammarco MC, Morici LA, et al. *In situ* Treatment With Novel Microbiocide Inhibits Methicillin Resistant *Staphylococcus aureus* in a Murine Wound Infection Model. Front Microbiol. 2020 Jan 23;10:3106. doi: 10.3389/fmicb.2019.03106.
5. Duvansky VA, Eliseenko VI, Shin EF. Influence of photodynamic therapy on the reparative processes of purulent wounds. Proceedings of the 3rd International Congress «Wounds and wound infections.» Moscow;2016:99–100.
6. Belyaeva OA, Luneva AG, Kryzhevsky VV, Karol IV, Zavadetskaya EP, Oleinik EA, Krivenko EA. [Cytological monitoring of the healing of purulent wounds during their treatment using application sorption and NO-therapy. Laboratory diagnostics]. Eastern Europe. 2018;7(3):374–383. [Article in Russian].
7. Muñoz-Sepeda PA, Blatun LA, Paskhalova YuS. The effectiveness of ultrasonic cavitation in the treatment of purulent-necrotic complications of the diabetic foot syndrome. Proceedings of the 4th International Scientific and Practical Congress «Wounds and wound infections.» Moscow;2018:123–124.
8. Conner-Kerr T, Oesterle ME. Current perspectives on therapeutic ultrasound in the management of chronic wounds: a review of evidence. Chronic Wound Care Management and Research. 2017;4:89-98.