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EXPERIMENTAL EVALUATION OF THE EFFECTIVENESS OF THE USE OF A GEL OF RARE-STITCHED ACRYLIC POLYMERS WITH NATURAL ANTIMICROBIAL PEPTIDES IN THE TREATMENT OF BORDERLINE SKIN BURNS

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Abstract. The use of natural antimicrobial peptides with high antibacterial activity against most microorganisms, including antibiotic-resistant strains and polymicrobial associates (biological films), can be attributed to new methods of management of patients with burns. The experiment included 40 rats weighing 220–240 g, distributed into 4 groups depending on the treatment method: control (without treatment), wet-drying dressings with an antiseptic solution (iodopyron), "Levomekol" ointment, gel of rare acrylic polymers with natural antimicrobial peptides. The results were evaluated on the 4th, 7th, 14th and 28th days. The gel of rare-stitched acrylic polymers with natural antimicrobial peptides had the highest efficiency: the area of the experimental wound by the 28th day was reduced to 0.3 ± 0.1 cm^2 (12.6 times less than in the control group), the growth retardation zone of *Staphylococcus aureus* 209P was 34.9 mm, which is only 3.9 mm less than when using iodopyron, and the frequency of infectious complications and mortality were minimal — 10 and 5%, respectively.

Key words: deep burns; reparative regeneration; natural antimicrobial peptides; carbopol; rare-sewn acrylic polymers.

ЭКСПЕРИМЕНТАЛЬНАЯ ОЦЕНКА ЭФФЕКТИВНОСТИ ПРИМЕНЕНИЯ ГЕЛЯ РЕДКОСШИТЫХ АКРИЛОВЫХ ПОЛИМЕРОВ С ПРИРОДНЫМИ АНТИМИКРОБНЫМИ ПЕПТИДАМИ ПРИ ЛЕЧЕНИИ ПОГРАНИЧНЫХ ОЖОГОВ КОЖИ

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Резюме. Применение природных антимикробных пептидов с высокой антибактериальной активностью в отношении большинства микроорганизмов, в том числе антибиотикорезистентных штаммов и полимикробных

ассоциатов (биологические пленки), можно отнести к новым методам ведения больных с ожогами. В эксперимент включены 40 крыс массой 220–240 г, распределенных в 4 группы в зависимости от методики лечения: контроль (без лечения), влажно-высыхающие повязки с раствором антисептика (йодопирон), мазь «Левомеколь», гель редкосшитых акриловых полимеров с природными антимикробными пептидами. Результаты оценивались на 4, 7, 14 и 28-е сутки. Гель редкосшитых акриловых полимеров с природными антимикробными пептидами обладал наибольшей эффективностью: площадь экспериментальной раны к 28-м суткам сократилась с 200 см^2 до 0,3 ± 0,1 см^2 (в 12,6 раза меньше, чем в группе контроля), зона задержки роста *Staphylococcus aureus* 209P составила 34,9 мм (что лишь на 3,9 мм меньше, чем при использовании йодопирона), а частота инфекционных осложнений и летальности были снижены до 10 и 5 % соответственно.

Ключевые слова: глубокие ожоги; репаративная регенерация; природные антимикробные пептиды; карбопол; редкосшитые акриловые полимеры.

INTRODUCTION

Today, domestic or industrial burns are one of the most serious public health problems. According to available sources, about 265,000 fatal burn injuries are registered annually in the world [12]. In the Russian Federation, burns occupy the 6th place (2.4 %) in the structure of traumatism, accounting for 2.1 cases per 1 thousand adults [2]. According to the Central Research Institute of Health Care Organisation and Informatisation, in 2014 more than 287 thousand people suffered from burns [7], 30% of them had extensive superficial burns and/or deep lesions and required hospital treatment [5].

Provision of care to victims with skin burns is a complex that must take into account all the features of pathophysiological processes characteristic of this type of injury [3]. The current wide range of wound healing agents available to specialists often does not meet these requirements. Annual statistical data show that the effectiveness of standard treatment of superficial and borderline burns remains low. This is due to the growing resistance of wound microflora to modern antibacterial drugs and, as a consequence, the reduction of available methods of treatment of the burned patients [11].

One of the possible solutions to this problem is the use of hydrogels with natural antimicrobial peptides (NAPs). NAPs are a heterogeneous population of molecules that actively participate in the processes of innate and acquired immune response. They have different physical, chemical and biological characteristics that allow them to effectively destroy cell membrane, leading to its necrobiosis [9]. This is done by selective accumulation of NAPs on the surface of microorganism with its subsequent death [1]. The results of studies indicate a pronounced bactericidal effect of this group of peptides compared to traditional antibiotics, as well as their ability to neutralise toxic cellular decay products formed during necrosis [10]. These molecules have great potential despite the limited knowledge about mechanisms of the effect of NAPs and the resistance of various microorganisms to them. Their combined use with hydrogels that form a moist environment in the wound area allows to create a complex product that optimises the wound process [4]. The introduction of these technologies into the practical activity of surgical hospitals providing care to patients with skin burns will improve the results of treatment of this category of patients.

AIM

To experimentally evaluate the efficacy of rare cross-linked acrylic polymer gel with natural antimicrobial peptides in the treatment of skin burns.

MATERIALS AND METHODS

The experimental study was carried out in the research laboratory of experimental surgery of the St. Petersburg State Pediatric Medical University. 40 small laboratory animals (rats) of both sexes of the Wistar line weighing 220-240 g were included in the experiment. Depending on the treatment used method, the animals were divided into 4 equal groups: the control group (without treatment), wet-drying dressings with antiseptic (iodopyron), levomekol ointment, rare cross-linked acrylic polymer gel with NAPs (Department of Insect Biopharmacology and Immunology of the Saint-Petersburg State University). All manipulations with animals were performed with general anaesthesia (Zoletil 100 drug) and complied with the requirements of ethical standards approved by legislative acts and international conventions. Modelling of skin burns was performed according to our own original method [8]. The total area of the experimental burn wound was 16 cm^2 (10 % of the rat's body surface). To control skin temperature, an electrothermocouple sensor of a multimeter was placed on the depilated skin of the animal's back, which was used to determine the temperature of the skin and the metal heating plate. Exposure time was 10 s at the skin surface temperature of 95–97 °C. Dressings of experimental burn wounds were performed

every day. To assess the effectiveness of wound-healing preparations, the appearance of wounds was evaluated daily, the nature of the discharge, the presence and type of granulation were noted, and the terms of scab rejection and wound healing were recorded. The effect of drugs on wound healing was assessed using the planimetric method of L.N. Popova with determination of the wound area and calculation of the healing index according to the formula:

$$\frac{(S-S_n)\cdot 100}{S\cdot T}$$

where S — the wound area at the previous measurement, mm^2; S_n — the wound area at this measurement, mm^2; T — the interval between measurements, days.

In normal wound process, the rate of wound healing is quite stable and averages about 4% per day. Slowdown of the healing rate may indicate the development of complications [6].

Biopsy specimens for histological examination were taken on the 3rd, 13th and 18th day. The study of antibacterial activity was performed using the disc-diffusion method on *Staphylococcus aureus 209P culture.*

The obtained data were processed using conventional methods of variation statistics. The parametric Student's t-test was used to assess the reliability of the obtained results. The alternative hypothesis was accepted at $p < 0.05$.

RESULTS

The main goal of the local treatment of burns is to optimise the course of reparative processes and, consequently, to reduce the duration of skin integrity restoration. The results of planimetric analysis of the dynamics of burn wound regeneration are presented in Table 1.

According to the data presented in Table 1, the treatment method based on the application of rare cross-linked acrylic polymer gel with NAPs showed the highest efficiency on the developed burn model. This method reduced the wound area to 0.3 cm^2 by the 28th day of the study, which is 92.1 % ($p < 0.01$) less compared to the control group. A similar trend was observed when evaluating the effectiveness of hydrogel in relation to traditional methods of burn wound management. Compared to the results of using wet-drying dressings with antiseptic solution (iodopyron) and Levomekol ointment, application of hydrogel with NAPs accelerated the intensity of reparative regeneration by 86.9 ($p < 0.05$) and 81.2 % ($p < 0.05$), respectively.

For an in-depth evaluation of the healing process of the experimental burn wound, a comparative analysis of the healing index was additionally performed (Fig. 1).

It was found that during the whole period of treatment, except for the 14th day, the gel of rare cross-linked acrylic polymers with NAPs was the most effective. By the end of the 2nd week of hydrogel application the index of wound healing was 3.5 %/day, which is 10.3 times (18.6 %) less compared to the results of using wet-drying dressings with antiseptic solution (iodopyron) and Levomekol ointment. The maximum value of this index (5.6 %/day) among all observations was recorded on the 28th day of the study in the group of animals treated by the application of hydrogel with NAPs.

It is known that burn wounds are characterised by a high frequency of infectious complications due to contamination by pathogenic microflora. This necessitates the mandatory

Table 1

Dynamics of burn wound healing in experimental groups

The experimental group	An average wound area, cm^2 (M ± m)			
	The 7th day	The 14th day	The 21st day	The 28th day
The control group (without treatment)	14 ± 1,9	8,3 ± 2,0	5,7 ± 1,6	3,8 ± 1,2
Wet-drying dressings	12,7 ± 1,9[1]	5,7 ± 2,1[1]	4,6 ± 2,2	2,3 ± 1,6[1]
"Levomekol" ointment	12,1 ± 1,8[1]	4,6 ± 1,7[1]	3,6 ± 1,5[1]	1,6 ± 0,9[1]
Hydrogel with NAPs	11,8 ± 2,7[1]	3,7 ± 1,0[1,2]	1,5 ± 0,7[1,2,3]	0,3 ± 0,3[1,2,3]

Notes. [1] — differences with the control group are significant ($p < 0.05$); [2] — differences with the "Wet-drying dressings" group are significant ($p < 0.05$); [3] —differences with the "Levomekol ointment" group are significant ($p < 0.05$)

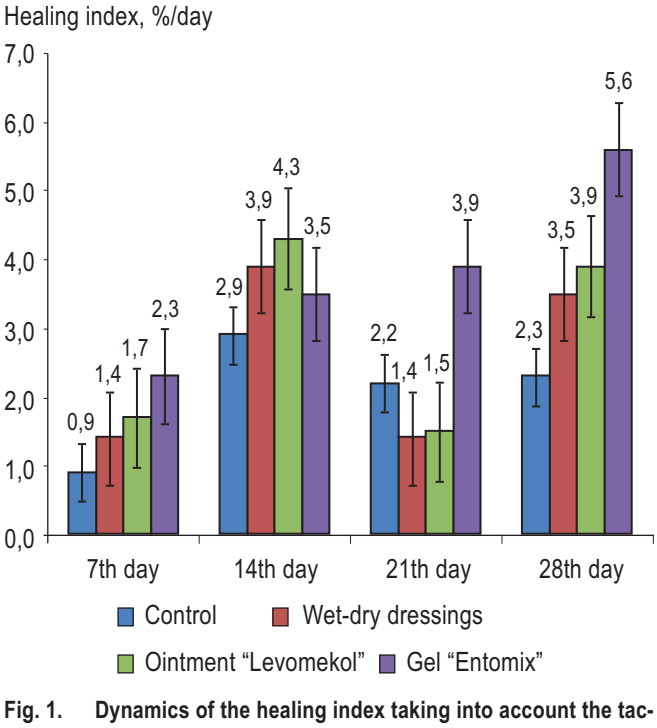

Fig. 1. Dynamics of the healing index taking into account the tactics of treatment of the experimental wound

inclusion of a local antibacterial component in the system of treatment of this type of trauma. The results of bactericidal activity evaluation of rare cross-linked acrylic polymer gel with NAPs against Staphylococcus aureus 209P are shown in Figure 2.

The results demonstrate that rare crosslinked acrylic polymer gel with NAPs was less effective against *Staphylococcus aureus* 209P than iodine-containing antiseptic drugs (iodopyron), 34.9 and 38.6 mm, respectively. The least antibacterial effect was demonstrated by "Levomekol" multicomponent ointment — 15.8 mm. Despite the less pronounced bactericidal effect of hydrogel with NAPs relative to the most frequently used antiseptic agent, the latter does not allow providing optimal conditions for the course of the wound process throughout the entire period of treatment, which causes its low wound-healing potential.

During the experiment, the frequency of infectious complications and mortality rates in the experimental groups were assessed (Table 2).

The data of Table 2 show that in the group of animals treated with rare cross-linked acrylic polymer gel with NAPs the frequency of infectious complications was minimal — 10 % of cases (2 observations). At the same time, one lethal outcome was noted. The use of wet-drying dressings and "Levomekol" ointment was less effective. Purulent inflammation was observed in 30 and 25 % of cases, respectively. In the control group (without treatment), the incidence of complications of the wound process was 55 %, the mortality rate was 20 %.

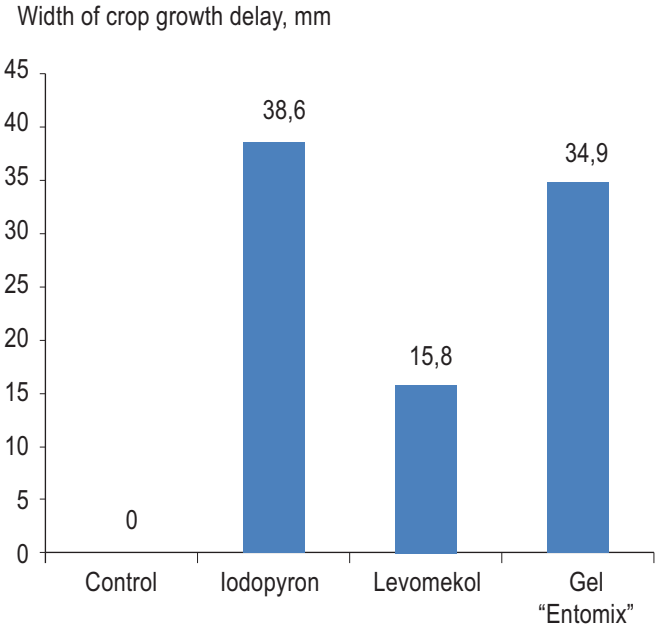

Fig. 2. Results of comparative evaluation of antibacterial activity of the investigated treatment methods against *Staphylococcus aureus* 209P

Table 2

The frequency of infectious complications and mortality in the experimental groups

The experimental group	Frequency of infectious complications, %	Mortality, %
The control group (without treatment)	55,0	20,0
Wet-drying dressings	30,0	10,0
"Levomekol" ointment	25,0	5,0
Hydrogel with NAPs	10,0	5,0

CONCLUSION

The use of rare cross-linked acrylic polymer gel with NAPs (entomix) in the treatment of experimental skin burns allowed to accelerate reparative regeneration in the area of soft tissue defect by 86.9 % ($p < 0.05$). Minimal incidence of infectious complications and lethality were also noted with its use. Thus, hydrogel with NAPs complex allows not only to effectively protect the wound from bacterial flora, but also to create optimal conditions for stimulation of reparative properties of the organism itself. The development of an algorithm for the treatment of borderline skin burns using this wound-healing agent will improve the result of treatment for this category of patients.

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

Competing interests. The authors declare that they have no competing interests.

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Experiments with animals were carried out in accordance with international rules (Directive 2010/63/EU of the European Parliament and of the Council of the European Union of September 22, 2010 on the protection of animals used for scientific purposes).

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. Все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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