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CLINICAL EVALUATION OF THE EFFECTIVENESS OF THE GEL OF RARE-CUT ACRYLIC POLYMERS WITH A COMPLEX OF NATURAL ANTIMICROBIAL PEPTIDES FLIP-7 IN THE TREATMENT OF BORDERLINE SKIN BURNS

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ABSTRACT. The effectiveness of the treatment of borderline skin burns using a gel of rare-sewn acrylic polymers with natural antimicrobial peptides FLIP-7 was evaluated. A total of 60 burned patients hospitalized in the Burn Center of Saint Petersburg Scientific Research Institute for First Aid named after I.I. Janelidze were examined during the period from 2019–2021. The following were evaluated: the general condition of patients, planimetric indicators, as well as the results of microscopic examination of smears-prints from the wound surface. This drug was established to be effective in the treatment of borderline skin burns from the first day of use. The burn wound areas upon use of FLIP-7 gel compared to wet-drying dressings and water-soluble ointment levomekol decreased by 6.25% and 8.6%, respectively. A complete restoration of the integrity of the skin was noted by the 7th treatment day, against the background FLIP-7 gel use. A complete restoration of skin integrity was noted by the 7th day of treatment, against the background of FLIP-7 gel use. The application of a hydrogel with a complex of natural antimicrobial peptides was demonstrated to have a pronounced antibacterial effect against most pathogenic microorganisms. Hence, the number of burn wound infection decreased by 35% by the 4th day from the treatment initiation. There was a possibility of reducing the analyzed indicator to 10% by the end of the week of local treatment with FLIP-7 gel, which indicates almost complete elimination of pathogenic microflora from the burn wound. It was proven that the humid environment conditions formed by hydrogel can stimulate the reparative regeneration processes. This was confirmed by the results of microscopic examination of smear prints, indicating a statistically significant increase in the concentration of fibroblasts on the wound surface by the 4^{th} day of observation compared with the results obtained before treatment initiation with FLIP-7 gel. Not a single case of side effect or undesirable phenomenon was recorded during course of the clinical study. The gel of rarestitched acrylic polymers with natural antimicrobial peptides FLIP-7 is an effective means of local treatment of borderline skin burns, which provides not only optimal conditions for the course of reparative regeneration processes, but also actively eliminates pathogenic microflora, including polyresistant microorganisms strains.

Keywords: skin burn; dermal lesions; humid environment; pathogenic microflora; biofilms; natural antimicrobial peptides; reparative regeneration; polyresistant strains of microorganisms.

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КЛИНИЧЕСКАЯ ОЦЕНКА ЭФФЕКТИВНОСТИ КОМПЛЕКСА ПРИРОДНЫХ АНТИМИКРОБНЫХ ПЕПТИДОВ FLIP-7 ПРИ ЛЕЧЕНИИ ПОГРАНИЧНЫХ ОЖОГОВ КОЖИ

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Резюме. Оценивается эффективность лечения пограничных ожогов кожи с использованием геля редкосшитых акриловых полимеров с природными антимикробными пептидами FLIP-7. Обследованы 60 обожженных, госпитализированных в ожоговый центр Санкт-Петербургского научно-исследовательского института скорой помощи им. И.И. Джанелидзе в период с 2019 по 2021 г. В ходе работы оценивались общее состояние пациентов, планиметрические показатели, а также результаты микроскопического исследования мазков-отпечатков с раневой поверхности. Установлено, что данное лекарственное средство при лечении пограничных ожогов кожи эффективно уже в первые сутки применения. При этом площадь ожоговой раны при использовании геля FLIP-7 по сравнению с использованием влажно-высыхающих повязок и мази на водорастворимой основе левомеколь сократилась на 6,25 и 8,6% соответственно. К 7-м суткам лечения на фоне применения геля FLIP-7 отмечается полное восстановление целостности кожного покрова. Продемонстрировано, что аппликация гидрогеля с комплексом природных антимикробных пептидов обладает выраженным антибактериальным эффектом в отношении большинства патогенных микроорганизмов. Так, уже к 4-м суткам от момента начала лечения количество обожженных с инфицированной раной снизилось на 35%. К исходу недели местного лечения гелем FLIP-7 удалось уменьшить анализируемый показатель до 10%, что свидетельствует о практически полной элиминации патогенной микрофлоры из ожоговой раны. Доказано, что условия влажной среды, формируемые гидрогелем, позволяют стимулировать процессы репаративной регенерации. Это подтверждается результатами микроскопического исследования мазков-отпечатков, свидетельствующих о статистически значимом увеличении концентрации фибробластов на раневой поверхности к 4-м суткам наблюдения по сравнению с результатами, полученными до начала лечения гелем FLIP-7. В ходе клинического исследования не зафиксировано ни одного случая побочного эффекта или нежелательного явления. Гель редкосшитых акриловых полимеров с природными антимикробными пептидами FLIP-7 является эффективным средством местного лечения пограничных ожогов кожи, которое не только обеспечивает оптимальные условия для течения процессов репаративной регенерации, но и активно элиминирует патогенную микрофлору, в том числе полирезистентные штаммы микроорганизмов.

Ключевые слова: ожог кожи; дермальные поражения; влажная среда; патогенная микрофлора; биопленки; природные антимикробные пептиды; репаративная регенерация; полирезистентные штаммы микроорганизмов.

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BACKGROUND

In the treatment of borderline skin burns, major tasks include the optimization of the wound process and the provision of effective treatment of wound infection [1, 2]. The latter is of particular importance because burns have a high risk of purulent and inflammatory complications and systemic infectious process caused by multiresistant strains of microorganisms [3, 4].

The main component of the conservative management of borderline skin burns is a local treatment strategy based on the current phase of the wound process [5, 6]. In accordance with the International Classification of Diseases, 10th Edition of 2014, medical institutions in the Russian Federation use a three-degree classification of burns, where degree I implies epidermal burns; degree II, dermal burns; and degree III, deep necrosis of all layers of the skin and underlying tissues. Wet-to-dry dressings with antiseptic solutions (1% solution of iodopyrone, iodinol, chlorhexidine, etc.), detergents (miramistin), and ointments on a water-soluble polyethylene glycol base (levosin, levomekol, etc.) are used for treatment. However, this technique causes excessive traumatization of the wound surface during dressings and increases the risk of an allergic reaction. Moreover, the frequent use of one type of antiseptics leads to a gradual decrease in their efficiency because microorganisms form protective biofilms. This necessitates a detailed and individual approach when choosing a local treatment strategy [7-9].

This problem can be solved by employing biosurgical innovations. The development of this field enabled the isolation of a complex of natural antimicrobial peptide fly larvae immune peptide 7 (FLIP-7), which includes defensins, cecropins, diptericins, and proline-rich peptides [10, 11]. The interaction of these peptides ensures total destruction of the matrix and various types of pathogenic bacteria. Moreover, it is not toxic to the host cells and immune system. The effective application of this complex in suppressing biofilm formation by gram-positive and gram-negative bacteria at various stages of their life cycle was confirmed in laboratory studies on microbial cultures conducted at the Entomology Department of St. Petersburg State University. The FLIP-7 gel can be used not only to eliminate actively pathogenic microorganisms from the wound but also to act as a synergist of antibiotics, increasing their bioavailability. Experimental studies have demonstrated the high efficiency of FLIP-7 peptides against most opportunistic gram-positive and gram-negative microorganisms, including multiresistant strains. This complex destroys actively biological films, overcoming collective and individual resistance, and slows down their formation [12]. The optimization of the wound process is achieved by adding a gel of lightly crosslinked acrylic polymers to the formula, which functions as a FLIP-7 carrier, providing favorable conditions for reparative regeneration. The application of the resulting hydrogel wound dressing based on FLIP-7, a complex of natural antimicrobial peptides, in skin burns will increase the treatment efficiency.

The study aimed to evaluate the efficiency of the treatment of borderline skin burns using the FLIP-7 gel of lightly cross-linked acrylic polymers with natural antimicrobial peptides.

MATERIALS AND METHODS

This clinical study enrolled 60 patients with burns hospitalized at the Burn Center of the I.I. Dzhanelidze Saint Petersburg Research Institute of Emergency Medicine between 2019 and 2021. All patients provided informed consent voluntarily before the start of treatment. Patients aged 19–60 (mean age, 42.2) years and had a burn duration of no more than 4 days were included. Hydrogel coating with FLIP-7 (State Registration Certificate for a Medical Product: No. RZN 2020/12585 dated October 17, 2020) is approved for use in clinical practice. This product complies with the requirements of GOST R 50444-92, GOST 33756-2016, TU 32.50.50-006-72500079-2020, and a set of design documentation AFM.72500079.

The gel of lightly cross-linked acrylic polymers with natural antimicrobial peptides (entomix) was applied on the wound surface, which has a dermal injury nature and an area of approximately 200 cm². To assess the resistance ability of the tissue to this medicinal product, a daily planimetric assessment of this area was performed. The treatment strategy of using this drug included pretreating the wound with a neutral solution (physiological saline solution), drying it with a sterile wipe, and then applying a thin layer of the hydrogel. The study was performed for 7 days, during which the general condition of the patients and microbiological and planimetric indicators were evaluated. For 7 days, the patient's general condition (temperature and blood pressure), results of laboratory tests (general blood test), local status of the wound process (according to a specially developed rating scale), and planimetric indicators were analyzed. The dynamics of reparative regeneration was assessed by the healing index, which was calculated according to a special equation:

$$(\text{V3}) = \frac{(S - S_n) \times 100}{S \times T},$$

where S is the wound area during the previous measurement (mm²), Sn is the wound area for a given measurement (mm²), and T is the interval between measurements (days).

Research materials were analyzed using Microsoft Excel and SPSS Statistics version 17.0. To analyze the veracity of the results, a non-parametric Mann – Whitney U-test was used for unrelated samples. The alternative hypothesis was confirmed at p < 0.05.

RESULTS AND DISCUSSION

The management of wounds in a humid environment using the FLIP-7 gel of lightly cross-linked acrylic polymers containing natural antimicrobial peptides, has been established to significantly accelerate the healing time, which indicates its high efficiency. Thus, on day 1 of using the FLIP-7 gel, the burn surface area reduced to 180 cm², which was 6.25% and 8.6% less compared with the results of using wet-to-dry dressings (1% iodopyrone solution) and levomekol ointment (Table 1). This tendency continued on days 2, 3, and 4. By day 7, the highest intensity of reparation was noted, which ensured complete restoration of the skin in most cases. In the comparison groups, where wounds were treated with wet-to-dry dressings and levomekol ointment, these indicators were 44 (34; 51) cm² and 19 (16; 33) cm² at the end of the study,

The FLIP-7 gel of lightly cross-linked acrylic polymers with a complex of natural antimicrobial peptides has

pronounced antibacterial effects against most gram-positive and gram-negative microorganisms and enables the active destruction of biological films, which is the main factor of protection against antimicrobial therapy.

Before the application of the FLIP-7 gel, 19 (95%) patients with burns were found to have wound infections. In total, six species of pathogenic microorganisms were detected, the most common of which was Staphylococcus aureus (n = 12, 60%; Table 2). By day 4, the analyzed indicator decreased in 12 (60%) patients. At this control point, the microbiological presentation indicated a uniform species distribution of microorganisms. By the end of the study, burn wounds were almost completely epithelialized, and the results of microbiological studies indicated the nearly complete absence of pathogenic strains.

Microscopic examinations of the cellular composition on the wound surface enabled an in-depth study of the wound process characteristics. The cytological examination results of imprint smears indicated the efficiency of the FLIP-7 gel

Table 1. Dynamics of borderline skin burns healing **Таблица 1.** Динамика заживления пограничных ожогов кожи

| Parameter - | Burn wound area, cm ² , Me (Q25; Q75) | | | | | | | | |
|---------------------|--|-------------------|-------------------|------------------|----------------|----------------|----------------|--|--|
| | Day | | | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | |
| Wet-to-dry dressing | 197 (196; 199) | 188 (183; 192) | 141 (132; 150) | 102 (88; 112) | 78 (70; 91) | 57 (35; 70) | 44 (34; 51) | | |
| Levomekol ointment | 192 (188; 198) | 167 (154; 177) | 116 (101; 125) | 78 (54; 93) | 55 (41; 75) | 38 (19; 45) | 19 (16;33) | | |
| Hydrogel FLIP-7 | 180 (162; 191) | 145 (162; 191) | 91 (66; 128) | 57 (32; 80) | 24 (12; 38) | 4 (2; 8) | 0 (0; 2) | | |

Table 2. Effectiveness of the complex of natural antimicrobial peptides FLIP-7 in relation to pathogenic strains of microorganisms **Таблица 2.** Эффективность комплекса природных антимикробных пептидов FLIP-7 по отношению к патогенным штаммам микроорганизмов

| Parameter | | Number of cases | | | |
|---|---------|-----------------|--------|--|--|
| rarameter | Day 0 | Day 4 | Day 7 | | |
| Staphylococcus aureus | 12 | 3 | 2 | | |
| Coagulase negative staphylococcus | 7 | 3 | 0 | | |
| Enterococcus spp. | 3 | 2 | 0 | | |
| Klebsiella pneumoniae | 1 | 2 | 0 | | |
| Corynebacterium spp | 1 | 2 | 0 | | |
| Streptococcus pyogenes | 0 | 1 | 0 | | |
| Number of patients with infected burn wounds, n (%) | 19 (95) | 12 (60) | 2 (10) | | |

Table 3. Dynamics of the number of leukocytes and fibroblasts in smears, taking into account the chosen treatment method **Таблица 3.** Динамика количества лейкоцитов и фибробластов в мазках-отпечатках с учетом выбранной методики лечения

| Parameter | Number of cells in the field of view, units, Me (Q25; Q75) | | | | | | |
|---------------------|--|--------|--------|-------------|--------|--------|--|
| | leukocytes | | | fibroblasts | | | |
| | Day 0 | Day 4 | Day 7 | Day 0 | Day 4 | Day 7 | |
| Wet-to-dry dressing | 4 | 4 | 2 | 0 | 0 | 1 | |
| | (3; 4) | (3; 4) | (1; 3) | (0; 1) | (0; 1) | (1; 2) | |
| Levomekol ointment | 4 | 3 | 1 | 0 | 1 | 2 | |
| | (3; 4) | (3; 3) | (1; 2) | (0; 1) | (1; 2) | (1; 2) | |
| Hydrogel FLIP-7 | 4 | 3 | 0 | 0 | 2 | 0 | |
| | (3; 4) | (1; 4) | (0; 0) | (0; 1) | (2; 4) | (0; 0) | |

in the treatment of borderline skin burns. Thus, if before the start of the treatment all patients had a high leukocyte count, then by day 4, the leukocyte count in the FLIP-7 gel group would have decreased. In the comparison groups (wet-to-dry dressings and levomekol ointment) at this control point, the changes were insignificant. By the end of day 7 of treatment with the FLIP-7 gel, the burn wounds were completely epithelialized (Table 3).

The quantitative analysis of the fibroblast population showed a similar tendency. For illustrative purposes, a clinical case is presented below.

Clinical case. Patient A., aged 55, was admitted to the Burn Center of the I.I. Dzhanelidze Saint Petersburg Research Institute of Emergency Medicine with a diagnosis of degree 16%/II hot water burn of the trunk, limbs, and

external genitalia. The provision of specialized medical care was delayed for 3 days. *Status localis* showed burn wounds on an area of approximately 16% of the body surface, represented by a de-epithelialized dermis with a coating of fibrin, exfoliated fixed epidermis, and areas of a thin light-brown scab. After signing an informed consent, the patient was included in the clinical study.

Status localis on day 1 of hospitalization in a dressing room showed moderate serous, purulent discharge from the wounds. No necrotic sites and signs of perifocal inflammation were noted. Moderate edema and wound bleeding were observed. The pain syndrome severity was significant. Epithelialization was sluggish and marginal (Fig. 1). The burn wound was washed with physiological saline solution and dried with a sterile wipe. The FLIP-7 gel

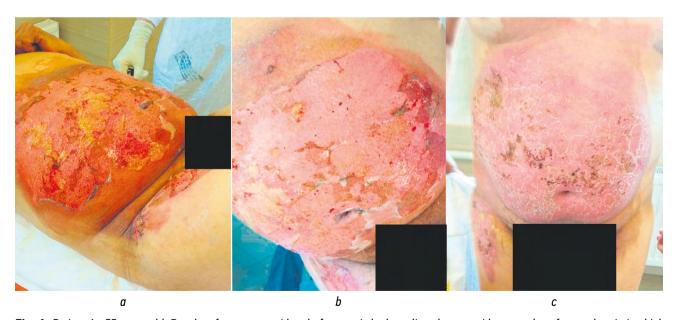


Fig. 1. Patient A., 55 years old. Results of treatment with gel of rare-stitched acrylic polymers with a complex of natural antimicrobial peptides FLIP-7: a — appearance of the wound—1st day; b — appearance of the wound — 4th day; c — appearance of the wound — 7th day of treatment, complete epithelization of the burn

Рис. 1. Пациент А., 55 лет. Результаты лечения гелем редкосшитых акриловых полимеров с комплексом природных антимикробных пептидов FLIP-7: a — внешний вид раны — 1-е сутки; b — внешний вид раны — 4-е сутки; c — внешний вид раны — 7-е сутки лечения, полная эпителизация ожога

was applied to the wound surface. In addition, a general blood test was performed, and inoculation from the wound surface and an imprint smear were taken. In a previous study, no clinically significant deviations in the parameter analyzed were noted. *Staphylococcus aureus* and *Streptococcus pyogenes* strains were verified in the wound discharge, with a concentration of 10⁵ and 10⁴ colony-forming units (CFU), respectively. Microscopic examination revealed an inflammatory cytogram with high counts of leukocytes, single eosinophils, and cellular detritus.

On day 2, the FLIP-7 gel was repeatedly applied. A moderate amount of serous discharge from the wound was noted. No edema was registered. The pain syndrome intensity decreased. The wound bleeding was moderate. Epithelialization was active and mixed. On day 3 of FLIP-7 gel application on the wound defect area, minor soreness persisted during mechanical impact. The discharge was serous, with moderate bleeding. Active mixed epithelialization continued. On day 4, the wound was actively epithelialized. The discharge was mild and serous. No bleeding or soreness was noted. Control analysis of the general clinical blood test and microbiological inoculation of the wound discharge was performed. An imprint smear was taken from the wound surface for cytological examination. The results of the control analysis indicated the absence of clinically significant deviations in the parameters analyzed. The wound microflora was represented by Staphylococcus aureus at a concentration of 10³ CFU. Microscopic examination

revealed a moderate count of leukocytes and fibroblasts in the field of view, and single eosinophils were also visualized. The cytogram was regenerative. On days 5 and 6, a small amount of serous discharge was noted, with signs of active mixed epithelization. By the end of the week (day 7), complete epithelialization of the burn wound was registered. In the control general clinical blood test, no significant deviations in the parameters analyzed were noted.

CONCLUSION

The use of the FLIP-7 gel of lightly cross-linked acrylic polymers with natural antimicrobial peptides (entomix) provides favorable conditions for stimulating the reparative regeneration of damaged tissues. By the end of the follow-up (day 7), the burn wound area was 89.4% and 93% less, respectively, compared with the results of wet-to-dry dressings and levomekol ointment. Natural peptides in the FLIP-7 biocomplex demonstrated a pronounced antimicrobial effect. By day 4, the frequency microorganisms decreased from 95% to 65%, and by the end of the week, pathogenic strains were registered only in 10% of the cases. The application of the FLIP-7 gel reduces the amount of wound discharge and decreases the severity of perifocal inflammation and soft tissue edema. The introduction of this technique for the treatment of borderline burn wounds will improve the results of the treatment of these patients.

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