

The Study of the Specific Activity of the Gel Containing Chitosan-Myramistin-Chymopsin Complex on the Linear and Planar Wounds' Model

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Abstract

Objective: The objective of the study was to assess the reparative and antibacterial properties of the gel containing chitosan-myramistin-chymopsin on the model of the linear and planar wounds in rats. **Materials and Methods:** Histological study has been performed on the material of the cutaneous flaps sized 1.5 x 1.0 x 0.5 cm, stained with eosin, from 48 animals divided into 6 groups: 8 rats with linear wound treated with CMCC-based gel (LWG), 8 rats with planar wound treated with test gel (PWG), 8 rats with planar wound and comparator agent (PWCA), 8 rats with linear wound and comparator agent (LWCA). The animals were withdrawn from the experiment, and the material was taken on the 8th day in case of the linear wound and on the 35th day in case of planar one. **Results:** The wound healing-reparation degree was assessed by the following morphological features: The presence/absence of the wound canal and necrosis, granulation tissue and its maturity, epithelialization, inflammatory response and the nature of the inflammatory infiltrate (leucocytic, lymphoid, macrophagal), as well as the changes in the extracellular matrix or fibrosis severity. **Conclusion:** Thus, based on reparation signs, the more effective tissue reparation had been observed in groups with gel, comparator agent and PWR, the least effective - in the LWR group, which was probably due to the accession of secondary infection and festering development. In the groups treated with gel and comparator agent the average reparation level had been revealed, and in case of the linear wound the gel proved to be more effective, compared to the comparator agent, and in planar wounds' group the level of reparation was equally high.

Key words: Chitosan-myramistin-chymopsin complex, development of wound healing drugs, models of linear and planar wounds, wound treatment gel

INTRODUCTION

To treat the wounds of various etiologies, the application of combined drugs is advisable, among which the use of gels is preferable since they do not disturb the liquid balance and gas exchange, have pH from 4.0 to 6.0, close to the pH of the skin; they are easily applied and evenly distributed across the surface, able to bind exudate, prevent the return migration of microorganisms into the wound, have a cooling effect due to water evaporation, contribute to the processes of the wound granulation and epithelization, release well the active substances from the base, provide deeper penetration into the tissues, increase the duration of action of the active components, and are effective during 18–24 h.^[1-3]

The base plays an essential role in the gel, and the natural biopolymers such as chitosan, which is obtained from the shells of crustaceans, are best used for this purpose. Such a base maintains a moist environment and gas exchange in the wound, provides for the creation of an acidic environment

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that promotes reparative processes, and shows antibacterial and antioxidant effects.^[4-8]

In addition, high-molecular-weight chitosan has an immunostimulating effect and can be used as an adjuvant of other drugs, as part of the vaccines.^[9-11] Hemostatic effect of chitosan has been noted, which is associated with the electrostatic interaction of negatively charged cellular membranes of erythrocytes with positively charged chitosan molecules.^[12]

One of the promising directions in the development of wound healing drugs is to create multicomponent drugs influencing various stages of the wound process - those with antibacterial, lytic, anti-inflammatory, analgesic, osmotic effect, preventing secondary infection, providing moist environment, growth and protection of granulation tissue, stimulating the proliferation, and the formation of the epithelium, thus demonstrating the synergism phenomenon of active substances and the basis.^[13,14]

It is often necessary to also use antiseptic solutions in the treatment of wounds since the festering wounds are identified in 35–40% of surgical patients.^[1,12,15-19] Chlorhexidine (Bio Farm Combinat, Russia), benzyldimethyl [3-(myristoylamino) propyl] ammonium chloride monohydrate (for example, Miramistin [Infamed, LLC, Russia]), povidone-iodine+potassium iodide (for example, Iodopirone drug (YuzhFarm, LLC, Russia)); however, synthetic agents are not always able to influence the wound microflora.^[13,20]

According to the latest data of P.A. Fedosov, the components of the gel containing high molecular chitosan, taurine, and allantoin are compatible in physicochemical terms. At the same time, each of the components taken separately has wound healing effects. The effective wound healing concentrations of taurine - 4.0% and allantoin - 0.5% have been found in the model of a planar full flap wound. Based on rheological and biopharmaceutical studies, the optimum chitosan content of 1–3% has been established in the gel dosage form. Chitosan gel with taurine and allantoin has low toxicity and is effective in the treatment of planar wounds in mice, of linear wounds in rats, and of thermal wounds.^[21]

The wound healing reparation degree was assessed by the following morphological features: The presence/absence of the wound canal and necrosis, granulation tissue and its maturity, epithelialization, inflammatory response and the nature of the inflammatory infiltrate (leukocytic, lymphoid, and macrophagal), as well as the changes in the extracellular matrix or fibrosis severity.

The degree of epithelization of the discussion zone reflects the reparation effectiveness. With good healing, the expressed epithelization of the wound surface is found, as well as the absence of purulent inflammation in the wound canal and of the wound canal itself, the presence of granulation tissue with

signs of maturation, and proliferation of fibroblasts. Changes of surface epithelium are associated with reactive changes and are non-specific in nature.

Granulation tissue appears in the wound several days after dissection of the skin and is most pronounced on day 7 after trauma. First, the immature granulation tissue is found, being represented by inflammatory infiltrate cells such as leukocytes, macrophages, lymphocytes, plasmocytes, newly formed vessels, as well as fibroblasts. As it matures, the number of cells and vessels decreases and that of the extracellular matrix increases, which can also lead to the scar development on the skin. The angiogenesis severity in the wound may indicate symptoms of granulation tissue or the presence of a focus of chronic inflammation. If the granulation tissue resorbs and the wound surface is epithelialized without an apparent defect, it is referred to the wound healing by primary tension. In addition, the severity and the speed of maturation of granulation tissue in the wound may reflect both effective and ineffective reparation. The formation of collagen fibers/connective tissue is typical for reparations by substitution with the formation of a connective tissue scar. In case of festering, the wound reparation takes place by means of secondary tension, i.e., by substitution with the scar formation.

Necrotic detritus is characteristic for the initial stage of reparation, and as the wound heals, it must disappear from the surface of the lesion. The presence of detritus on day 7 after the skin injury also indicates inefficient reparations. It is also possible to evaluate the reparation effectiveness by the presence of signs of inflammation. The detection of acute purulent inflammation in wounds in the groups under study was seen as an unfortunate sign that characterized the development of bacterial infection and indicative of the reparation ineffectiveness. The presence of leukocytes in the infiltrate is indicative of an acute inflammatory reaction in tissues. The more leukocytes are present in the infiltrate, the more pronounced the exudative purulent inflammation is, and hence the reparation is ineffective.

The involvement of subcutaneous tissue and subcutaneous musculature in the inflammatory process indicates the depth of the lesion.

The objective of the study was to assess the reparative and antibacterial properties of the gel containing chitosan-miramistin-chymopsin on the model of linear and planar wounds in rats.

MATERIALS AND METHODS

Histological study had been performed on the material of the cutaneous flaps sized 1.5 cm × 1.0 cm × 0.5 cm, from 48 animals divided into six groups: Eight rats with LWG, eight rats with PWG, eight rats with planar wound and a comparator agent substance PWCA, and eight rats with LWCA [Table 1]. The animals were withdrawn from the experiment, and the

Table 1: Research groups

| Groups | Amount |
|---------------------------------------|--------|
| Linear wound. CMCC-based gel (LWG) | 8 |
| Planar wound. CMCC-based gel (PWG) | 8 |
| Linear wound. Comparator agent (LWCA) | 8 |
| Planar wound. Comparator agent (PWCA) | 8 |
| Planar wound. Control (PWR) | 8 |
| Linear wound. Control (LWR) | 8 |

CMCC: Chitosan-myrastin-chymopsin complex, LWG: Linear wound treated with chitosan-myrastin-chymopsin complex-based gellinear wound treated with chitosan-myrastin-chymopsin complex-based gel, PWG: Planar wound treated with test gel, LWCA: Linear wound and comparator agent, PWCA: Planar wound and comparator agent, PWR: Pressurised water reactor, LWR: Light water reactor

material was taken on the 8th day in case of the linear wound and on the 35th day in case of planar one.

Outbred rats, females, aged 10–12 weeks, had body weight at the beginning of the study 160–180 g (males), KrolInfo, LLC. Epicutaneously (as the main route of administration planned for clinical use), CMCC (miramistin content - 0.05; chimopsyn - 0.2) was administered: Daily, once a day, epicutaneously, for 7 days on a linear wound model; during 35 days on the planar wound model; on the 8th day - for a linear wound; and on the 35th day - for the planar wound, a guillotine.

Linear wound model

The study of the wound healing action on the model of a linear wound lasts 8 days. Linear wounds in rats represent a longitudinal incision of skin and subcutaneous fat along the midline of the back of 3–3.5 cm in length. Before surgery, the wool along the spine to the width of 300 mm and the length of 700 mm is cut off. Then, “Zoletil 100” (“Virbac santé animale,” France) - the dissociative anesthetic approved for the use on the territory of the Russian Federation - is injected intramuscularly. After the anesthesia of the animal, the wounding starts. Then, the edges of the wound are brought together, by applying three seams (thread silk) at an equal distance from each other.

Starting from the day of surgery, the gel and comparator agent are applied for 7 days on the surface of the wound of an animal. Daily monitoring of experimental animals and assessing the healing of linear wounds (appearance, wound size, and scar size) are performed during 8 days. On the first and the last day of the experiment, the masses of the test animals are measured. In addition, microbiological monitoring is carried out on the 3rd, 5th, and 8th days.

Euthanasia of the test animals should be performed on the 8th day followed by a blood sampling for hematology research and the cutaneous flaps drawing for histology and wound tensiometry.

Planar wound model

The study of the wound healing action on the model of a planar wound lasts 35 days. Before surgery, wool and an underhair are cut out in the dorsum area. Then, the cutaneous flap with an area of 400 mm² is also cut out (also removing subcutaneous fatty tissue) using a special stencil under intramuscular anesthesia with “Zoletil 100” (“Virbac santé animale,” France). Planar wound is left open throughout the observation period.

Starting from the day of surgery, the gel and comparator agent are applied for 34 days on the surface of the wound of an animal. Furthermore, daily monitoring of the condition of the animals tested is carried out and the degree of healing of the planar wound is evaluated (by tracing the patterns of wounds, weighing them), and the thermometry inside and around the wound is carried out for 35 days. The masses of the test animal are measured once a week. Measurement of the mass of animals on the first and last day of the experiment is necessary. Moreover, microbiological control is made on the 5th, 8th, and 35th days. The euthanasia of the test animals should be carried out on the 35th day followed by a blood sampling for hematology research and the cutaneous flap drawing for histology.

After the excision of cutaneous flaps from the wound region, the material is placed in a 10% neutral formalin and then poured into paraffin. Paraffin blocks are cut to serial 4 μm thick slices, which are fixed on slide plates and incubated in the thermostat at 37°C for 12 h. The resulting preparations are dewaxed, rehydrated, stained with hematoxylin and eosin, and enclosed under coverslips.

The wound healing degree is estimated by the reparation index, which is calculated by the difference between the “positive” and “negative” signs of reparation. The “positive” signs include: Granulation tissue and its maturity, monocytic infiltrate, the presence of fibroblasts, angiogenesis, wound surface epithelization, and the absence of fibrosis. The presence of the wound canal, leukocyte infiltration, foci of necrosis, and fibrosis in the cutaneous flap is regarded as “negative” sign.

Morphological study results

In the group with LWG, no erosion was detected, a wound canal filled with immature granulation tissue was found, and a weak inflammatory infiltrate represented by leukocytes and lymphocytes was found at the edges. The wound surface was epithelialized. Flat epithelium with the vacuolar interface dermatitis and weak acanthosis is shown in Figure 1.

The cutaneous flaps from the PWG group at the place of injury were also epithelialized, granulation tissue with a large number of fibroblasts was found in the superficial layers of the dermis, and weak inflammatory infiltrate was found in the edges and was represented by lymphocytes, plasmocytes, and single leukocytes. Flat epithelium with the vacuolar interface dermatitis and acanthosis is shown in Figure 2.

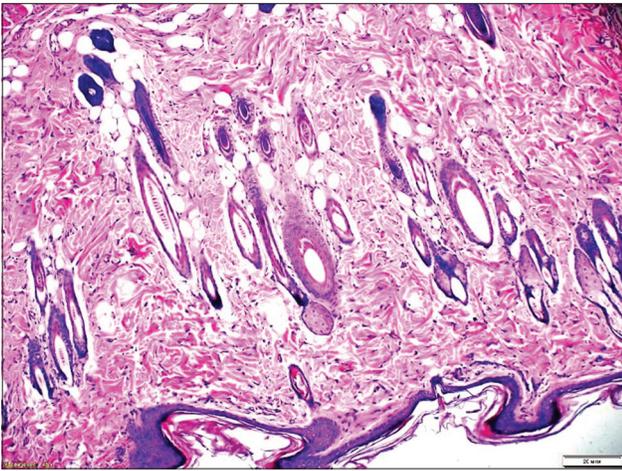


Figure 1: The results of the morphological study of the cutaneous flap of the linear wound treated with chitosan-myramistin-chymopsin complex-based gel group (magnification $\times 40$)

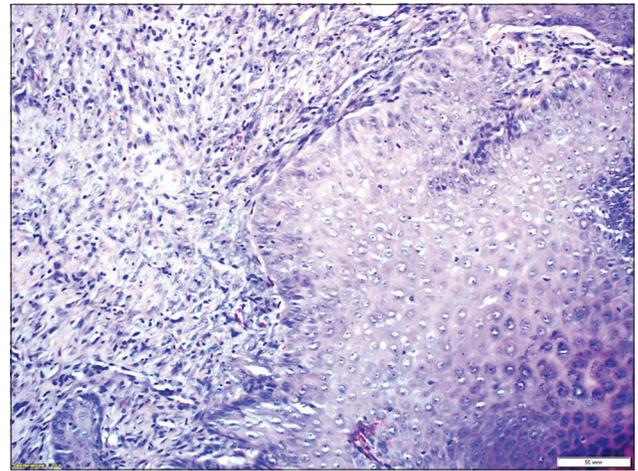


Figure 4: The results of the morphological study of the cutaneous flap of the linear wound and comparator agent group (magnification $\times 20$)

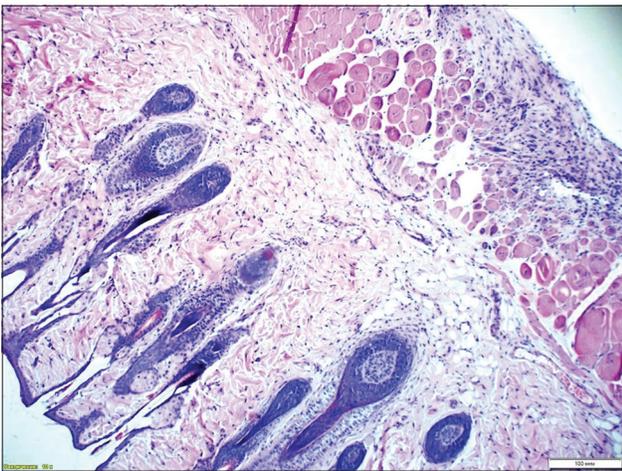


Figure 2: The results of the morphological study of the cutaneous flap of the planar wound treated with test gel group (magnification $\times 40$)

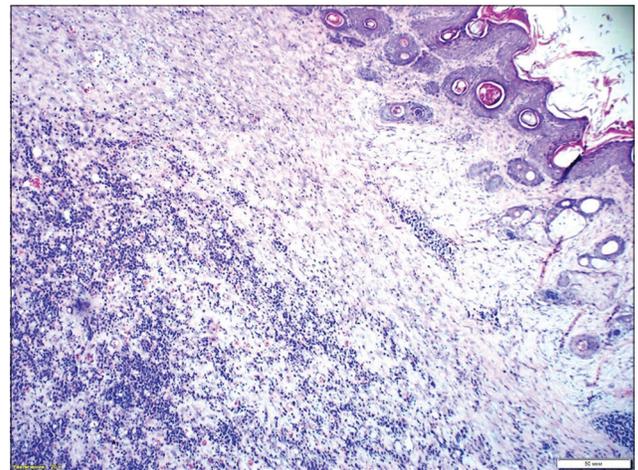


Figure 5: The results of the morphological study of the cutaneous flap of the linear wound treated with chitosan-myramistin-chymopsin complex-based gel group (magnification $\times 20$)

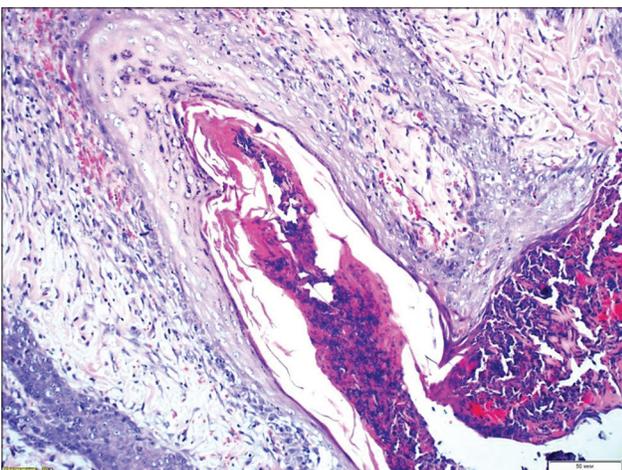


Figure 3: The results of the morphological study of the cutaneous flap of the planar wound and comparator agent group (magnification $\times 40$)

In the group with PWCA, small erosion was detected, epithelized at the edges, as well as the mature granulation tissue in the underlying layers, and an inflammatory infiltrate represented by leukocytes and lymphocytes at the edges. Flat epithelium with the vacuolar interface dermatitis and leukopedesis phenomena is shown in Figure 3.

The cutaneous flaps with linear wound and comparator agent: The wound canal was epithelialized on the surface, it was filled with granulation tissue all the way through, and moderate monocyte infiltrates with an admixture of leukocytes at the edges. Flat epithelium with the vacuolar interface dermatitis phenomena and acanthosis is shown in Figure 4.

In LWG and PWG [Figure 5] in the area of the former wound canal and wound, the remains of granulation tissue, small lymphocytic infiltration with an admixture of single

leukocytes were observed. The wound surface at the edges was epithelialized; the erosion of the small size was detected in the central part. In a number of LWG cases, the festering in the region of the wound canal was observed [Figure 6].

In linear wounds, granulomatous tissue reactions with the formation of non-specific granules of foreign bodies were detected in the area of suture remains.

Thus, all the wounds treated with the gel and the preparatory agent were healed by primary tension, whereas in the control, in some cases, this process occurred with the development of purulent liquefaction - i.e. by secondary tension. In the pressurised water reactor (PWR) group, the wounds were healed without complications by primary tension [Figures 7 and 8].

CONCLUSION

Thus, based on reparation signs, the more effective tissue reparation had been observed in groups with gel, comparator agent and PWR, and the least effective in the light water reactor group, which was probably due to the accession of

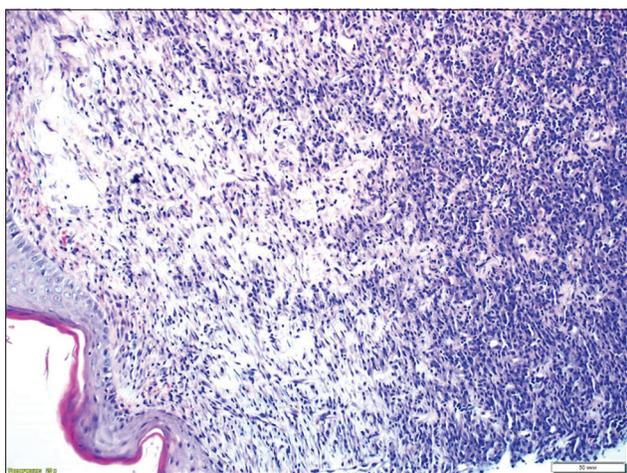


Figure 6: The results of the morphological study of the cutaneous flap of the linear wound treated with chitosan-myramistin-chymopsin complex-based gel group; the festering in the region of the wound canal (magnification $\times 20$)

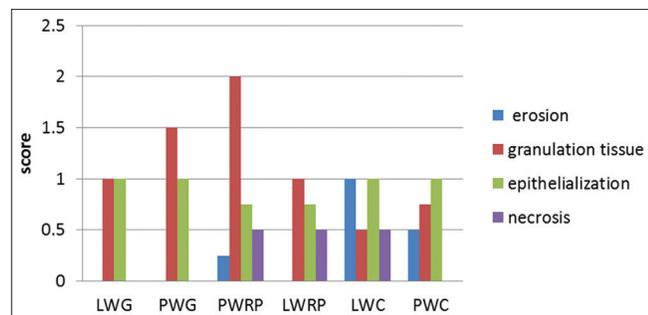


Figure 7: Morphological characteristics of wounds

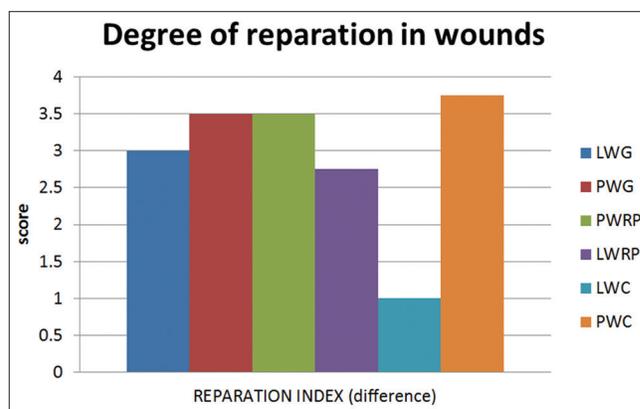


Figure 8: Severity of reparation in wounds

secondary infection and festering development. In the groups treated with gel and comparator agent, the average reparation level had been revealed, and in case of the linear wound, the gel proved to be more effective, compared to the comparator agent, and in planar wounds' group, the level of reparation had been equally high.

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