The Study of the Wound Healing Activity of the Gel with a Comprehensive Therapeutic Effect

Liliana Lyubanovna Brkich, Andrey Anatolevich Nedorubov*, Natalia Valeryevna Pyatigorskaya, Galina Eduardovna Brkich, Elena Sergeevna Odintsova

Institute of Translational Medicine and Biotechnology, Sechenov First Moscow State Medical University, Trubetskaya Street, 8-2, Moscow, 119991, Russian Federation

Abstract

AIM: The study was aimed at researching the specific wound healing activity of the drug with a comprehensive therapeutic effect based on derivatives of glucosamine and acrylic polymers to treat the infected wounds of various origins on a model of a planar infected wound.

METHODS: The model of septic wounds in rats as per the method of P.I. Tolstyk was used during the study of the specific activity of the drug with a comprehensive therapeutic effect based on derivatives of glucosamine and acrylic polymers for the treatment of infected wounds. The infection was performed with the S. aureus and E. coli strains. The study lasted 18 days, and during this period no full scarring occurred. The wound diameter was chosen as the effectiveness criterion. The planimetric method was used to assess the course of the wound process in experimental animals.

RESULTS: The obtained data prove the specific action of the drug with a comprehensive therapeutic action based on derivatives of glucosamine and acrylic polymers to treat the infected wounds of various origins. The study has shown that bacterially infected wounds healed worse than noninfected ones. Both types of wounds — infected and uninfected ones — healed faster when applying the test drug or Levomekol ointment.

CONCLUSION: On the model of a planar infected wound, the developed drug with a comprehensive therapeutic action has shown better wound healing effect compared with the Levomekol reference drug.

Introduction

The drug with a comprehensive therapeutic action based on derivatives of glucosamine and acrylic polymers to treat the infected wounds of various origin is a translucent gel based on hydroxypropyl cellulose and polyacrylamide, with the inclusion of chitosan, hemopexin, miramistin and lidocaine hydrochloride as pharmaceutical agents (Brkich, Pyatigorskaya, 2017) [1].

The drug for the treatment of infected wounds has four types of pharmacological effects, namely necrolytic, antimicrobial, wound healing and anaesthetic ones.

The necrolytic effect is ensured by hemopexin, providing a lysing effect on septic substrates and cleansing the wound surface from purple-necrotic masses. When applied to tissue, hemopexin splits fibrinous formations and necrotic tissue structures, and blood clots. It also thins exudate and viscous secret. Himopexin restores microcirculation in the wound walls, improves metabolic processes, which is clinically manifested by a decrease in local inflammation.

Chitosan is a carrier of the enzyme complex. It provides a prolonged therapeutic effect of himopexin and has a wound healing effect.

The antimicrobial action is ensured by the
presence of Miramistin antiseptic (benzyl dimethyl [3- (myristoylamino) propyl] ammonium chloride monohydrate). The compound has a pronounced bactericidal action against aerobic and anaerobic bacteria, gram-positive (Staphylococcus, Streptococcus, Bacillus subtilis, Bacillus anthracoides) and gram-negative organisms (Shigella, Pseudomonas aeruginosa, Escherichia coli, Salmonella), as well as against hospital strains multiresistant to antibiotics. Miramistin provides antimicrobial and fungicidal action, enhances the functional activity of immune cells, while stimulating local (nonspecific) immunity, accelerates the wound healing process, reduces the resistance of pathogenic microorganisms to antibiotic therapy, and activates protective reactions at the application site through the activation of the absorbing and digestive functions of phagocytes.

The analgesic effect is due to the presence of lidocaine anaesthetic, which has a local anaesthetic effect, blocks potential-dependent sodium channels, thereby preventing the generation of impulses in the endings of sensory nerves and conduction of impulses along nerve fibres. The anaesthetic action of lidocaine is 2 – 6 times stronger than that of novocaine and procaine. When applied locally, it dilates blood vessels and has no irritating local action.

The study was aimed at researching the specific wound healing activity of the drug with a comprehensive therapeutic effect on a model of a planar infected wound.

Material and Methods

The drug is a gel for external use, containing per 100 g: himopsin – 0.2 g, miramistin – 0.05 g, chitosan – 2 g, lidocaine – 0.1 g, polyacrylamide – 0.1 g, hydroxypropylmethylcellulose – 2 g, glycerin – 5.0 g, and water – up to 100 g.

A group of 45 male rats of the Wistar line, weighing 200 – 240 g, (Stolbovaya branch of the FSBUN NCBMT FMBA of Russia) were used in the study.

The bacterial strains of the American Type Culture Collection (ATCC) obtained from the ACM (All-Russian Collection of Microorganisms) of Moscow were used in the experiment. The species composition was represented by the following microorganisms: Escherichia coli (ATCC 25922); Staphylococcus aureus (ATCC 6538-R).

The antibacterial effect of the studied objects was studied by the "wells" method. Bacteria were cultivated in L-broth at 37°C for 20 hours. Then, the number of cells per 1 ml of the initial suspension was determined using the Koch method. For further research, dilutions were used, providing the medium contamination on a Petri dish of 10⁴ and 10⁵ CFU/ml, which corresponded to the initial dissemination of the wound.

From the obtained dilutions, previously thoroughly mixed, bacteria were sown on the surface of the agar plate in a Petri dish with a sterile pipette in the amount of 0.1 ml. The volume of the applied suspension was distributed over the surface of the medium with a sterile spatula.

The septic wound was modelled for the animals under anaesthesia under sterile conditions as per the P.I. Tolstykh method (1976) [2]. For this purpose, the skin with subcutaneous tissue 25 mm in diameter was dissected into on the antiseptic-treated back section shayed from wool. A gauze plug containing 1.2 x 10⁶ microbial bodies of the S. aureus daily culture or 2.6 x 10⁶ E. coli was introduced into the resulting wound, and the wound was sutured. On the following day (24 hours), after modelling, the abscess with all the characteristic signs of inflammation was formed in all animals. After the stitches' removal, the upper skin flap was removed, the gauze plug was removed, and the purulence was evacuated. The area of the original wound was determined by applying a contour to a transparent film.

Treatment of the wound with the test drug or Levomekol reference product was started 24 hours after the septic wound modelling. The drugs were applied for 18 days. During the treatment, the diameter of the wound was measured every three days. Planimetric method was used to assess the course of the wound process in experimental animals.

Results

The animals were divided into groups, in accordance with the doses of the injected substance (Table 1).

| No. | Infection type | Drug | Number of animals | Drug dose, g/kg |
|-----|----------------|------|-------------------|----------------|
| 1.  | S. aureus      | Gel, 0.2 g/200 g | 3♂  | 1               |
| 2.  | E. coli        | Gel, 0.1 g/200 g | 3♂  | 5               |
| 3.  | S. aureus      | Levomekol, 0.2 g/200 g | 3♂  | 1               |
| 4.  | E. coli        | Levomekol, 1 g/200 g | 3♂  | 5               |
| 5.  | S. aureus      | Without drug     | 3♂  | -               |
| 6.  | E. coli        |                     | 3♂  | -               |
| 7.  | S. aureus      |                     | 3♀  | -               |
| 8.  | E. coli        |                     | 3♀  | -               |
| 9.  | S. aureus      |                     | 3♀  | -               |
| 10. | E. coli        |                     | 3♀  | -               |
| 11. | Without infection | Gel, 0.2 g/200 g | 3♂  | 1               |
| 12. | Gel, 1 g/200 g | 3♂  | 5               |
| 13. | Levomekol, 0.2 g/200 g | 3♂  | 1               |
| 14. | Levomekol, 1 g/200 g | 3♂  | 5 g/kg          |
| 15. | Without drug   | 3♂  | -               |

After modelling a septic wound, rats in all groups of the test drug, the Levomekol reference
drug, and the wounds without drugs were sluggish in the first two days of the study. The wound at the beginning of the study is presented in Figure 1.

![Image of wound at the beginning of the study](image)

**Figure 1: Wound at the beginning of the study**

The wound diameter during the treatment was measured planimetrically every three days. The data are shown in Table 2.

| No. | Infection type | Drug          | Wound size, cm |
|-----|----------------|---------------|----------------|
|     |                | Days of infection |                 |
|     |                | 1   | 3   | 6   | 9   | 12  | 15  | 18  |
| 1   | S. aureus      | Test drug, 0.2 g/200 g | 2.7 | 2.5 | 2.1 | 2.0 | 1.2 | 1.0 | 0.5 |
|     |                | Average: | 2.6 | 2.5 | 2.0 | 1.8 | 1.0 | 0.7 | 0.3 |
| 2   | E. coli        | Test drug, 0.2 g/200 g | 2.3 | 2.3 | 2.0 | 1.9 | 1.3 | 1.1 | 0.6 |
|     |                | Average: | 2.3 | 2.3 | 2.3 | 2.2 | 1.2 | 1.0 | 0.5 |
| 3   | S. aureus      | Test drug, 1 g/200 g | 2.6 | 2.6 | 2.1 | 2.1 | 1.3 | 1.0 | 0.7 |
|     |                | Average: | 2.6 | 2.6 | 2.4 | 2.1 | 1.8 | 1.1 | 0.7 |
| 4   | E. coli        | Test drug, 1 g/200 g | 2.4 | 2.4 | 2.2 | 2.1 | 1.2 | 1.0 | 0.6 |
|     |                | Average: | 2.3 | 2.3 | 2.1 | 2.0 | 0.9 | 0.8 | 0.3 |
| 5   | S. aureus      | Levomekol, 0.2 g/200 g | 2.8 | 2.6 | 2.4 | 2.0 | 1.4 | 1.1 | 0.9 |
|     |                | Average: | 2.8 | 2.6 | 2.5 | 2.2 | 1.2 | 1.0 | 0.8 |
| 6   | E. coli        | Levomekol, 0.2 g/200 g | 2.5 | 2.4 | 2.2 | 2.0 | 1.5 | 1.1 | 0.7 |
|     |                | Average: | 2.5 | 2.4 | 2.2 | 2.0 | 1.4 | 1.1 | 0.6 |
| 7   | S. aureus      | Levomekol, 1 g/200 g | 2.7 | 2.5 | 2.2 | 2.0 | 1.3 | 1.1 | 0.8 |
|     |                | Average: | 2.7 | 2.5 | 2.3 | 2.1 | 1.3 | 1.0 | 0.8 |
| 8   | E. coli        | Levomekol, 1 g/200 g | 2.5 | 2.3 | 2.3 | 2.0 | 1.4 | 1.1 | 0.9 |
|     |                | Average: | 2.5 | 2.4 | 2.4 | 2.1 | 1.5 | 1.1 | 0.8 |
| 9   | S. aureus      | Without drugs | 2.6 | 2.5 | 2.2 | 1.8 | 1.6 | 1.2 |
|     |                | Average: | 2.6 | 2.6 | 2.5 | 2.4 | 1.5 | 1.3 | 1.0 |
| 10  | E. coli        | Without drugs | 2.7 | 2.8 | 2.5 | 2.3 | 1.7 | 1.5 | 1.1 |
|     |                | Average: | 2.7 | 2.8 | 2.5 | 2.3 | 1.7 | 1.5 | 1.1 |
| 11  | Without infection | Test drug, 0.2 g/200 g | 2.4 | 2.4 | 2.3 | 2.0 | 1.3 | 1.2 | 0.8 |
|     |                | Average: | 2.4 | 2.4 | 2.3 | 2.0 | 1.0 | 0.9 | 0.6 |
| 12  | Test drug, 1 g/200 g | 2.7 | 2.6 | 2.3 | 2.0 | 1.3 | 1.0 | 0.7 |
|     |                | Average: | 2.7 | 2.6 | 2.4 | 1.9 | 1.3 | 1.0 | 0.6 |
| 13  | Levomekol, 0.2 g/200 g | 2.6 | 2.5 | 2.4 | 1.8 | 1.4 | 1.2 | 1.1 |
|     |                | Average: | 2.6 | 2.5 | 2.7 | 2.6 | 1.4 | 1.2 | 1.0 |
| 14  | Levomekol, 1 g/200 g | 2.7 | 2.6 | 2.4 | 1.9 | 1.4 | 1.1 | 0.7 |
|     |                | Average: | 2.7 | 2.6 | 2.4 | 1.9 | 1.4 | 1.1 | 0.9 |
| 15  | Without drugs | 2.5 | 2.5 | 2.3 | 2.0 | 1.3 | 1.0 | 0.8 |
|     |                | Average: | 2.5 | 2.5 | 2.3 | 2.0 | 1.3 | 1.0 | 0.8 |

**Table 2: The result of measuring the size of the wound during the study**

**Discussion**

Levomekol was used as a reference drug. In the international practice, it has been known since the 70s as "Chloramphenicol + Methyluracil", and is a combined drug for local administration, having an anti-inflammatory effect. It is also active against gram-positive and gram-negative microbes (staphylococci, Pseudomonas bacilli and e-coli), penetrates tissues without damaging biological membranes and stimulates regeneration processes (Azuma, 2015; Dai, 2011) [3], [4]. Levomekol is widely used in surgical practice for any type of tissue damage, mainly infected ones, with mixed microflora in the first purulonecrotic phase of the wound process (Brkich et al., 2018; Shipovskaia, Zudina, Fomina, 2015) [5], [6].

The model of septic wounds in rats as per the P.I. Tolstyk method was used during the study of the specific activity of the drug with a comprehensive therapeutic effect based on derivatives of glucosamine and acrylic polymers for the treatment of infected wounds (Tolstyk et al., 2013) [2]. The infection was performed with the S. aureus and E. coli strains. The study lasted 18 days, and during this period no full scarring occurred. The wound diameter was chosen as the effectiveness criterion.

The bacteriologically infected wounds healed worse than the uninected ones. On the 18th day of the study, the average diameter of the wounds infected with S. aureus (group 9) or E. coli. (group 10) was 1.1 cm, and in group 15 – 0.97 cm.

The healing rate of an uninfected and untreated wound was 0.1 cm/day (Table 2). The average diameter of the wounds without infection to which the preparations were applied on the first day of the study was 2.66 cm (groups 11 – 15). The rate of wound healing without infection after the application of the test drug was 0.105 cm/day for a dose of 0.2 g/200 g (group 11), and 0.111 for a dose of 1 g/200 g (group 12). The rate of wound healing with the
Application of Levomekol was 0.105 cm/day for a dose of 0.2 g/200 g (group 13), and 0.107 cm/day for a dose of 0.2 g/200 g (group 14). The rate of healing of the uninfected wounds when applying a larger amount of the test or the reference drug was slightly higher. In general, the rate of healing of the uninfected wounds when applying the test drug was 1.11 times greater than the healing of the uninfected untreated wounds, and 1.07 times greater for the reference drug.

The size of the wounds on day 1 of the study after the infection with S. aureus in five groups of animals (1, 3, 5, 7, 9) averaged 2.63 cm. The dynamics of healing are presented in Figure 2.

The size of the wound on the 18th day of the study without the use of drugs was 1.1 cm. On the 18th day of the experiment, after applying the test drug to the wounds, the wound diameter decreased to 0.5 cm (dose of 0.2 g/200 g of the live weight) and 0.6 cm (dose of 1 g/200 g of the live weight). The wound healing rate was 0.117 cm/day for groups 1 and 3, which suggests that increasing the drug dose by more than 0.2 g per 200 g of the live weight lacked greater healing effect on wounds infected with S. aureus. For group 9 (healing with infection, without applying gels), the wound healing rate was 0.082 cm/day. The use of the studied gel increases the wound healing rate 1.43 times compared to the group of animals with the infected wounds, on which the gel was not applied.

The size of the wounds on day 1 of the study after the infection with E. coli in five groups of animals (2, 4, 6, 8, 10) averaged 2.63 cm. The dynamics of healing are presented in Figure 3.

The size of the wound on the 18th day of the study without the use of drugs was 1.1 cm, as in case of infection with E. coli (healing rate – 0.077 cm/day). On the 18th day of the experiment, after applying the test drug to the wounds, the wound diameter decreased to 0.46 cm (dose of 0.2 g/200 g of the live weight) and 0.5 cm (dose of 1 g/200 g of the live weight). The wound healing rate was 0.105 cm/day for group 2 and 0.104 for group 4, which suggests that increasing the drug dose by more than 0.2 g per 200 g of the live weight lacked greater healing effect on the wounds infected with E. coli. The use of the studied gel increases the wound healing rate 1.36 times compared to the group of animals with the infected wounds, on which the gel was not applied.

After applying the Levomekol reference test drug to the wounds infected with S. aureus, on the 18th day of the experiment, the wound diameter decreased to 0.8 cm (dose of 0.2 g/200 g of the live weight) and 0.76 cm (dose of 1 g/200 g of the live weight). The data are presented in Figure 4.

The wound healing rate was 0.102 cm/day for group 5 and 0.104 for group 7, which suggests that increasing the drug dose by more than 0.2 g per 200 g of the live weight lacked greater healing effect on the wounds. The use of Levomekol increases the healing rate of the wounds infected with S. aureus 1.27 times compared to the group of animals with the infected wounds to which the gel was not applied.

In case of the wound infection with S. aureus, the healing rate in the group with the test drug was higher than in the group with the reference Levomekol drug by 1.2 mm/day in diameter. The wound healing rate in the group with the test drug was statistically significantly higher than that in the group of animals.
with the infected and untreated wounds (group 9).

After applying the Levomekol reference test drug to the wounds infected with S. aureus, on the 18th day of the experiment, the wound diameter decreased to 0.8 cm (dose of 0.2 g/200 g of the live weight) and 0.76 cm (dose of 1 g/200 g of the live weight.). The data are presented in Figure 5.

The wound healing rate for groups 6 and 8 was 0.098 cm/day. The use of Levomekol increased the healing rate of the wounds infected with E. coli 1.27 times, as well as of the wounds infected with S. aureus, compared to the group of animals with the infected wounds, on which the gel was not applied, indicating the same effectiveness of Levomekol on this model of septic wounds.

In case of the wound infection with E. coli, the healing rate in the group with the test drug was higher than that in the group with the reference Levomekol drug by 1.2 mm/day in diameter. The wound healing rate in the group with the test drug was statistically significantly higher than that in the group of animals with the infected and untreated wounds (group 10).

**Discussion**

The obtained data prove the specific action of the drug with a comprehensive therapeutic effect based on derivatives of glucosamine and acrylic polymers to treat the infected wounds of various origins. The study has shown that the bacterially infected wounds healed worse than the noninfected ones. Both types of wounds — infected and uninfected ones — healed faster when applying the test drug or Levomekol ointment. When infected with S. aureus and E. coli, the studied drug showed the best result in wound healing compared with Levomekol. Increasing the dose of drugs more than 0.2 g per 200 g of the live weight lacked greater healing effect both in S. aureus and in E. coli infection.

On a model of a planar infected wound, the developed drug with comprehensive therapeutic effect has shown better wound healing effect compared with the Levomekol reference drug.

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