Assessment of wound-healing activity of zinc oxide nanoparticles

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ABSTRACT

The article presents the results of studying the effect of a complex of zinc oxide nanoparticles on the reparative regeneration of an experimental wound. The analysis of data from planimetric studies (changes in the area of the wound in dynamics, the rate of wound healing, the daily change in the area of the wound) showed a high wound healing activity of the drug, statistically significantly superior to the indicators in the comparison group. Zinc oxide nanoparticles, in addition to regenerating, have an antimicrobial effect and prevent secondary contamination of the wound, which also contributes to full-fledged reparative regeneration. It has been proven that topical application of the drug in animals with experimental conditionally aseptic wounds provides pronounced regenerating and antibacterial effects. This drug can be used to treat skin and soft tissue injuries of various etiologies. The use of the drug for local wound treatment at all stages of complex treatment will reduce the duration of systemic antimicrobial therapy, avoid the development of side effects, significantly reduce the cost of expensive antibacterial drugs, and avoid the formation of resistance of microflora to the systemic antibiotics used.

Keywords: Nanoparticles, Zinc, Rats, Regeneration, Wound-healing activity

Introduction

Optimization of the processes of reparative regeneration of skin and soft tissue injuries caused by various mechanical, thermal, and other factors (purulent-inflammatory processes, dystrophic, etc.) remains an urgent scientific and practical task [1-3].

The use of nanomaterials is a promising direction for the creation of new drugs with a wound-healing effect [4, 5]. Metal nanoparticles have physicochemical properties that differ both from the properties of massive metal objects and from the properties of individual atoms [6]. When ingested into living organisms, nanoscale metal particles cause a biological response that differs from the action of the traditional ionic form of the elements [7].

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Topical preparations based on metal nanoparticles are used for topical treatment (water-soluble ointments, sorbents, enzymes, foam and film-forming aerosols, hydrocolloids, alginates, wound coatings, modified suture materials, etc.) [8-11].

The study of the properties of metal nanoparticles has shown their wound-healing activity, and regenerating and bactericidal properties, which makes their use promising for the treatment of purulent inflammatory complications [12-14].

Ultrafine zinc has a pronounced antibacterial effect, zinc ions released from the surface of nanoparticles inhibit the action of respiratory enzymes, which leads to the activation of free oxygen and damage to the bacterial cell [15, 16].

The auxiliary powdered component actively absorbs blood and wound exudate, which are a nutrient medium for bacteria [17, 18]. Unlike regenerating and antibacterial creams and ointments, powder-based preparations do not create films on the wound, which provides free oxygen access to damaged tissue cells, accelerating the natural healing processes [19]. The drugs have a pronounced draining and deodorizing effect, due to the removal of intradermal pressure, due to their sorption properties, analgesia of damaged skin areas occurs [20].

It is relevant to assess the effect of topical application of zinc nanoparticles in combination with starch on the healing dynamics of an experimental full-layer wound. The work aimed to evaluate the effect of a complex preparation consisting of zinc nanoparticles and an auxiliary powdered component on the reparative regeneration of an experimental wound.

Materials and Methods

Zinc nanoparticles obtained using plasma technology were used in the work. Based on them, a drug for the regeneration of soft tissues with an antibacterial effect has been developed, having a powdered form and containing zinc nanoparticles, as well as sterile starch as a powdered base.

The experiment was conducted on 40 white mongrel rats weighing 170 ±20 g. The studies were conducted by the Helsinki Declaration of 1975 and its revision in 1983. The model of a full-layered wound was obtained as follows: after pretreatment of the skin, under aseptic conditions, under anesthesia, on a fur-shaven area in the interscapular region, the skin with subcutaneous tissue in the form of a 2×2 cm square (400 mm^2) along the contour previously stenciled [21]. The wound surface of the experimental group was treated once with 300 mg of a complex powdered preparation with zinc nanoparticles.

The appearance of the wound with the applied complex preparation is shown in **Figure 1**.

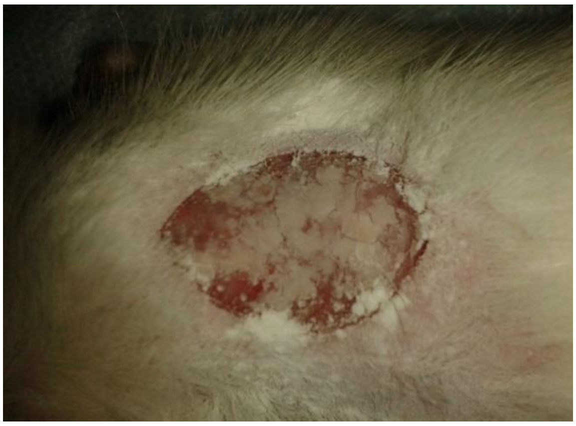


Figure 1. Model of a full-layer wound with a complex preparation based on zinc nanoparticles (the first day)

Statistical analysis allowed us to confirm the reliability of the results obtained. The normality of the distribution of quantitative indicators was checked using the Kolmogorov – Smirnov criterion, the coefficients of asymmetry, and kurtosis. The differences between the samples were evaluated using the Student's t-test since the variables corresponded to a normal distribution. The following statistical indicators were used in the analysis: n is the number of observations; M is the arithmetic mean; m is the standard error; p is the confidence coefficient. The results were considered reliable at $p<0.05$, which meets the requirements for biomedical research.

Results and Discussion

The analysis of data from planimetric studies (dynamics of wound area, wound healing rate, daily decrease in wound area) reflecting the activity of reparative regeneration of the experimental wound was carried out. The study of the dynamics of the area of a conditionally aseptic wound in experimental animals is given in **Table 1**.

Table 1. Change in the area of the wound surface in experimental animals

Day	The area of the conditionally aseptic wound in experimental animals, M, mm^2	
	Comparison group, n=20	Experimental group, n =20
1	425.1±11.3	412.8±21.6
3	395.3±10.7	211.4±17.3 (p<0.01)
5	438.7±15.3	74.3±8.4 (p<0.001)
7	218.4±9.3	36.8 ±3.2 (p<0.001)
10	156.1±7.3	0.0±0.0 (p<0.001)
14	22.9±7.3	0.0±0.0 (p<0.001)

Note: p is the confidence level of the differences in the indicators about the comparison group.

The use of the complex drug had a pronounced effect on wound regeneration at all times of control measurements, by the 10th day of the study, complete wound healing was observed in the experimental group, while in the comparison group, the healing percentage was 61.2%.

In five animals of the comparison group, an increase in the wound area by 30-35% was noted about baseline values on the 5th day of the study. During bacteriological examination in these animals, E. coli in the amount of $7 \cdot 10^6$ CFU/ml was isolated and identified from the wound discharge. Thus, secondary infection of the wound with intestinal autoflora was observed, leading to an increase in the wound surface and a slowdown in the reparative regeneration of the wound.

In all animals of the group treated with a powdered preparation based on zinc nanoparticles, dynamic healing of the experimental wound occurred against the background of the absence of secondary infection of the wound.

The analysis of the daily decrease in the area of the experimental wound in animals of the experimental group and the comparison group was carried out, the results of which are shown in **Figure 2**.

An analysis of the daily decrease in the area of wounds in dynamics in experimental animals showed that the maximum decrease in the area of the experimental wound ($p<0.001$) occurred under the action of a complex preparation on the 3rd-5th day after wound modeling – 28.3±3.9%, then on the 5th-7th day, the average daily decrease in the area of wounds decreased slightly to 18.7±2.8% This is because some of the animals had complete wound healing. The daily decrease in the area of experimental wounds in the experimental group at all periods was significantly higher than in the comparison group, where the indicators had significantly lower values (p<0.01), did not exceed 8.2 ± 1.2% per day, and tended to increase by the end of the observation period.

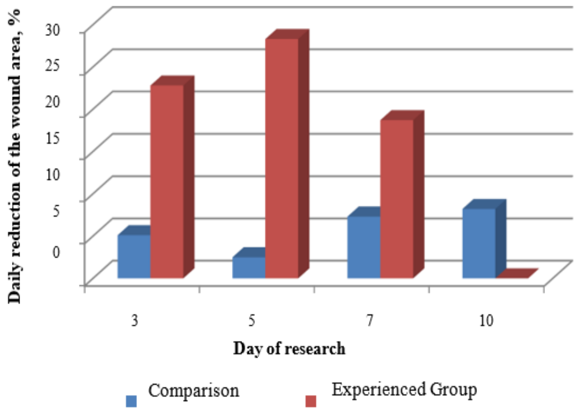


Figure 2. Daily decrease in the area of experimental wounds (in %)

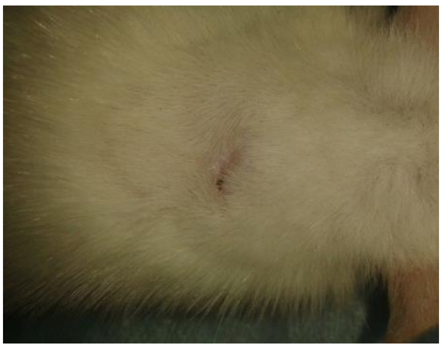
One of the planimetric characteristics of the reparative regeneration of an experimental wound is the rate of wound healing (mm/day). The healing rate of a conditionally aseptic wound during treatment with a complex drug significantly exceeds similar indicators (p<0.001) in the comparison group, which is reflected in **Table 2**.

Table 2. The healing rate of conditionally aseptic wounds (mm/day) in experimental animals (M±m)

Day	The healing rate of conditionally aseptic wounds (mm/day) in experimental animals (M±m)	
	Comparison group, n=20	Experimental group, n=20
3	23.3±3.6	54.3±7.1 (p<0.01)
5	14.1±8.3	85.2±2.4 (p<0.001)
7	12.3±3.5	39.6±4.4 (p<0.001)
10	21.2±6.7	0 (p<0.001)

Note: p is the confidence level of the differences in the indicators about the comparison group.

The analysis of the wound area healing rate statistically reliably demonstrates the difference between animal groups (p<0.001) at all follow-up periods, which proves a positive effect on the reparative regeneration of the complex preparation **(Figure 3)**.



a)

b)

Figure 3. The experimental wound of an animal of the experimental group a) and the comparison group b) on the 10th day of the study

The study of planimetry indicators: dynamics of the wound area, wound healing rate, and daily reduction of the wound area showed high wound healing activity of the developed complex preparation. Zinc nanoparticles included in the preparation, in addition to regenerating, have an antimicrobial effect and prevent wound infection, which is important for full-fledged reparative regeneration. The drug does not require wound–damaging removal from the wound surface, and creates optimal conditions for the action of the main therapeutic components - zinc nanoparticles, causing their total effect to exceed the activity of the initial components. The choice of a complex drug for the treatment of infected and purulent wounds is considered etiologically and pathogenetically justified due to its activity at a high level of bacterial contamination with antibiotic-resistant gram-positive and gram-negative microorganisms.

Conclusion

The developed preparation based on zinc nanoparticles has a pronounced stimulating effect on the reparative regeneration of soft tissues and can be used for various types of damage to the integumentary tissues. The use of the drug for local wound treatment at all stages of complex treatment will reduce the duration of systemic antimicrobial therapy, avoid the development of side effects, significantly reduce the cost of expensive antibacterial drugs, and avoid the formation of resistance of microflora to the systemic antibiotics used.

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Conflict of interest: None

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Ethics statement: The protocol for experiments with laboratory animals complied with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

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