Original Article

Effect of Drugs Based on Silver, Copper, and Zinc Nanoparticles on Skin Wound Healing in Rats

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Abstract

Modern medicine cannot do without the use of new technologies, including nanotechnology. They make it possible to achieve significant improvements in the diagnosis and treatment of various diseases, as well as increase the effectiveness of prevention. In particular, in recent years, more and more attention has been paid to the use of silver, zinc, and copper nanoparticles in medicine. They have unique properties, such as antimicrobial, antiviral, and anti-inflammatory effects, which allows them to be used to fight various infections and inflammatory processes. This scientific paper examines the effect of medical treatment of a fresh wound with preparations based on silver nanoparticles and an alloy of copper and zinc in the example of laboratory rats. Visual observation of the wound healing process was carried out, as well as morphological and hematological parameters of blood serum were studied. During the experiment, it was found that the treatment of a fresh wound with a preparation based on silver nanoparticles led to the fastest wound healing. And treatment with a preparation based on copper and zinc alloy nanoparticles significantly reduced the recovery time. The toxicological safety of the use of both drugs has been proven.

Keywords: Wound healing, Silver nanoparticles, Zinc and copper alloy nanoparticles, Morphological blood analysis, Biochemical blood analysis

Introduction

The main types of damage to the skin are wounds and burns [1, 2]. A wound is a violation of the integrity of the skin and mucous membranes, which can be accompanied by damage to deep-lying tissues: muscles, joints, bones, and internal organs [3].

Burn is a type of tissue damage caused by the action of hot steam, fire, high temperature, or the action of certain chemicals (for example, alkalis and acids) [4]. The danger of burns lies in the fact that during the burn, innervation of the entire burn surface occurs, and blood circulation is disrupted, which prevents rapid healing [5]. The effect of any damaging agent on the skin causes protein coagulation and, as a consequence, the death of epithelial cells (necrosis) [6]. Preparations for the treatment of any type of burn should have an antiseptic, wound-healing, and anti-inflammatory effect, as well as protect the burning surface from secondary infection and protect the cell from damaging effects and activation of metabolic processes, improve blood supply to tissues, stimulate wound healing [7].

The main dangers of any injury are bleeding and purulent complications as a result of germs entering the wound [8]. They get into it together with the wounding projectile – this is the primary microbial infection of the wound. A second time after injury, microbes can enter the wound from the surrounding skin, from touching the wound with dirty hands and other objects [9]. To prevent complications after injury, the most important thing is timely and proper first aid - a set of measures that temporarily eliminate the causes that threaten the life and health of the wounded, as well as to exclude the development of dangerous complications [10].

To date, the search for fundamentally new wound-healing agents effective in the treatment of purulent-inflammatory infections during injury is relevant. Nanotechnology is a promising field of science and technology, which occupies a strong place in the medical field [11]. They make it possible to create and use devices, systems, and materials with unique properties and effectiveness, which is especially important in the fight against various diseases [11]. One of the most

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promising areas of application of nanotechnology in medicine is the use of metal nanoparticles, such as silver, zinc, and copper [12, 13]. These materials have high biocompatibility and can be used to create functional nanomaterials that can be used in the diagnosis and treatment of various diseases [14]. For example, silver nanoparticles are used to create antimicrobial drugs that are highly effective against bacteria, viruses, and fungi [15, 16]. In addition, they can be used in the creation of new types of medical materials, such as bandages, surgical pads, or suture materials, which can disinfect wounds and shorten their healing time [17, 18].

Zinc and copper nanoparticles are effective antioxidants and can be used in the treatment of various diseases associated with damage to cells and tissues of the body. They can be used as components in new drugs for the treatment of various diseases, such as heart and vascular diseases, oncological diseases, diseases of the nervous system, and others [19-22].

Thus, the use of metal nanoparticles in medicine has a huge potential for improving the quality of life of patients, improving the effectiveness of treatment, and reducing healthcare costs. In this article, we will consider scientific research on the use of silver, zinc, and copper nanoparticles in medicine, as well as the prospects for their use in the future.

Materials and Methods

Experimental confirmation of the possibilities of a wound healing agent containing silver, copper, and zinc alloy nanoparticles for the restoration of epithelial tissues was carried out on laboratory rats. A preliminary study of the effect of silver, copper, and zinc alloy nanoparticles on Escherichia coli bacteria was also conducted. For this purpose, the intensity of bioluminescence of Escherichia coli bacteria under the action of nanoparticles of different concentrations was determined. In the course of the study, the following was determined: silver nanoparticles at a concentration of 0.003 M cause 80% quenching of the glow of the Escherichia coli biosensor. For copper and zinc alloy nanoparticles, a concentration of 0.00019 M causes a similar reaction. The toxicity of nanomaterials was determined after 180 minutes of contact with Escherichia coli. Based on these data, 2 aerosol sprays were prepared, one of which contained Ag nanoparticles and the other Cu/Zn alloy nanoparticles.

The studies were conducted on laboratory rats that were kept on a standard diet for laboratory animals according to the rules of laboratory practice when conducting preclinical studies in the Russian Federation [23]. The experiments were conducted in accordance with the requirements of the humane treatment of animals.

For the experiment, six-month-old male rats weighing 180 g were selected, among which three groups were formed by the method of pairs of analogs: control 1, experiment 1, and experiment 2 of 10 individuals in each group. For 14 days, the experimental animals were in the preparatory period, their feeding was carried out with compound feed based on a wheat-barley feed mixture of 70%, with a content of 172.3 g / kg of crude protein.

The weighing of nanoparticles was carried out on laboratory scales MS105DU (Mettler Toledo, USA).

Preparation of the nanoparticle preparation was carried out in a physical solution on an ultrasonic dispersant (f-35 kHz, N-300 W, A-10 μA), by dispersion for 30 minutes.

The maintenance and nutrition regime was the same in all experimental groups. The studies were conducted for 14 days.

Experimental wounds up to the muscle layer were inflicted on all laboratory animals under standard subcutaneous anesthesia. The injuries, 10 mm long, were inflicted with the help of surgical instruments. In the control group, wounds were treated with saline solution. In Experimental Group 1, a solution of silver nanoparticles (C=0.003 M) was applied to the wounds. In Experimental Group 2, a solution of copper and zinc alloy nanoparticles (C=0.00019 M) was applied to the wounds. During the accounting period, observations were recorded on the first, seventh, and fourteenth days of the experiment.

The criteria by which the fixation was performed are as follows: assessment of the appearance and size of the wound surface, and morphological and biochemical blood analysis. Based on the analysis of the data obtained, conclusions were formulated as to how the nanoparticles of the metals taken affect wound healing.

Results and Discussion

The animals of Experimental Group 1 treated with a solution of silver nanoparticles had faster healing of the wound surface during all the study periods than in the comparison group (control group) and in Experimental Group 2, which is due to the pronounced antibacterial effect of silver nanoparticles. The results of wound healing in the control group and the Experimental group 2 are shown in **Figure 1**. **Table 1** shows the dimensions of the wound surface, of all the groups studied.

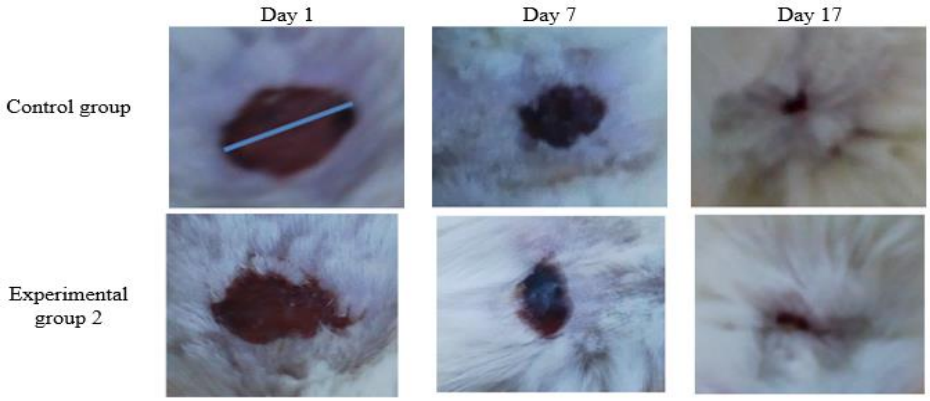

Figure 1. Results of wound healing in the control group and experimental group 2

At the next stage, it was proved that silver nanoparticles and copper-zinc alloy nanoparticles in this concentration (C =0.003 M) do not have a toxic effect on a living organism. For this purpose, morphological and biochemical blood analysis was carried out.

Table 1. Dynamics of the size of the wound surface of the studied animals

Day	Control group	Experimental group 1	Experimental group 2
Day 1	9.5	8.5	9
Day 7	0.65	0.45	0.5
Day 14	0.45	0.3	0.35

A blood sample was taken from the tail vein of laboratory animals for analysis. The data were processed using the URIT-2900 Vet Plus automatic hematology analyzer and entered in the table. **Table 2** shows that the drugs used did not have a toxic effect on the body, because all indicators were close to the control values and were within the normal range for the wound.

Table 2. Morphological parameters of blood serum

Index	Control group			Experimental group 1			Experimental group 2		
	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14
Red blood cells, 10^{12}/l	9.3±3.5	9.5±0.1	9.1±0.2	9.8±1.2	9.8±0.5	9.7±0.9	9.6±0.4	9.6±0.3	9.5±0.4
Hemoglobin, g/l	163±3	163.5±8.5	160±8.5	164±3.0	157±8	157±6	162±4	159±3	158±4
Leukocytes, 10^9/l	7.6±0.4	7.3±0.2	6.8±0.5	7.8±0.4	7.1±0.8	7.3±0.6	7.7±0.4	7.4±0.4	7.3±0.5
Platelets, 10^9/l	384±3	382±3	387.5±2	387.5±4.5	385.5±7	383±8	385±4	386±6	383±7

ASaT (aspartate aminotransferase), ALaT (alanine aminotransferase), and LDH (lactate dehydrogenase) are enzymes that are widely used in medical practice to diagnose damage to organs and tissues in the body [24]. With toxic effects on the body, cell cytolysis (destruction) occurs and these enzymes enter the bloodstream, their concentration increases, which is detected by laboratory methods [25].

Biochemical analysis of blood serum was carried out on an automatic biochemical analyzer SS-T240 (manufacturer – Dirui Industrial Co., Ltd., China) using commercial biochemical kits for veterinary medicine DiaVetTest (manufacturer – Russia) and commercial biochemical kits Randox (manufacturer – USA). Dirui CST240 analyzer (norms: ALaT: 20-92 U/l; ASaT: 150-300 U/l; LDG: 1241-1750 U/L).

Table 3. Biochemical parameters of blood

	Control group			Experimental group 1			Experimental group 2		
	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14
ALaT, U/l	45.3±3.5	50.7±4.9	43.8±3.2	46.8 ±1.2	52.3±1.1	46.5±1.1	44.6±1.8	54.3±1.6	44.2±2.4
ASaT, U/l	209.2± 23.5	202.8± 7.6	195.8± 5.7	212.9± 12.1	198.2± 6.2	193.2± 4	210± 18.6	199.6± 9	194.2± 12.2
LDG, U/l	1494.6± 72.3	1537.5± 104	1571± 65.5	1500± 53	1540.1± 94	1568± 117.1	1502.7± 46.8	1529.6± 82.6	1864.5± 61.5

Table 3 shows that silver nanoparticles did not have a toxic effect on the body throughout the experiment. All indicators were close to the control values.

The analysis of data on the reduction of wound area in experimental animals under the influence of a solution of silver nanoparticles (Experimental Group 1) and copper and zinc alloy nanoparticles (Experimental Group 2) showed their positive effect on the healing process of experimental wounds, exceeding the rate of wound healing in the Control group; toxic effects on the body were not detected. It should be noted, however, that the treatment of wounds with silver nanoparticles provides faster healing.

Conclusion

In the course of the study, the physicochemical, and regenerative properties and the possibility of using a wound-healing agent with metal nanoparticles for the treatment of wounds and burns were studied. The laboratory experiment proved that the use of the drug with silver nanoparticles or with copper and zinc alloy nanoparticles creates favorable prerequisites for accelerated restoration of the structure and functions of the skin and opens up wide possibilities for using this drug for first aid. It has been established that these nanoparticles have bactericidal properties, therefore, a wound-healing effect, which makes their study promising in terms of optimizing the regeneration of soft tissue damage.

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