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Dynamics of Microbial Contamination of the Experimental Purulent Wound During Topical Treatment with Agents Containing Copper Nanoparticles

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Abstract: The effect of topical application of copper and zinc nanoparticle suspensions on the dynamics of bacterial contamination of the experimental purulent wound was studied. A model of purulent wound infected with polyantibiotic-resistant clinical strains of *Staphylococcus aureus* isolated from orthopedic trauma patients was obtained in 30 white male rats. The use of copper nanoparticle suspension ensured rapid elimination of the infectious agent contaminating the wound; zinc nanoparticles had a weaker antibacterial effect: the *St. aureus* culture was disseminated until day 12 (until day 20 in the control group). The quantitative study of microbial contamination of 1 g of wound tissue demonstrated that it decreased to 10^2 CFU/g in the experimental animals treated with copper nanoparticles. The use of zinc nanoparticles to treat the wound resulted in a lesser decrease in the count of microbial bodies: the CFU/g value decreased to 10^5 CFU/ml on day 5, which also reliably differed from the control group, where an increase in the degree of bacterial contamination of the wound was observed at this time. The studies revealed the high antibacterial activity of copper nanoparticle suspension against clinical polyantibiotic-resistant strains of *St. aureus* under conditions of experimental purulent would.

Key words: Nanoparticles · Copper · Zinc · Experimental wound

INTRODUCTION

The general antibacterial therapy for pyoinflammatory postoperative and post-traumatic complications requires the introduction of antibiotics at high doses, which negatively affects the patient's organism, namely, impairs the immune system, causes dysbacteriosis and mycotic lesions [1]. All the methods for introduction of antibacterial agents fail to ensure sufficient concentration of antibiotics in the wound during a long period because of hemodynamic and morphological changes in the pathological nidus and the adjacent tissues, reducing treatment effectiveness and promoting the selection of polyantibiotic-resistant strains [2, 3].

The complex pathogenesis of the wound process stipulates the necessity for multidirectional effect of drugs. The use of modern drugs for topical treatment of wounds with antimicrobial and regenerating components allows one to make the most efficient use of antibacterial agents and to reduce the duration of the systemic antibacterial therapy to a significant extent. The unique properties of nanomaterials and their biological activity can be used to design a novel class of antibacterial and wound healing agents [4, 5, 6]. The mechanisms of biocidal activity of metal nanostructures have not been completely elucidated yet; the most probable of them include depletion of ATP synthesis [7, 8], disturbance of DNA replication [9, 10], oxidative impairment of cellular structures and changes in permeability of the cell membrane [11, 12].

This study was aimed at investigating the dynamics of bacterial contamination of the experimental purulent wound during topical application of copper and zinc nanoparticle suspensions.

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MATERIALS AND METHODS

Copper and zinc nanoparticles synthesized at the Plasmochemical Complex of the State Research Institute of Chemistry and Technology of Elementorganic Compounds (Moscow, Russia) were used. The experimental studies of the effect of metal nanoparticles on the wound process were conducted using 30 white male rats (170 30 g). All animals were kept in individual cages. The keeping and feeding conditions of the animals were identical in all the experimental groups. All the studies were carried out in compliance with the 1975 Declaration of Helsinki and its revision in 1983.

The purulent wound model was obtained as follows [13]: after the preliminary treatment of skin, the skin and subcutaneous fat in the form of a 2 × 2 cm square (400 mm^2) was excised in the shaved interscapular region along the contour drawn using a template in anaesthetized rats under aseptic conditions. The edges and bottom of the wound were crushed with a Kocher clamp. The wound was plugged with a gauze tampon with a suspension of 1-day-old culture of *St. aureus* at a dose of $5•10^9$ CFU/ml of microbial bodies in 0.5 ml of physiological saline. The wound was completely sutured. Methicillin-resistant clinical staphylococcus strains derived from orthopedic trauma patients were used. A wound with all typical signs of pyoinflammation was formed in the interscapular region on day 3. Edema and hyperemia of the skin around the wound and slight swelling were observed; pus discharged in some animals.

Sterile sheets moistened with 1.0 ml of copper and zinc nanoparticle suspension in isotonic solution (at concentration of 0.01 mg/ml) were daily placed onto the wound surface according to the subdivision of animals into groups.

The bacteriological examination of a purulent wound included the qualitative and quantitative study of the dynamics of wound microflora on days 3, 5, 7 and 14 after the wound had been formed. The biopsy material (0.2–0.4 g) was collected from the purulent wound surface. The material was suspended in an isotonic NaCl solution followed by dilution to 10^9 CFU/ml. A 100-µl sample for each dilution was seeded onto a solid nutrient medium Nutrigen Agar (Becton Dickinson, USA); *St.aureus* was isolated using the conventional methods. The strains were identified using a BBL Auto Reader BD microbiological analyzer (Becton Dickinson, USA). The results of screening using MeReSa Agar Base (MRSA Alert Kit) (Becton Dickinson, USA) and agar base for identification of methicillin-resistant *St.aureus* (HiMediaLaboratories, India) were used to determine whether a strain was methicillin-resistant or not. Furthermore, the sensitivity of the selected strains to antibiotics was determined using the Sensi-disc systems (Becton Dickinson, USA).

The number of microorganisms per 1 g of biomaterial was calculated using the formula:

$$N = n•P•K•10,$$

where N is the number of colonies per 1 g of biomaterial;
n is the number of colonies grown in a Petri dish;
p is factor for recalculating to 1 g of biomaterial;
K is the degree of dilution of the material;
10 is the factor for recalculating the inoculation dose.

The results of the study were statistically processed to calculate the mean values, mean errors and the correlation coefficient. The significance of differences between the mean values was assessed using the Student's t-test.

RESULTS AND DISCUSSION

During the bacteriological study of the wound discharge, only the *St. aureus* monoculture was obtained, while no other saprophytic flora disseminated. When zinc and copper nanoparticles were used, the *St. aureus* culture disseminated until day 12 and day 5, respectively. The *St. aureus* culture was observed in the control group until day 20.

The quantitative determination of the content of microbial bodies in the wound is important for diagnosing and predicting the course of the wound process. Exceeding of the critical level of bacterial contamination demonstrates that the course of the wound process is unfavorable and is a diagnostic indicator of the transition of the local purulent process to the generalized form.

Table 1 lists the results of determining the quantitative composition of the wound microflora per 1 g of biopsy material.

The microbial contamination of 1 g of the tissue of animals in the experimental groups in the case when copper nanoparticle suspension was used decreased to 10^2 CFU/g on day 5; this figure was reliably lower than that in animals in the control group (Table 1).

Table 1: Quantitative determination of microorganisms per 1 g of tissue in different groups of experimental animals (CFU/g, *M±m*)

Experimental groups	Day 3	Day 5	Day 7	Day 14
Control group *n=10*	$(6.3\pm1.8)\cdot10^6$	$(8.3\pm2.1)\cdot10^9$	$(7.9\pm2.3)\cdot10^8$	$(5.3\pm1.4)\cdot10^5$
Zinc nanoparticle suspension *n=10*	$(3.2\pm2.5)\cdot10^6$ P(1-2)<0.05	$(5.1\pm2.3)\cdot10^5$ P(1-2)<0.05	$(8.6\pm1.8)\cdot10^3$ P(1-2)<0.05	$(3.1\pm2.3)\cdot10^2$ P(1-2)<0.05
Copper nanoparticle suspension *n=10*	$(6.2\pm3.1)\cdot10^4$ P(1-3)<0.05	$(4.5\pm1.8)\cdot10^2$ P(1-3)<0.05	No growth	No growth

Note: p – confidence level of differences in indicators as compared to the control group

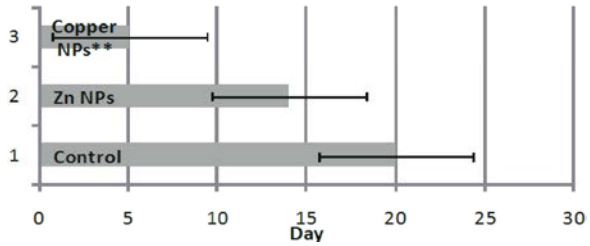

Fig. 1: Dynamics of contamination of the experimental wounds with *St.aureus* during therapy

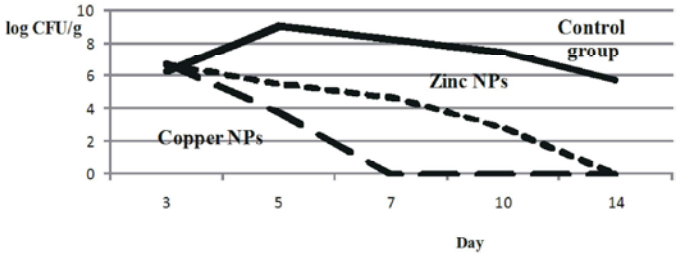

Diagram 1: Dynamics of contamination of the wound (log CFU/g) depending on the agent used: 1 – control group; 2 – zinc nanoparticle suspension; 3 – copper nanoparticle suspension

The use of zinc nanoparticles for wound management to a lesser extent reduced the number of microbial bodies (the number of CFU/g on day 5 decreased to 10^5 CFU/ml). In the control group of animals, bacterial contamination of the wound on day 5 was 10^9 CFU/g; i.e., it has significantly increased as compared to day 3. The changes were statistically reliable (P<0.05). The number of CFU/g in the control group was appreciably high during the entire observation period: 10^9–10^5. The dynamics of elimination of the agent contaminating the wound under the effect of metal nanoparticles and in the control group is shown in Diagram.

In summary, the use of copper nanoparticle suspension ensures rapid elimination of the infectious agent contaminating the wound as compared to the group of animals treated with zinc nanoparticles and to the control group. These data have been verified by the *in vitro* studies, where the pronounced antibacterial effect of copper and zinc nanoparticle suspensions at low concentrations (0.001–0.1 mg/ml) on clinical *St. aureus* strains has been demonstrated [14, 15]. Elimination of the bacterial causative agent for purulent process is important for improving the reparative regeneration and reducing the surface area of the experimental wound. The studies have demonstrated that copper nanoparticle suspension exhibits high antibacterial activity against polyantibiotic-resistant clinical *St. aureus* strains in the experimental purulent wound.

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