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Regeneration of Conventionally Aseptic Experimental Wounds under the Action of Copper Nanoparticles

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Abstract: Optimization of reparative regeneration of conventionally aseptic experimental full-thickness wounds in rats on exposure to a suspension of copper nanoparticles was studied. Healing of the experimental wound in test group animals was accompanied by fast decrease in the wound area according to the data of planimetric measurements (change in the wound area, wound healing rate, daily decrease in the wound area) and by normalization of haematological and biochemical parameters. Study of haematological parameters (total white blood cell count, differential white blood cell count) for different groups of animals demonstrated different dynamics and levels of general inflammatory parameters with respect to the normal state. The epidermal thickness in the wound area and the ratio of epidermal to dermal thickness were determined in the dynamics by morphometric investigation of the wound surface characteristics. Histological evaluation of the wound surface tissues was performed (structural components of the connective tissue, blood-vessel arrangement, scar tissue formation). A beneficial effect of copper nanoparticles on the metabolic processes in rats was demonstrated, in addition to the reduced time of the reparative regeneration of conventionally aseptic wounds, in particular, clear-cut preventive effect against secondary infection in the wound, which is confirmed by laboratory data. The suspension of copper nanoparticles can be recommended for therapy of epidermal tissue injuries.

Key words: Experimental full-thickness wound · Copper nanoparticles

INTRODUCTION

Optimization of reparative regeneration of soft tissue injuries caused by various mechanical, thermal and other factors (suppurative inflammations, dystrophic processes, etc.) remains a topical scientific and practical task [1]. Patients with suppurative inflammatory diseases account for about 40% of surgical patients. Post-surgical suppurative complications develop in 30% patients on average. These data indicate that the surgical infection problem is topical and yet unsolved and acquires increasing socioeconomic role [2,3].

Metal nanoparticles have a clear-cut antibacterial action [4,5]. The mechanisms by which metal nanostructures damage bacterial cells are not entirely clear. The following mechanism are accepted most generally: absorption of metal ions and subsequent suppression of ATP synthesis and violation of DNA replication; oxidative damage of cell structures and change in the bacterial membrane permeability [6-9].

Copper plays an important role in the vital activity of organisms; it catalyzes complete tissue regeneration and promotes healing of wounds [9,10,11]. Despite the obvious prospects for investigations in this area, only scattered data on the effect of copper nanoparticles on wound healing are available from the literature.

Goal of the work: evaluation of the effect of a suspension of copper nanoparticles on the reparative regeneration of a conventionally aseptic experimental wound.

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MATERIALS AND METHODS

Highly dispersed copper nanopowders synthesized on the plasmachemical unit of the branch of the RF Federal State Unitary Enterprise "State Research Institute for Chemistry and Technology of Organoelement Compounds" (Moscow) were used. The nanoparticles were obtained from copper of brand PMS1 as per GOST 4960–75 by the plasma process.

Sixty white male rats, weighing 130-140 g, were used as test subjects. A full-thickness skin wound measuring 400 mm^2 was formed in the interscapular region of each rat.

The rats were divided into 3 groups each comprising 20 rats:

- Group 1 (control)-intact animals;
- Group 2 (reference)-rats with a full-thickness skin wound, which healed naturally;
- Group 3 (test)-rats with a full-thickness skin wound that were treated with the tested agent.

The purulent wound model was obtained in the following way [12]: after skin pretreatment, a 2×2 cm (400 mm^2) square was excised in the skin and subcutaneous tissue along the contour drawn using a stencil in the shaved interscapular region of anaesthesized rats under aseptic conditions.

Sterile drapes wetted with 1.0 mL of a copper nanoparticle suspension in isotonic salt solution (0.1 mg/mL) were applied every day on the wound surface in conformity with subdivision of animals into groups.

The bacteriological examination of the purulent wound included quantitative monitoring of the wound microflora on the 1st, 3rd, 5th, 7th and 14th day of treatment.

All animals had wounds of the same shape, area and position, which was important for the subsequent comparison and analysis of the wound healing process.

The treatment efficiency was evaluated using experimental, planimetric, haemotological, histological, microbiological and statistical methods.

The L.N. Popova planimetric method was used, which is based on recording the rate of decrease in the wound surface area with time: a sterile polymer plate is applied on the wound and the wound contour is depicted on the plate. The wound contours are transferred to transparent films and scanned on a HP Scanjet 3970 scanner (China). The wound area was calculated using ImageJ software.

The reduction the wound during 24 h in percent was determined by formula (1):

$$\frac{(S - S_n).100}{S.t} \qquad (1)$$

where S is the wound area found in the preceding measurement; S_n is the wound area found in the current measurement; t is the number of days between the first and current measurements.

The rate of wound healing was determined by the M. G. Markaryan and G.Ts. Sarkisyan formula (2):

$$HR = \frac{H - H_1}{n} \qquad (2)$$

where HR is the healing rate of the wound area per day; H is the initial wound area; H_1 is the current wound area; n is the number of days.

The haemotological characteristics were measured on a Micros 60 ABX analyzer (France).

The histological evaluation of the wound surface tissues was performed by staining the tissue specimens with Alcian Blue in order to visualize acid glycosaminoglycans and by Mallory's trichrome to visualize collagen. The intensity of epithelialization was evaluated by the morphometric measurement of the epidermal thickness using an ocular micrometer.

The statistical treatment of the results was done using Statistica 6.0 software designed for evaluation of the results of medical and biological measurements and calculating the arithmetic mean (M), the root-mean-square deviation (σ), the mean error of the arithmetic mean (m), the validity coefficient (t) and the probability factor (p).

RESULTS

In all experimental series, the mean area of the wounds on the 1st day after modeling of the conventionally aseptic wounds, was 400 mm^2 according to the planimetric data. The change in the wound surface area in the test group and reference group with time is presented in Table 1.

The study demonstrated slow healing of the experimental wounds in reference-group animals; no complete healing was observed on the 14th day in any of the animals, the wound area decreasing by 52% during this period. In the test group animals, the wound area decreased by 86.4% by the 7th day and complete healing of the wounds occurred by the 14th day in all animals of

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

Day	Experimental wound area, M, mm^2	
	Reference group, n=20	Application of the suspension of copper nanoparticles n =20
1st	392.9±3.1	412.8±8.1
3rd	412.3±5.2	314.7±10.2* p<0.001
5th	384.1±11.5	107.5±14.2*** p<0.001
7th	291.4±14.3	47.3 ±7.3*** p<0.001
10th	223.7±9.5	8.4±1.7*** p<0.001
14th	159.9±18.2	0.0

Note: p is the confidence level for the differences between the test and reference groups.

Table 2: Changes in the differential white blood cell count in rats of different groups

Day	Series	Total WBC count	Band neutrophils	Segmented neutrophils	Lymphocytes
14th	Control group	$11.9±5.7•10^9$/L	4.1±1.5	23.4±5.1	64.7±5.5
	Reference group	$25.3±4.1•10^9$/L p_{1-2}□0.05	18.5±3.4p_{1-2}□0.05	39.3±4.2 p_{1-2}□0.05	37.5±3.8p_{1-2}□0.05
	Test group	$17.5±4.6•10^9$/L p_{2-3}□0.05	9.2±3.8 p_{2-3}□0.001	26.5±3.9 p_{2-3}□0.05	45.3±4.4 p_{2-3}□0.05

p_{1-2} is the confidence level of the differences between the reference group and control group characteristics;
p_{2-3} is the confidence level of the differences between the test group and reference group characteristics.

Table 3: Skin layer thickness of experimental animals in the wound region (μm) on the 21st day

Characteristics	Groups of animals		
	Norm n=20	Reference group n=20	Test group n=20
Epidermal thickness	27.4±3.2	48.1±5.4	25.8±4.5
Dermal thickness	97.4±8.6	137.8±12.5	105.3±11.0
Epidermis/derma	0.3	0.35	0.2

the group. Thus, application of a suspension of copper nanoparticles had a pronounced stimulating action on the planimetric characteristics in test group animals.

For three animals of the reference group, the wound area was found to increase by 12-24% on the 5h day of the test with respect to the initial value. A bacteriological assay showed the presence of *E. coli* and *St. aureus* in amounts of $3•10^3$ CFU/mL in these experimental animals. The wound edges and bottoms were hyperemic and edematous. Thus, secondary infection of the wound took place, resulting in an increase of the wound surface area. In all animals of the group treated by copper nanoparticles, fast healing of the experimental wound and no secondary infection were observed.

One of planimetric characteristics of the reparative regeneration of experimental wounds is the wound healing rate (in mm per day); the results indicate that the maximum wounds healing rate on the 5th day (103.7 mm per day) is substantially higher in the test group than in the reference group (32.4 mm per day) ($p<0.001$).

Analysis of the daily reduction of the wound area in percent demonstrated that the application of copper nanoparticles induced the most pronounced reduction of the experimental wound area, by 24%, on the 7 to 10th day after wound modeling and after that the average daily reduction of the wound area somewhat decreased to 15%, due to the fact that the wounds in some of the animals have been completely healed. The difference between the two groups as regards this characteristic was statistically significant ($p<0.001$) for any observation time, which proves the high efficiency of copper nanoparticles as a suspension for the reparative regeneration.

Studies of the haemotological parameters in the test group and reference group of rats demonstrated different dynamics and different levels of general inflammatory parameters with respect to the normal state (intact animals) (Table 2).

Reference-group animals developed a clear-cut inflammatory response, manifested as a considerable increase in the content of segmented and band white blood cells and also the total white blood cell count as compared with test-group animals, which were treated with a suspension of copper nanoparticles and in which these values were close to the normal level by the 14th day.

Morphometric measurements of the wound surface were made to determine the variation of the epidermal thickness in the experimental wound and the epidermal to dermal thickness ratio in reference group animals and test group animals treated with the suspension of copper nanoparticles (Table 3). The pattern of arrangement of blood vessels and the rate of hair coat restoration were measured, which are important characteristics of the efficiency of copper nanoparticles and reparative regeneration of the experimental wound. In the epidermis of test group rats, the blood vessel were found to be ordered unlike the reference group rats. In addition, pronounced fibroblast proliferation was noted in the areas of active blood circulation of test animals.

On the 21st day of observation, complete skin structure with formed oil glands was noted in test animals, whereas in the reference group, scar tissue formed characterized by accumulation of sulfated glycosaminoglycans. The structural components of the connective tissue in the wound area of test rats were formed by horizontally arranged mature collagen. In the rats of reference group, metachromasy sites and chaotic arrangement of collagen fibers or more rarely, vertical arrangement along the vessels, were noted in the same time instants.

CONCLUSIONS

The beneficial influence of a suspension of copper nanoparticles on the reparative regeneration of conventionally aseptic experimental wounds in rats was demonstrated, in particular, copper nanoparticles have a clear-cut preventive effect against secondary infection of the wound, which is confirmed by laboratory data. A suspension of copper nanoparticles can be recommended for treating epidermal tissue injuries.

REFERENCES

1. Gostishschev, V., 2007. Infections in surgery. GEOTAR-Media, pp: 768.
2. Huang, S.S. and R. Platt, 2003. Risk of Methicillin-Resistant Staphylococus Aureus Infection After Previous Infection or Colonization. Clin. Infect. Dis., 36: 281-285.
3. Copsgrove, S.E., G. Sakoulas, E.N. Perecevich, *et al.,* 2003. Comparison of Mortality Associated with Methicillin-Resistant and Methicillin Susceptible Staphylococcus aureus Bacteremia; a Meta-Analysis. Clin. Infect. Dis., 36: 53-59.
4. Glushchenko, N.N., O.A. Bogoslovskaya and I.P. Ol'khovskaya, 2002. Physico-Chemical Regularities of the Biological Action of Highly Dispersed Metal Powders. Chem. Phys., 21(4): 79-85.
5. Arsent'eva, I.P., E.S. Zotova, G.E. Folmanis, *et al.,* 2007. Certification and Application of Metal Nanoparticles as Biologically Active Agents, Nanotechnics. Special issue: "Nanotechnology for Medicine", 2(10): 72-77.
6. Weir, E., A. Lawlor, A. Whelan and F. Regan, 2008. The use of nanoparticles in anti-microbial materials and their characterization. Analyst, 133(7): 835-845.
7. Singh, M., S. Singh, S. Prasad and I.S. Gambhir, 2008. Nanotechnology in Medicine and Antibacterial Effect of Silver Nanoparticles. Digest Journal of Nanomaterials and Biostructures, 3(3): 115-122.
8. Chen, Z., H. Meng, G. Xing, *et al*., 2006. Acute toxicological effects of copper nanoparticles *in vivo*. Toxicology Letters, 163: 109-120.
9. Diaz-Visurraga, J., A. Garcia and G. Cardenas, 2010. Morphological changes induced in bacteria as evaluated by electron microscopy. In: Microscopy: Science, Technology, Applications and Education, Eds., A. Méndez-Vilas and J. Díaz. Badajoz, Spain: Formatex, pp: 307-315.
10. Ruparelia, J.P., A.K. Chatterjee, S.P. Duttagupta and S. Mukherji, 2008. Strain specificity in antimicrobial activity of silver and copper nanoparticles. Acta Biomaterialia, 4: 707-716.
11. Nel, A.E., L. Mädler, D. Velegol, T. Xia, E.M.V. Hoek, S. Somasundaran, F. Klaessig, V. Castranova and M. Thompson, Understanding biophysicochemical interactions at the nano-bio interface. Nature Materials, 8: 543-557.
12. Datsenko, B.M., 1995. Theory and Practice of Topical Treatment of Purilent Wounds (Drug Therapy Problems). Kyiv: Zdirovia.