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Effect of a Complex Preparation on Regeneration of the Experimental Soft Tissue Wound

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Abstract: The effect of a complex preparation based on low molecular weight chitosan biopolymer and containing plasmochemically synthesized copper and zinc nanoparticles on healing of the experimental full-thickness conditionally aseptic and infected wounds was studied in 90 male white rats. The planimetric and morpho-histochemical studies characterizing the regenerative processes were carried out. The complex preparation exhibited a pronounced effect on the dynamics of planimetric indices in animals with both noninfected and purulent wounds. The effect of the complex preparation on connective tissue regeneration was revealed and found to be associated with affecting both the fibrillar structures and glycosaminoglycans, which optimize cell proliferation as well as formation of fiber structures and granulation tissue. The preparation was proved to have a stimulating effect on reparative regeneration processes characterized by changes in structure and metabolism of the connective tissue and by the recovery of its morphofunctional features.

Key words: Wounds In Experimental Animals · Ultra-Dispersed Metal Powders · Chitosan · Regeneration

INTRODUCTION

The healing of lesions caused by various mechanical, thermal and other factors (pyoinflammation, dystrophic processes, etc.) remains a topical problem of both fundamental and practical medicine [1].

The introduction of high doses of antibiotic agents disturbs the immune function, causes dysbiosis and mycotic lesions. The use of modern wound coatings to treat pyoinflammatory diseases of soft tissues allows one to correct metabolic dysbalance, to remove low molecular weight and particularly toxic compounds, while retaining the regeneration factors and protecting the organism and the wound surface against infection.

There is a need for designing new wound coatings that would possess a combination of properties, such as absorption activity, protection against microorganism penetration with simultaneous gas permeability, absence of toxicity and irritating properties, ability to release antimicrobial agents and compounds that facilitate regeneration [2].

A number of studies have been devoted to the investigation of reparative regeneration of soft tissues under the effect of locally applied wound healing composites based on natural compounds (in particular, chitosan) [3-8]. Within the context of the reparative process, antibacterial, anti-inflammatory and anti-oxidant effects are the most significant properties of this natural biopolymer, which have been confirmed both experimentally and clinically.

Compositions based on biopolymers and metal nanoparticles have become widely recognized [9]. It is a known fact that the joint use of metal nano-powders and chitosan mutually enhances the biological effects for the wound surface requiring healing [10].

Despite the undoubtedly high potential of the studies devoted to reparative activity of metal nanoparticles, the data regarding the effect of copper and zinc nanoparticles on wound healing in literature are scarce [11-13].

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A natural polysaccharide chitosan possesses polyfunctional properties and is widely applied in medicine. Moreover, chitosan and its derivatives are environmentally friendly components of pharmaceutical systems [14].

It is a topical task to study the effect of complexes based on copper and zinc nanoparticles and natural biopolymers on reparative regeneration of a full-thickness skin wound in experimental animals.

This work was aimed at studying the effect of the complex preparation based on chitosan and containing copper and zinc nanoparticles on reparative regeneration of full-thickness skin wounds, including purulent ones.

MATERIALS AND METHODS

Low molecular weight chitosan, copper and zinc nanoparticles (dispersion of 30-40 and 30-70 nm, respectively) obtained using plasma technology were used for the experiments.

The planimetric method proposed by L.N. Popova was used to obtain the objective indices of wound healing. This method is based on recording the rate of reduction of the wound surface area with time: a sterile plate made of transparent polymer is positioned over the wound; the wound contour is drawn on the plate. The image is processed by a computer to calculate the wound surface area. The experiments were carried out using 90 white male rats (180±30 g).

The distribution of the animals over groups is shown in Table 1.

The full-thickness wound model was obtained as follows. After preliminary treatment, skin and subcutaneous fat in the shaved area in the interscapular region of anaesthetized rats was excised in the form of a 2×2 cm (400 mm^2) square under aseptic conditions.

The wound surface in the experimental group of animals was subjected to a single treatment with 1 g of the complex preparation consisting of chitosan and copper and zinc nanoparticles. As it interacts with the wound exudate, the preparation forms a 2 mm thick hydrogel layer that is required to completely cover a wound of this area and to form an elastic 1 mm thick film that would tightly adjoin the wound surface.

Control studies were carried out during the entire surveillance period. The following parameters of the course of wound process were taken into account: the presence and character of an inflammatory reaction, conditions of wound edges and bottom, time until the wound was clear of necrotic tissues and granulation tissue appeared, time of the onset of epithelization for the wound. The changes in these parameters were detected and expressed numerically; with allowance for the treatment duration, either planimetric or bacteriological methods of wound examination were used on days 3, 5, 7, 10 and 14.

The morpho-histochemical studies were carried out as follows: the tissues were fixed in neutral formalin, transferred to alcohols with a density gradient and embedded in paraffin wax. The resulting 5 µm thick slices were stained with hematoxylin and eosin to differentiate the tissue structure. Histochemical staining allowed one to detect glycosaminoglycans using Alcian Blue at pH 1.0 (highly sulfated glycosaminoglycans) and at pH 2.5 (the sum of sulfated glycosaminoglycans); collagen fibers using Van Gieson's trichrome and Masson's trichrome with Aniline Blue. Collagen and elastic fibers were also identified using the Weigert Van Gieson and Mallory's trichrome staining.

RESULTS AND DISCUSSION

The planimetric study has shown that the use of the complex preparation had a more pronounced effect on regeneration of the noninfected wound as compared to the comparison group at any points of control measurements. Complete healing of the wound was observed in all the animals of the experimental group by day 10 of surveillance, while in the comparison group the wound surface area was reduced only by 54%.

Table 1: Distribution of animals over groups in the experiment

Group	Treatment method	Number of animals
1	Control group (intact animals)	10
2	Comparison group (animals with a noninfected wound)	20
3	Experimental group (animals with a noninfected wound) treated with the complex preparation	20
4	Comparison group (animals with a purulent wound)	20
5	Experimental group (animals with a purulent wound) treated with the complex preparation	20
	TOTAL:	90

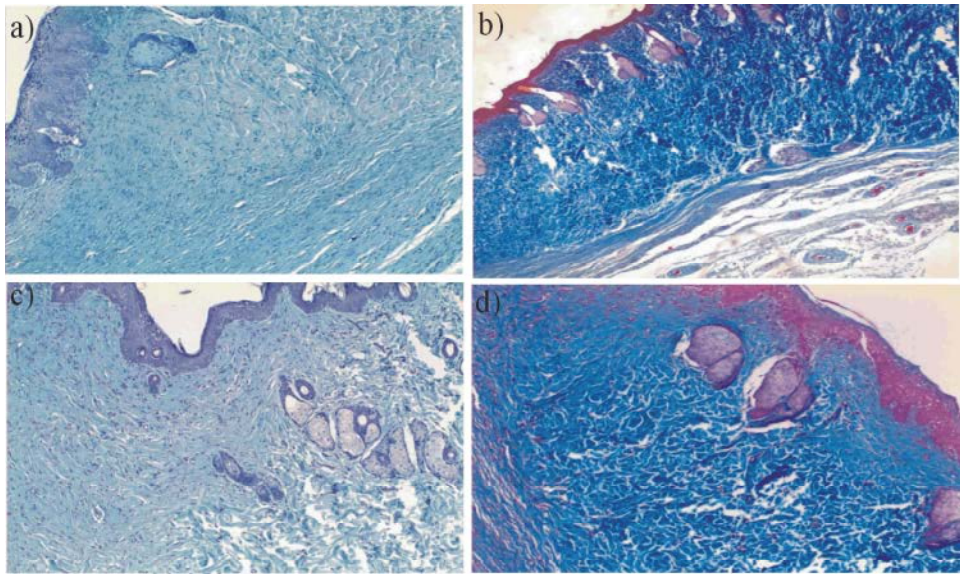

Fig. 1: Histo-morphological features of the wound area in the comparison groups: (a) comparison group with a conditionally aseptic wound, Alcian Blue staining, pH 2.5. 10x magnification; (b) comparison group with a conditionally aseptic wound, Masson trichrome staining, 10x magnification; (c) comparison group with a purulent wound, pH 2.5, 10x magnification, Alcian Blue staining; (d) comparison group with a purulent wound, Mallory staining, 10x magnification.

Animals with a purulent wound in the comparison group demonstrated slow healing. The purulent detached body part was observed in animals of this group on day 7; complete healing has been observed in none of the animals of this group. The wound surface area decreased by 32% by day 14. A reduction of the wound surface area by 86.4% was observed in animals with a purulent wound in the experimental group (the animals that received the complex preparation) was observed on day 7; the wound had healed completely by day 14.

Thus, the application of the complex preparation had a pronounced stimulating effect on the dynamics of planimetric indices in animals with both noninfected and purulent wounds.

The morpho-histochemical study has demonstrated that there was a tight commissure between skin and the underlying tissue and the wound crust was retained in rats in both comparison groups. The number of hair bulbs and oil glands per unit area of skin slice around the wound crust was considerably lower in these groups as compared to that in the corresponding experimental groups. Immature granulation tissue near the wound was rich in fibroblasts and was permeated to a significant extent by vertically oriented blood vessels. The collagen fibers were mostly irregularly disordered or (less frequently) oriented vertically (parallel to blood vessels). Sulfated (acidic) glycosaminoglycans were found in the dermis; areas of metachromasia were present (Fig. 1).

In animals of both experimental groups, skin was mobile; no commissures between skin and the underlying tissue were observed. The granulation tissue contained a considerably smaller amount of fibroblasts and blood vessels; the positive reaction to acidic glycosaminoglycans was expressed much less as compared to that in rats in the corresponding comparison groups; horizontal orientation of collagen fibers was detected. In general, all these facts indicated that the granulation tissue matured faster in rats of both experimental groups (Fig. 2).

The optimization of the reparative regeneration processes in the experimental wound under the effect of the complex preparation is characterized by changes in structure and metabolism of the connective tissue, which are directed at the recovery of its morpho-functional features. The complex preparation was proved to affect the functions of the connective tissue, both fibrillar structures and glycosaminoglycans that optimize the cell proliferation processes, formation of fibrillar structures and granulation tissue.

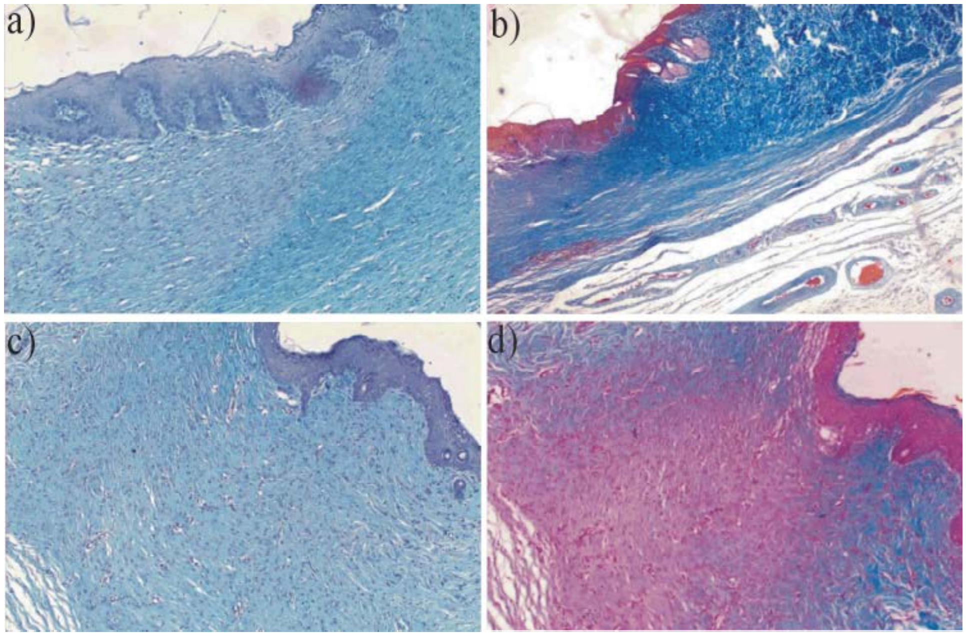

Fig. 2: Histo-morphological features of the wound area in the experimental groups:(à) experimental group with a conditionally aseptic wound; Alcian Blue staining, pH 2.5. 10x magnification; (b) experimental group with a conditionally aseptic wound, Masson trichrome staining, 10x magnification; (c) experimental group with a purulent wound, pH 1.0, 10x magnification, Alcian Blue staining; (d) experimental group with a purulent wound, Mallory staining, 10x magnification.

Thus, stimulation of the reparative regeneration processes in the experimental soft tissue full-thickness wounds (both conditionally aseptic and infected ones) in rats under the action of the original wound-healing composite comprising chitosan and copper and zinc nanoparticles and exhibiting antibacterial and anti-inflammatory effects is characterized by a pronounced positive effect on the condition of structural biopolymers in the matrix of the connective tissue.

CONCLUSIONS

The preparation containing copper and zinc nanoparticles and natural biopolymer chitosan has demonstrated a pronounced regenerative effect when applied locally in animals with conditionally aseptic and infected purulent wounds.

- A preparation possessing a regenerative effect for both conditionally aseptic and purulent wounds has been designed.
- The proposed combination of the components mutually enhances the effects, which has been proved by the experimental studies in animals with purulent wounds.

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