Effect of a Composition Containing Chitosan Gel and a Bioregulator from Blood Serum on Healing of Purulent Wounds in Mice

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We studied the effect of combined preparation on the basis of chitosan containing a bioregulator isolated from cattle serum in a concentration of 10^{-10} mg/ml on healing of purulent wounds in mammals *in vivo*.

Key Words: *healing of purulent wounds of the skin; bioregulators; blood serum; chitosan*

We studied the effects of a bioregulator from blood serum belonging to the group of membranotropic homeostatic tissue-specific bioregulators [2,3,5,7,8]. It was previously demonstrated that blood serum bioregulator in the form of aqueous solution produced a wound-healing effects on rabbit cornea and mouse skin *in vivo* [1,7].

Here we studied the effect of this bioregulator on healing of infected wounds.

MATERIALS AND METHODS

For preparing the test composition, 10 g chitosan was dissolved in 1 liter water in the presence of 3 g salicylic acid at 50°C. The solution was sterilized at 120°C for 5 min, cooled, and 10 ml aqueous solution of the bioregulator from cattle blood serum was added in a concentration of 10^{-9} mg/ml. The bioregulator was isolated as described elsewhere [5].

The experiment was carried out on male F1 mice weighing 20-22 g (n=36). The mice were narcotized with ether and a skin fragment with a diameter of about 1 cm was cut from the back of each mouse. The animals were divided into 4 groups (9 mice per group). Group 1 mice received no treatment (intact control). In group 2 mice (control), the wound immediately after surgery was contaminated with pus containing the following bacteria: *Staphylococcus aureus, Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans*. In group 3 mice, levomecol ointment (Nizhfarm, reference preparation) containing antibiotics levomycetin routinely used for the treatment of infected wounds was applied after infection. In group 4 mice, the test preparation was applied to the wound after infection. To arrest spreading of the inflammatory process, the serum bioregulator was included in chitosan-based gel.

Histological sections (7 μ) of the skin wounds were examined on days 3, 8, and 13 after trauma. The sections were stained with hematoxylin and eosin.

RESULTS

On day 3 after wound infliction, a non-healing wound without epithelium in the central zone was observed in all cases. Pronounced inflammation with neutrophilic infiltration was seen in the derma. The effects of the

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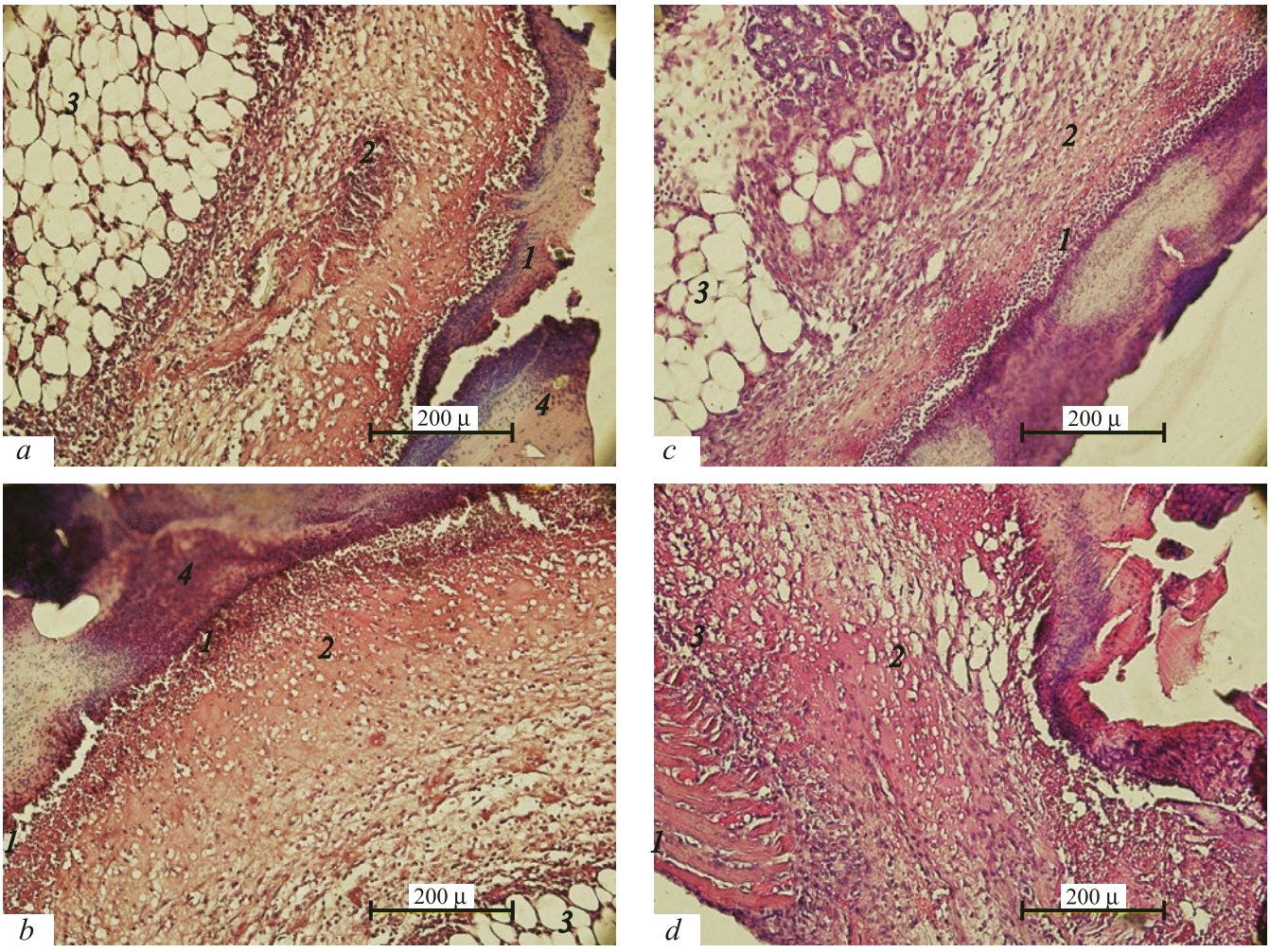

Fig. 1. Healing of skin wound in mice on day 3. Hematoxylin and eosin staining. *1*) inflammatory focus, *2*) derma, *3*) subcutaneous adipose tissue, *4*) crust. Here and in Figs. 2 and 3: *a*) wound infected with pus; *b*) wound without pus contamination; *c*) application of levomecol; *d*) application of chitosan gel containing serum bioregulator.

test preparations cannot be adequately evaluated at this stage (Fig. 1).

On day 8 after wound infliction, we observed incomplete epithelization of infected wounds, the crust adherent to the wound surface, inflammatory focus and numerous blood vessels in the derma, formation of fibrous scar, and poorly developed adipose tissue (Fig. 2, *a*).

In the wound not contaminated with pus, epithelization and crust separation from the epithelium were observed. In the derma, the formation of fibrous scar, minor inflammatory foci, and well-developed subcutaneous adipose tissue with gland ducts were seen (Fig. 2, *b*).

In purulent wound treated with levomecol, pronounced inflammatory reaction was observed in the derma. Epithelization was not completed and the crust tightly adhered to the wound; poorly developed adipose tissue and the formation of fibrous scar were observed (Fig. 2, *c*).

Application of the chitosan-based gel containing serum bioregulator on the wound led to its intensive epithelization and complete crust detachment. No inflammatory foci were seen in the derma, the formation of gland ducts and muscle elements was seen in the subcutaneous adipose tissue. (Fig. 2, *d*).

On day 13 after wound infliction and infection with pus, the appearance a focus of chronic inflammation in its central zone and the formation of the connective tissue scar in the derma were observed; no complete reepithelization was seen (Fig. 3, *a*).

In the control group (wounds without pus contamination), practically complete re-epithelization and only minor inflammation were noted. The derma was enriched with small capillaries, collagen fibers were arranged in dense cords parallel to the epidermis, *i.e.* the formation of fibrous scar was observed. Scar formation was also noted in the subcutaneous tissue, collagen fibers were somewhat looser than in the derma, but adipose cells were almost absent (Fig. 3, *b*).

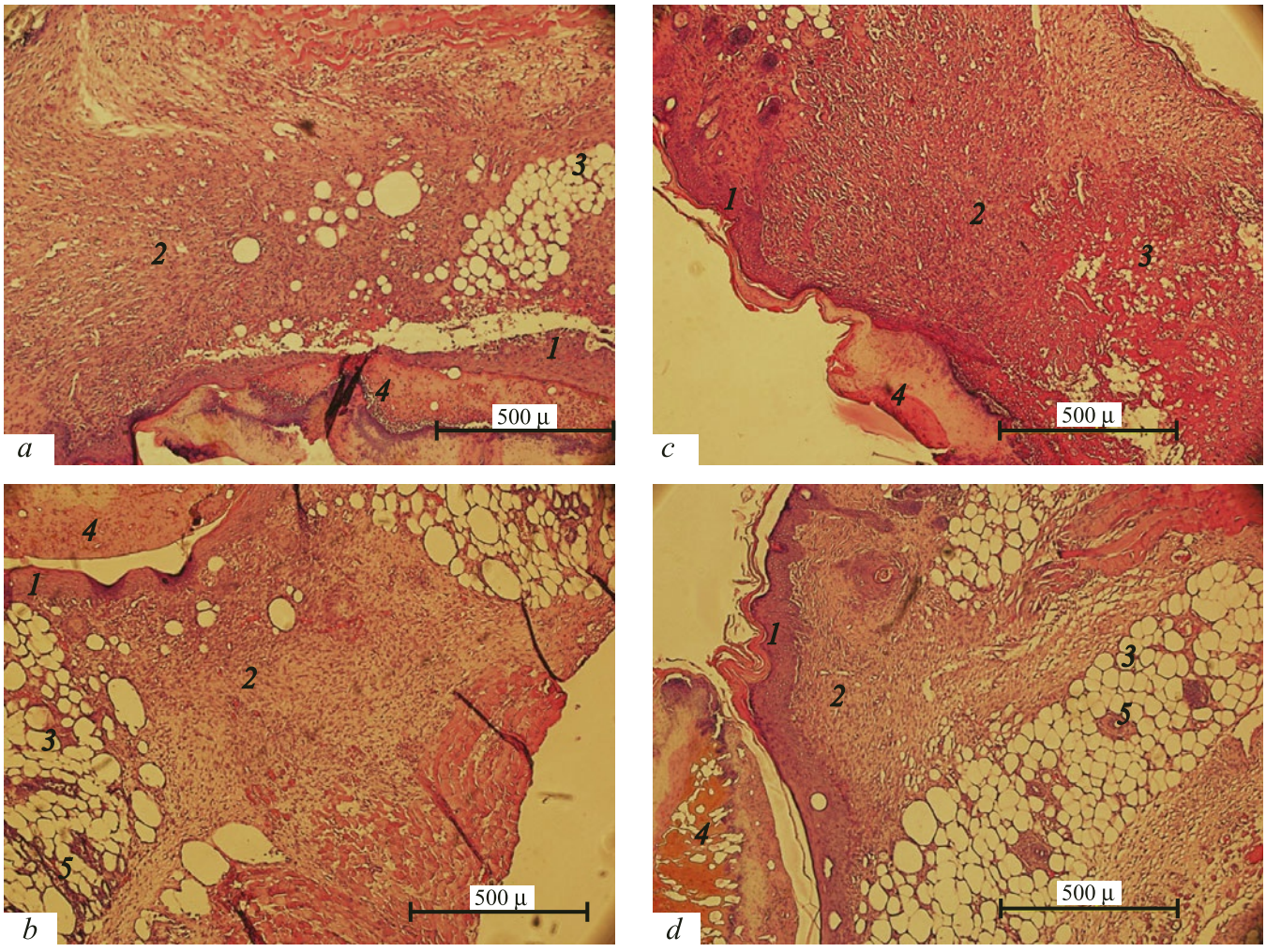

Fig. 2. Healing of skin wound in mice on day 8. Hematoxylin and eosin staining. *1*) epithelium, *2*) derma, *3*) subcutaneous adipose tissue, *4*) crust, *5*) gland ducts.

The wounds treated with levomecol were almost completely reepithelized, but the epithelium detached from the derma. Inflammatory foci were seen in the derma. Subcutaneous adipose tissue was poorly developed, adipose cells were absent. Fibrous scar formed (Fig. 3, *c*).

Application of chitosan gel containing serum bioregulator, pronounced retraction of the wound edges, and its complete reepitelization were noted (more pronounced that in groups 1 and 2); no epithelium detachment was observed. The structure of the derma was almost restored, no inflammatory foci were seen. Restoration of numerous gland ducts in the subcutaneous tissue and hair follicles in the derma was observed. The adipose tissue was well-developed. Partial restoration of muscle elements was noted somewhere under the wound. These findings attest to extremely high efficiency of the test composition in skin wound healing in mammals *in vivo*. The preparation not only stimulated regeneration in the skin wound, but also promoted the formation of morphologically restored tissue in the injured area (Fig. 3, *d*).

Thus the test chitosan-based gel containing serum bioregulator not only promotes tissue recovery in the injured area, but also arrests inflammation and limits the development of the infection process caused by pathogenic microflora of the pus.

It was found that chitosan-based composition containing serum bioregulator actively participates in the regeneration of skin wounds in mammals *in vivo*. It ensures practically complete recovery of the morphological structure of injured skin. This composition can be used for prevention of scar formation and healing of purulent skin wounds [4].

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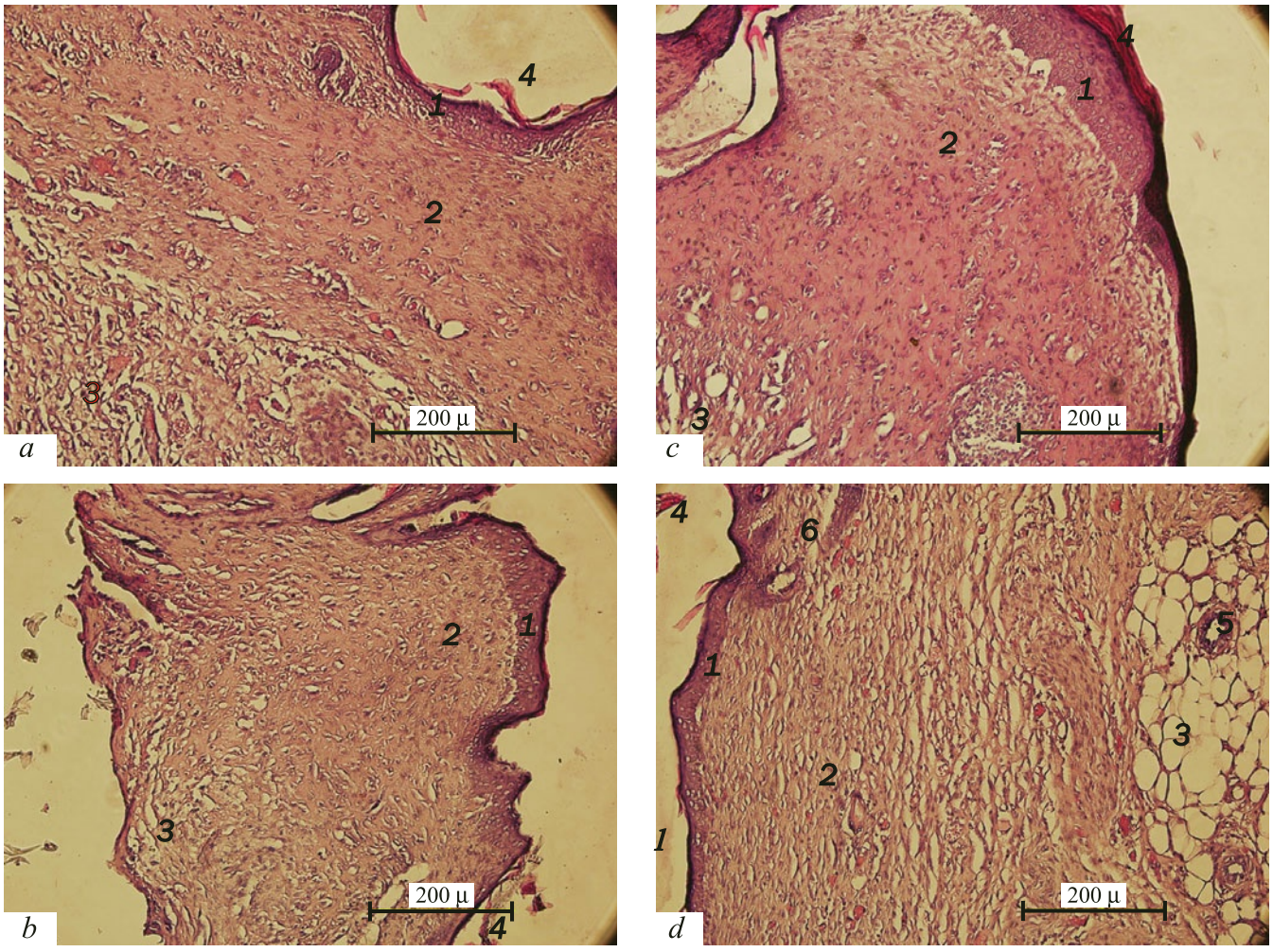

Fig. 3. Healing of skin wound in mice on day 13. Hematoxylin and eosin staining. *1*) epithelium, *2*) derma, *3*) subcutaneous adipose tissue, *4*) crust, *5*) gland ducts; *6*) hair follicles.

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