Burn-Healing Effects of a Composition Containing Chitosan Gel and a Blood Serum Bioregulator

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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 II-IIIa degree skin burns in mammals *in vivo*.

Key Words: *burns; bioregulators; blood serum; chitosan*

The incidence of thermal skin injuries now tends to increase. According to WHO data, burns rank 3rd among injuries. About 60 thousand people daily die from burns over the world. The problem of thermal injuries is extremely important for both civil and field military medicine. In the armory of burn-healing drugs, a special place is occupied by preparations based on inorganic and organometallic compounds, *e.g.* methyluracil, a representative of pyrimidine class [1]. Burn-healing compounds in the form of emulsions and gels are most effective, because they are easily applied and absorbed. Despite the wide spectrum of burn-healing pharmacological agents compounds includes, there are no preparations improving restoration of the damaged skin. These preparations should prevent wound infection, arrest the inflammatory process, and promote replacement of the lesion with the fibrous tissue. The search for new burn-healing agents is an urgent problem of biotechnology and medicine. Among bioactive substances stimulating regeneration and reparation in the skin, a new bioregulator isolated from blood serum should be mentioned. Previous studies showed that this bioregulator in low doses (10^{-10}-10^{-14} mg protein/ml) stimulated healing of skin wounds in mammals *in vivo* and produced a protective effect on skin cells from vertebrates *in vitro* [7].

Here we studied the burn-healing effects of a composition containing chitosan and serum bioregulator. Chitosan gel was chosen due to its bacteriostatic effect [6].

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, filtered, and 10 ml aqueous solution of the bioregulator from cattle blood serum in a concentration of 10^{-9} mg/ml was added. The bioregulator was isolated as described elsewhere [4].

Experiments were carried out on male Wistar rats weighing 200-280 g. Small-area skin burn was modeled by applying a tube (2-cm diameter) with boiling water onto a shaved skin area on the back for 30 sec. II-IIIA degree thermal injuries with an area about 4 cm^2 were thus modeled. The animals were divided into 4 groups (7 rats per group). The damaged skin surface was daily treated with the test composition on the basis of chitosan with bioregulator (group 1), chitosan gel without bioregulator (group 2), reference preparation solcoseryl eye gel (Solcoseryl, Solco Basel AG; group 3), or was left untreated (group 4). The animals were sacrificed on days 14 and 25 (ether narcosis), skin fragments from the damaged area were isolated

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and fixed in Bouin fixative. Histological 11-μ sections were routinely stained with hematoxylin and eosin.

RESULTS

Visual examination on day 1 after burn infliction showed bad general state of all animals (no differences between the groups were noted): burned surface was sharply edematous, waxy, hot, and hyperemied; exudation was observed. In the control group, edema aggravation and appearance of ulcerations filled with necrotic mass was noted on day 2. In all cases, signs of inflammation were seen in the burn wound: dilatation of dermal vessels under the wound surface and cell infiltration. The degree of these changes varied in different groups. By the end of the first week, the wounds in all animals were covered by a primary crust. In control rats, a wet slowly healing wound was seen on day 14 of the experiment. The dynamics of wound healing in group 2 little differed from that in controls, but was

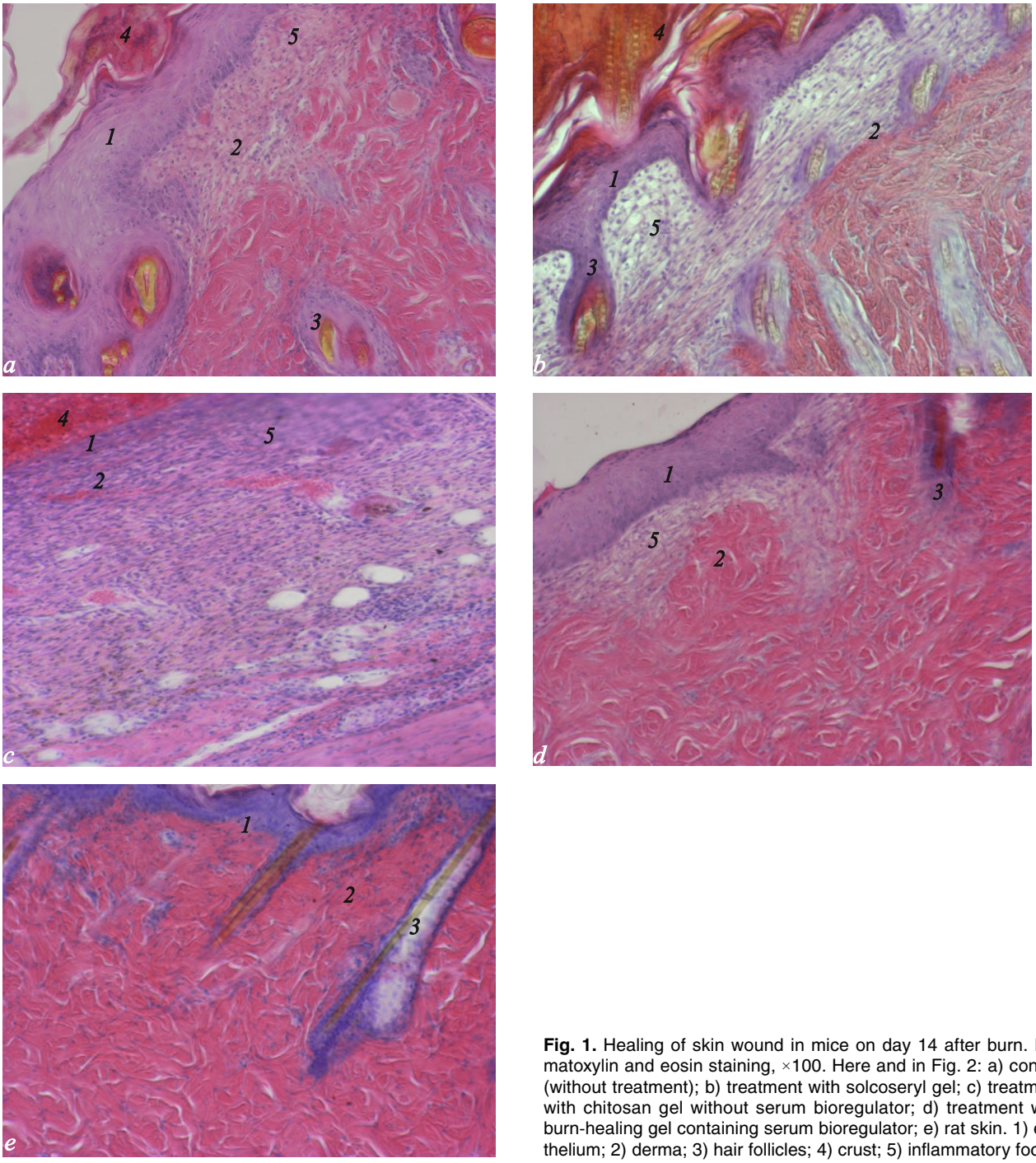

Fig. 1. Healing of skin wound in mice on day 14 after burn. Hematoxylin and eosin staining, ×100. Here and in Fig. 2: a) control (without treatment); b) treatment with solcoseryl gel; c) treatment with chitosan gel without serum bioregulator; d) treatment with burn-healing gel containing serum bioregulator; e) rat skin. 1) epithelium; 2) derma; 3) hair follicles; 4) crust; 5) inflammatory focus.

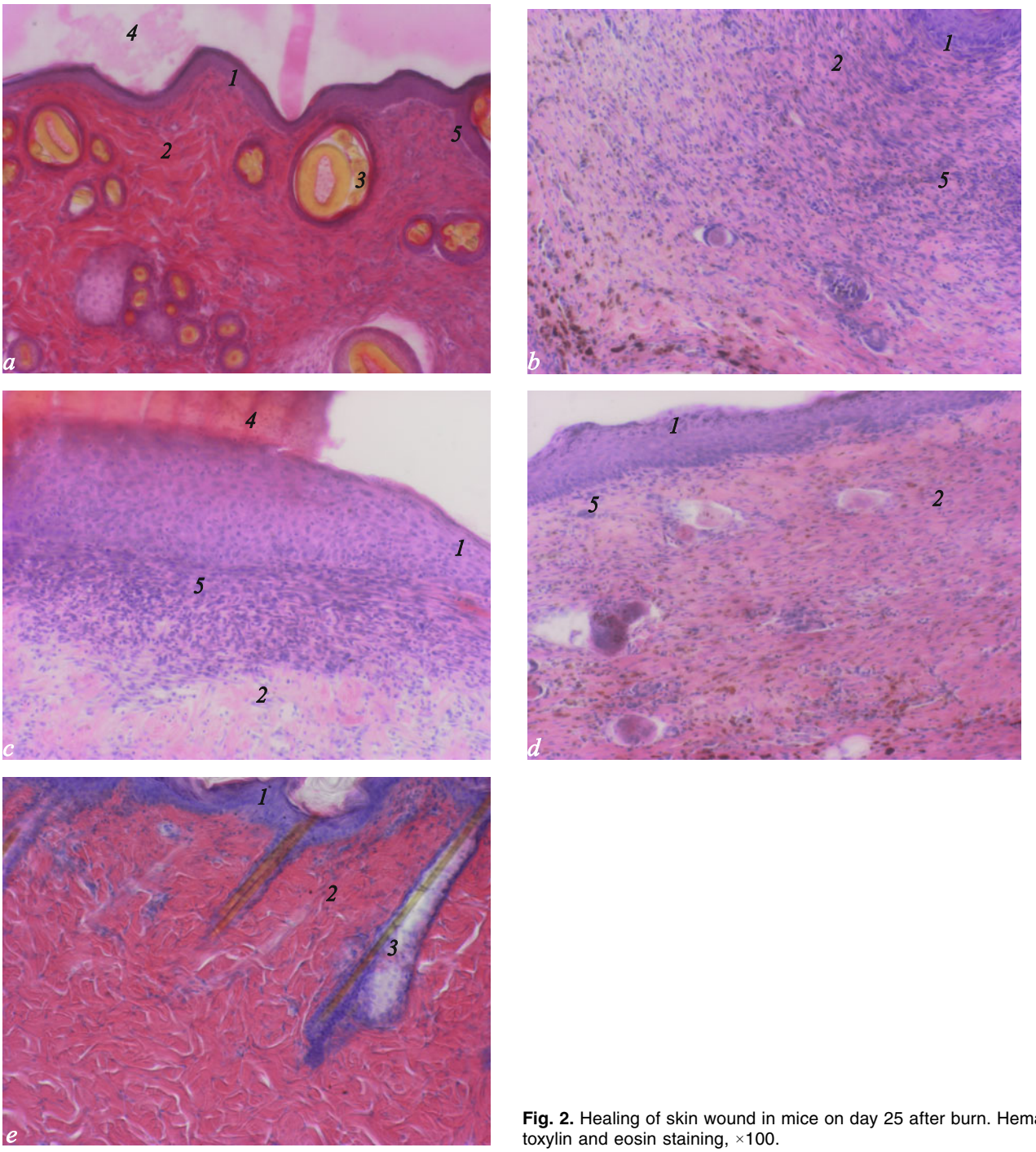

Fig. 2. Healing of skin wound in mice on day 25 after burn. Hematoxylin and eosin staining, ×100.

more effective in group 3 rats. The most complete reparation of the burn wound was observed on day 14 in group 1 animals treated with chitosan gel containing serum bioregulator. By day 25, the burned surface completely healed in rats of all groups.

On histological sections, the following picture was observed after wound healing.

In controls on day 14 after burn infliction, practically complete re-epithelialization was achieved; the crust loosely adhered to the skin. In the derma and muscular layer, minor inflammation was observed. Necrosis of hair follicles was seen; in the muscular layer, empty spaces between the muscle fibers appeared due to inflammation. Under the epithelium, accumulation of neutrophils and fibrocellular granulation tissue were observed (Fig. 1, *a*).

By day 25, a connective tissue cicatrix containing few neutrophils was formed. Necrosis of hair follicles and practically complete crust separation were found (Fig. 2, *a*).

In group 3 (treatment with solcoseryl gel), necrosis of hair follicles and practically complete re-epithelialization were observed on day 14, the crust loosely adhered to the epithelium. Pronounced inflammation with neutrophilic infiltration developed in the skin directly under the epithelium; in the muscular layer, inflammation resulted in appearance of cavities between the muscle fibers. In the granulation tissue, the cellular component predominated over fibers (Fig. 1, *b*).

On day 25, complete re-epithelialization with stratified epithelium and almost complete crust detachment were observed. In the derma, slight inflammation and hemosiderin granules were seen. Fibrous cicatrix formed; collagen fibers were not loose. No hair follicles were found in the damaged area (Fig. 2, *b*).

In group 2 (treatment with chitosan gel without bioregulator), re-epithelialization of the wound was not complete by day 14, the crust tightly adhered to the cell surface, inflammation with neutrophilic infiltration was seen in the wound under the epithelium. Cicatrix formed. The adjacent muscular layer was also involved in inflammation. No hair follicles were seen. The granulation tissue had fibrocellular structure (Fig. 1, *c*).

By day 25, epithelialization was completed, but pronounced inflammation with neutrophilic inflammation persisted in the derma. Fibrous scar tissue formed. The crust partially detached, but in some sites it tightly adhered to the epithelium (Fig. 2, *c*).

In group 1 (treatment with burn-healing gel containing cattle serum bioregulator), practically complete re-epithelialization with stratified epithelium was achieved by day 14 after burn. The crust partially detached and tightly adhered to the wound surface only in sites with incomplete epithelialization. The newly formed stratified epithelium was well developed, ingrowths into the derma (presumably formation of new hair follicles) were seen. No inflammation in the skin was observed, except small neutrophil infiltration foci under the epithelium. The muscular layer was well developed and preserved, the granulation tissue had fibrocellular structure (Fig. 1, *d*).

On day 25, almost complete crust detachment with complete epithelialization, minor inflammation in the derma, well developed muscular tissue, and hemosiderin depositions were seen (Fig. 2, *d*).

Thus, our findings attest to the burn-healing efficiency of the test chitosan gel containing serum bioregulator. Despite different mechanisms of skin lesions (mechanical trauma, burn, *etc.*), the test composition stimulated reparative processes and adjusted them to restoration of the tissue structure, thus preventing fibrous scar formation [3]. We believe that this effect is determined by the presence of serum bioregulator belonging to the group of membranotropic homeostatic tissue-specific bioregulators [2]. Previous studies showed that bioregulators of this group in very low concentrations can tissue-specifically stimulate reparative and restorative processes due to additional activation of cell. Analysis of these findings and our previous data on stimulation of reparative and restorative processes in the cornea, skin, bone, and cartilage tissue under the effect of serum regulator suggest that serum bioregulator additionally activates mesenchymal SC released from BM into the circulation and that these SC can participate in regeneration of mesenchymal tissues [5]. This assumption is the subject of further researches.

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