Effectiveness of Composition Based on Oxidized Dextran in the Treatment of Grade IIIB Skin Burns

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Grade IIIB skin burns were treated with a composition based on oxidized dextran with a molecular weight of 40 kDa (oxidation of 7% glucose residues). On day 32 after burn infliction and from the start of the treatment, the area of skin defect in rats was 30% less than in the group without treatment and by 2.3 times less than in rats treated with panthenol. In rats treated with dextran-based composition or panthenol, the eschar was absent on day 21 after the start of the treatment; by day 32, we found cells of surface epithelium, hair follicles, and sebaceous glands above the scar tissue that were absent in untreated animals; in rats treated with the composition, their number was higher by 2.5 times than in animals treated with panthenol. Treatment with the composition increased volume density (by 2.5 times) and numerical density (by more than 3 times) of blood vessels in the wound and reduced signs of inflammation and fibroplastic activity of fibroblasts in comparison with the corresponding parameters in untreated animals or animals treated with panthenol.

Key Words: burn; oxidized dextran; reparative regeneration; skin

Thermal burns are often associated with infectious and cicatricial complications that later lead to disability [1]. Grade IIIB burn usually heals with dense scar, which, depending on the area of burn, may result in the formation of contractures. It has been previously shown that oxidized dextran (OD) with lower oxidation degree than OD used in this work was captured by cells of different histogenesis including macrophages [4,5] and stimulated reparative cellular and intracellular responses in the liver and lungs [7], reparative regeneration of the skin [3] and had antifibrotic effect reducing scar area [3,5].

Here we studied the effect of OD-based composition on the processes of reparative regeneration in rat skin after infliction of grade IIIB burn to rats.

MATERIALS AND METHODS

Male Wistar rats weighing 190-210 g obtained from the Institute of Cytology and Genetics, Siberian Division of Russian Academy were used in the experiment. The animals were divided into 3 groups (9 animals per group). Group 1 consisted of untreated animals with grade IIIB burns; in group 2, rats with grade IIIB burns were treated with dextran-based composition (40 kDa) as follows: 5% aqueous solution of OD (7% glucose residues) [6], anesthetic lidocaine (0.04%) and a broad spectrum antibiotic metronidazole (0.1%). Group 3 animals (controls) were treated with dexpanthenol ointment (ANKERPHARM GmbH; Jenapharm GmbH). The animals were kept in isolation under standard vivarium conditions with free access to water and food.

Skin burn was inflicted under ether anesthesia as described elsewhere [2] in our modification. In brief, copper stamp with a diameter of 21 mm heated to 260°C was applied to the skin of the low back for 11 sec. The preparations were applied daily on the burn area. On days 21 and 32, skin specimens slightly exceeding the burn area were taken for histological examination to study the processes occurring at the edges of the burn injury. Skin samples from the bottom of the wound and wound edges were taken immediately after decapitation, fixed in 10% aqueous neutral formalin,
embedded in paraffin, and sections (5-7 μ) were sliced on HM 355S type microtome (Microm). Before slicing, the specimens in paraffin blocks were oriented in a plane perpendicular to the bottom of the burn wound and cut in the zone of maximum size (diameter) of the burn injury. The sections stained with hematoxylin and eosin were examined under an AxioStar plus light microscope (Carl Zeiss). To study the reparation process, the photos of burn injury were taken on days 3, 21, and 32 after burn infliction with a Nikon camera mounted on a tripod at a distance of 100 cm from the wound surface. Area of burn injury was measured on photographs using Corel Draw 10.0 software and expressed in mm². The photos taken on day 3 were used only to compare the size of the wound with those on day 21 and day 32. The examined structures were counted using an open test system of 16 squares 1600 μ² in total area at a final magnification of ×400. Leukocytes, lymphocytes, macrophages, mast cells, and fibroblasts were counted in the inflammatory infiltrate. To determine the functional state and activity of collagen synthesis by fibroblasts, the total volume of different types of collagens at the wound bottom was divided by the number of fibroblasts at the wound bottom.

The significance of differences between mean values of normally distributed data was assessed by Student’s t test using SPSS Statistics. The differences were significant at p<0.05.

RESULTS

Examination on the wound surface revealed an eschar on day 3 and a scar on days 21 and 32 in all groups. In rats of groups 1 and 3, it was dense with thick elevated edges. The scar had an irregular stellate shape in group 1 rats and had round shape corresponding to inflicted burn injury in group 3. In group 2, the scar was elastic, linear or rounded oval, the edges did not emerge above the skin surface. Signs of wound abscess (redness of the edges, swelling and viscous yellowish discharge from the surface) were reported in one animal in group 1 and one animal in group 3. On day 32, the area of burn wound in group 1 decreased by 3.1 times in comparison with day 3; in group 3, the scar area decreased only by 1.96 times and in group 2 by 4.86 times (Table 1). On day 32, the eschar was seen on the wound surface in group 1 and was absent in groups 2 and 3. Reparative regeneration of burn injury was completed between day 21 and day 32, but these processes markedly differed in groups 2 and 3. Collagen deposition on the wound bottom was observed in all groups, but hyalinosis foci indicating apparent trophic disorders were seen only in groups 1 and 3. On day 21, the wound area was covered with surface epithelium and the surface eschar absent in groups 2 and 3, while in the group 1, the epithelial defect persisted in the center of the wound and was covered with an eschar. In addition, hair follicles and gland were found under the epithelium in the injury area in group 2 rats on day 32 (Fig. 1). In our previous study, where the rats were treated only with less oxidized 40-kDa dextran (3-4% glucose residues), the eschar was rejected on day 10 [1]. In this study, the eschar in group 2 rats was rejected also on day 10, while in groups 1 and 3, eschar rejection was observed on day 14. This attests to increased risk of septic complications in groups 1 and 3.

On day 32, numerical density and volume density of vessels in group 2 were higher than in group 1 by 35 and 54%, respectively, and surpassed the corresponding parameters in group 3 by 3.1 and 3.2 times (Fig. 2). Consequently, the composition with OD more effectively stimulated angiogenesis. This, obviously, improved the trophism of the wound and contributed to better implementation of the reparative process (Table 1; Fig. 2). Similar effect (increased proliferation of

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**Fig. 1.** Numerical density of hair follicles and glands of the skin (Nai) in rats on day 32 after burn infliction.* p<0.05 in comparison with group 1.

**TABLE 1.** Area of Skin Injury (mm²) after Grade IIIb Skin Burn in Rats (M±m)

<table>
<thead>
<tr>
<th>Day of experiment</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>340.10±18.69</td>
<td>345.90±12.33</td>
<td>329.90±8.12</td>
</tr>
<tr>
<td>21</td>
<td>101.30±5.97</td>
<td>85.5±5.8*</td>
<td>162.40±13.32</td>
</tr>
<tr>
<td>32</td>
<td>108.8±7.2</td>
<td>71.20±4.37*</td>
<td>168.30±4.86</td>
</tr>
</tbody>
</table>

Note. Here and in Table 2: *p<0.05 in comparison with groups 1 and 3.