Effect of Oxidized Dextran on Reparative Regeneration of the Skin after Burn Injury

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In adult Wistar rats, a 3B degree skin burn was modeled and treated by daily application of 5% aqueous solution of oxidized dextran (molecular weight of 60 kDa) on the wound surface. In animals treated with oxidized dextran, neutrophil count in the connective tissue adjacent to the wound increased by day 5 and then decreased by day 21 after burn infliction; proliferation of fibroblasts was observed later than in untreated animals, in whom inflammation run a subacute course. Oxidized dextran increased the content of macrophages in the wound and surrounding connective tissue from days 14 to 21 after burn infliction and promoted effective and complete healing of the skin defect. Regeneration was realized mainly due to proliferation of keratinocytes at the wound edges and was completed by 7 days earlier than in untreated animals, in whom the area of injury by day 21 decreased by only 2 times (*vs*. 10 times in treated rats).

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

Skin burns are associated with a high risk of infection in the area of injury and, consequently, lower rate and quality of the repair processes, in particular, replacement of the lost skin with fibrous connective tissue and formation of keloid scars. Being a lysosomotropic substance oxidized dextran (OD) is captured by cells of different histogenesis [2,3]. It primes macrophages [7], stimulates reparative cellular and intracellular responses in the liver and lungs [5,6], and modulates fibroblast function and collagen production [2], which attests to nonspecific effects of OD on cells of different histogenesis. In light of this, it was hypothesized that the above properties of OD can be useful in the treatment of burn wounds.

Here we studied the effect of OD on inflammatory and reparative/regenerative processes in rats with modeled 3B degree skin burns.

MATERIALS AND METHODS

Wistar male rats weighing 190-210 g were used in the experiment. The animals were divided into two groups of 8 animals each. Group 1 comprised untreated animals with 3B degree skin burn; group 2 consisted of rats with 3B degree skin burns treated with OD. The animals were kept in isolation under standard vivarium conditions with free access to water and food.

Skin burn injury was modeled according to the method of A. A. Vlasov [1] modified by us. Copper plate (diameter of 21 mm) heated to 260°C was applied to the skin on the back near the pelvic area for 11 sec. In group 2 rats, the burned area was daily treated with 5% aqueous solution of OD [4] (molecular weight 60 kDa, room temperature 23-24°C). Skin samples slightly exceeding the burn area were obtained on days 1, 5, 14, and 21 after burn injury from animals of both groups to study the processes occurring at the edges of the burn injury. Burn modeling and skin sampling were performed under ether anesthesia. The animals were sacrificed by decapitation also under ether anesthesia.

Tissue samples were fixed in 10% neutral formalin and embedded in paraffin. The sections (6-7 μ)

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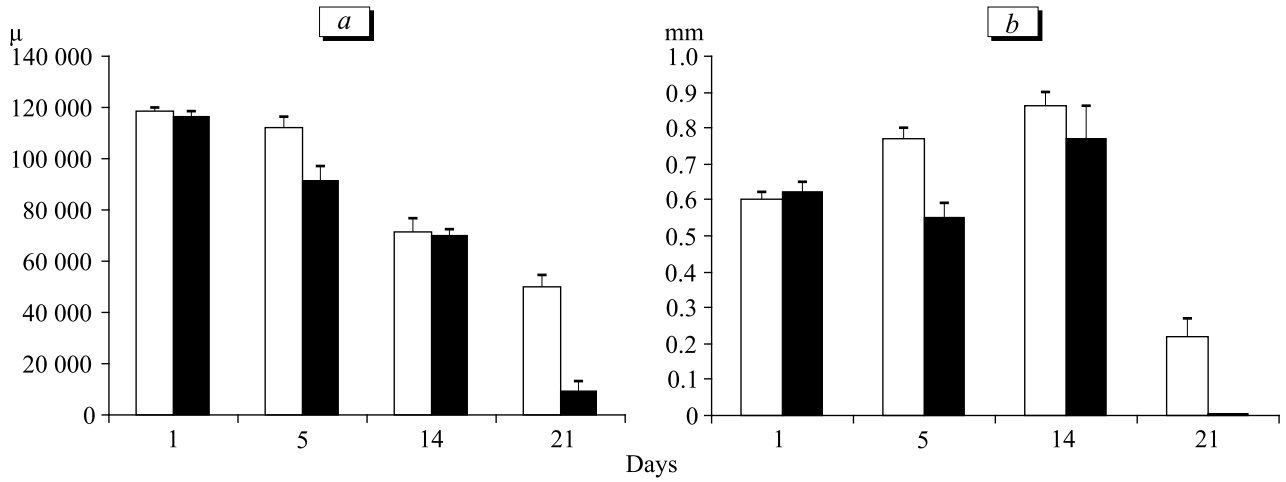

Fig. 1. Dynamics of surface area of burn wound (*a*) and depth of necrosis (*b*) in the skin after 3B degree skin burn modeling in Wistar rats. Here and in Fig. 2, 3: light bars: group 1; dark bars: group 2.

were sliced on a HM355S type microtome (Microm), stained with hematoxylin and eosin, and examined under an AxioStar plus light microscope (Carl Zeiss). On day 1 (immediately after burn infliction) and on days 5, 14, and 21 before skin sampling, the burns in both groups were photographed under ether anesthesia using a Samsung ES15 camera mounted on a tripod at a distance of 30 cm from the burn surface. The area of burn wound was determined by photographs using AxioImager software and expressed in pixels (μ). The depth of necrosis and area of edema in zones adjacent to the wound and at the bottom of the skin defect were measured by ocular micrometer at ×100. Before cutting sections, the skin specimens in blocks were oriented on the plane perpendicular to the bottom of the burn wound. The volume of skin injury at the burn area was calculated by multiplying the mean values of the burn wound area (μ) by the depth of the defect (mm) and expressed in relative units. Numerical density (Nai) of infiltrate cells was evaluated using a closed test system consisting of 16 squares at a final magnification of ×400. Leukocytes, lymphocytes, macrophages, mast cells, and fibroblasts were counted. The obtained data were expressed as a percentage to the total cell count at the zone of interest.

The means were compared by Student's *t* test using SPSS Statistics software. The differences were significant at $p<0.05$.

RESULTS

On day 1 after burn, the burn areas in groups 1 and 2 were similar (Fig. 1, *a*). On day 21, the burn area in rats treated with OD (group 2) was 5.4-fold smaller than in controls (group 1) and more than 10-fold smaller than on day 1. In untreated animals, the burn area decreased by only 2.3 times in comparison with that on day 1 (Fig. 1).

The depth of burn wound is an important risk factor of complications of the wound process (suppuration and spread of inflammation into the underlying and adjacent tissue). In rats treated with OD, the wound depth decreased more rapidly (Fig. 1, *b*); by day 21 in was practically flat and in some rats its depth was <1 mm. At the site of injury, only a rejected eschar was found. In untreated animals, wound depth decreased by about 2.5 times by day 21 (Fig. 1, *b*). Calculating of the volume of burn wounds in rats treated with OD on day 21 after injury showed that this value (absolute and relative) was negligibly small in comparison with that in untreated animals (Fig. 2). Therefore, we can assume that the burn wound in rats treated with OD was completely closed much earlier (by 7 days) than in untreated animals. The same result (healing of skin defect) in untreated rats can obviously be achieved after more than 21 days, because at this

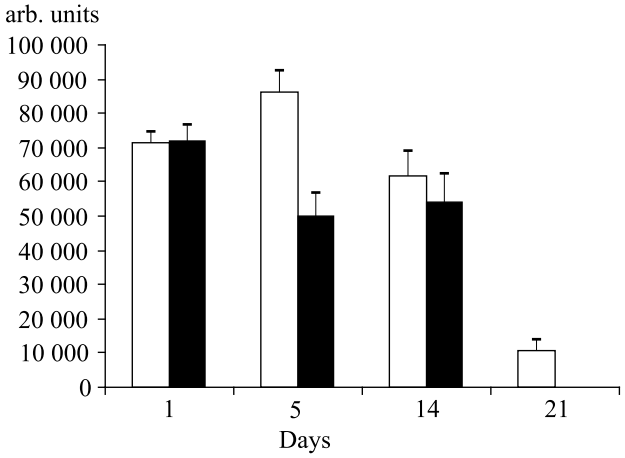

Fig. 2. Dynamics of 3B degree skin burn volume in Wistar rats.

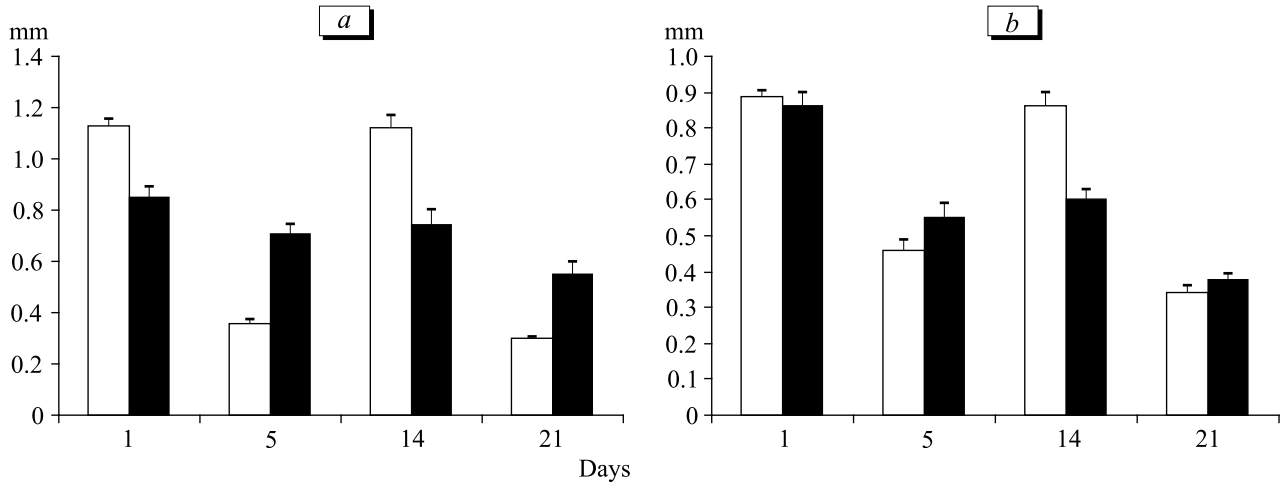

Fig. 3. Size of edema at the bottom (*a*) and edges (*b*) of burn wound in the skin after 3B degree skin burn modeling in Wistar rats.

term the skin injury decreased a little more than 2-fold in comparison with the initial value.

Edema is an obligatory attribute of inflammation; it characterizes the wound process and determines the risk for further inflammation. According to the histological examination, the rats in both groups exhibited serous exudative stage of inflammation.

Microscopy showed that in rats treated with OD edema persisted in the bottom and edges of the wound and was less than on day 1 after burn, but larger in the bottom of the wound than in rats of group 1 on day 21 after burn (Fig. 3, *a*). This is obviously due to the presence of a crust impeding outflow of the wound content. The size of edema at the edges of the wounds was identical in both groups on day 21 after injury (Fig. 3, *b*). The wound edges in rats treated with OD (group 2) were epithelialized and covered with several layers of keratinocytes. Granulation tissue islets retained under the epithelium, collagen fibers were observed, neutrophils, fibroblasts, and macrophages prevailed in the inflammation foci (Table 1). In group 1 (without treatment), the burn wound area was considerable on day 21 after injury (Fig. 1, *a*). The edges of the burn wound were hyperemic and edematous. Foci of granulation tissue and inflammatory infiltration with predominance of macrophages, neutrophils, and fibroblasts were observed. Size of edema in the wound edges was the same in both groups (Fig. 3; Table 1).

Initial concentration of neutrophils in the wound edges was similar in both groups (Table 1). Treatment with OD in group 2 rats activated phagocytic cells capturing OD in the wound [2,3,7] and probably in the skin adjacent to the site of burn injury. This was particularly evident on day 5 after injury and OD treatment. At this term, the concentration of neutrophils in these rats was higher than in untreated animals by 3.8 times. On day 21, it was 6.8-fold lower, whereas the relative content of neutrophils was still

TABLE 1. Cell Composition of Infiltrates in the Skin after Modeling 3B Degree Skin Burn in Wistar Rats (*M±m*)

Time after burn, days	Groups	Neutrophils	Lymphocytes	Macrophages	Mast cells	Fibroblasts
Day 1	group 1	58.02±4.14	2.32±1.07	9.98±1.23	7.10±1.37	22.56±3.54
	group 2	63.93±3.33	4.95±1.85	18.50±2.20*	5.93±1.75	6.67±1.07*
Day 5	group 1	15.97±2.06	2.28±0.81	35.05±2.35	14.92±3.65	31.75±2.23
	group 2	60.86±2.77	0.88±0.45	27.79±2.96	1.58±0.69*	8.86±1.55*
Day 14	group 1	22.66±2.71	3.56±0.75	33.71±2.82	17.13±1.88	22.92±2.11
	group 2	12.61±2.33*	4.04±0.92	40.62±1.92	15.15±2.54	27.56±3.71
Day 21	group 1	18.08±2.15	1.34±0.43	38.65±2.72	9.34±1.52	32.56±2.83
	2-я	2.63±0.83*	3.31±0.72*	43.27±2.30	11.98±1.58	38.79±2.87

Note. *$p<0.05$ in comparison with group 1.

high in group 1. This attests to subacute inflammatory reaction, because the concentration of macrophages and fibroblasts in the infiltrate in this group at the same term began to rise as soon as on day 5, but did not change until day 21. Along with persisting skin injury, this picture suggests delayed reparation. In contrast, in animals treated with OD the second stage of inflammation was manifested by apparent replacement of phagocytic cells in the infiltrates (neutrophils were replaced by macrophages). The content of macrophages increased from day 14 more rapidly than in untreated rats. At the same time, fibroblast proliferation was activated without inhibiting early proliferation of keratinocytes at the wound edges, which promoted epithelialization of the skin injury (Table 1; Fig. 1). We can assume that these positive effects are associated with lysosomotropic properties of OD [2,3] and its ability to activate phagocytic cells [2,3,7] involved in the processes of wound cleansing and producing growth factors promoting proliferation of keratinocytes and fibroblasts.

In both groups, the concentrations of lymphocytes and mast cells in infiltrates surrounding the burn wounds did not differ significantly except higher concentration of lymphocytes in rats treated with OD on experimental day 21. Moreover, a sharp decrease in the percentage of mast cells was also recorded in infiltrates on day 5 in rats treated with OD (Table 1).

Thus, OD used for the treatment of 3B degree skin burn in Wistar rats, activates phagocytic cells in the connective tissue surrounding the wound promoting effective wound cleansing and significantly accelerates (by at least one week in comparison with untreated animals) reparative regeneration of the skin mainly due to proliferation of keratinocytes at the wound edge, thus reducing the risk of fibrosis in skin defects.

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