EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Activity of Antioxidant Enzymes in the Wound in Patients with Deep Burns

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 147, No. 6, pp. 696-699, June, 2009
Original article submitted December 10, 2008

Analysis of wound discharge in children with deep burns over 3 weeks after the injury revealed gradual increase in catalase activity. The increase in activities of myeloperoxidase, glutathione-S-transferase, and catalase was maximum in patients with the most severe burns. Local complications were observed during the period of maximum myeloperoxidase activity, while the beginning of epithelialization was associated with its reduction. Analysis of wound impressions confirms long-term persistence of neutrophils in the wound.

Key Words: *burns; surgical necrectomy; antioxidant enzymes; myeloperoxidase*

Severe burns induce systemic activation of circulating neutrophils and development of oxidative stress in children when the area of the burn is larger than 15% [3]. The wound is the main source of inflammation in these cases. Surgical treatment of burn wounds consists in early removal of the burn crust with subsequent autodermoplasty. An obligatory condition for transplantation of the skin flap is maturation of the granulation tissue, usually, paralleled by epithelialization (marginal and islet).

Activities of fibroblasts and keratinocytes in the wound are regulated by many factors, primarily cytokines and reactive oxygen forms [10,11]. Phagocytic cells (neutrophils and macrophages) protecting the wound from infection release superoxide, hydroxyl radicals, H_2O_2 as a result of "respiratory burst". Hydrogen peroxide stimulates activity of keratinocytes and wound healing; in a high concentration it impedes the formation of the granulation tissue and epithelialization [9]. The status of the granulation tissue determines the efficiency of subsequent transplantation of the skin transplant and rate of epithelialization, in other words, the terms of skin recovery. It is therefore essential to define the approaches to biochemical evaluation of the wound status during surgical treatment and criteria for directed selection of drugs for wound treatment.

We evaluated the antioxidant status of wound discharge in children with severe burns during surgical treatment.

MATERIALS AND METHODS

Twenty-eight children with IIIB-IV degree burns involving more than 15% body surface were urgently hospitalized at G. N. Speransky Pediatric Clinical Hospital No. 9. Surgical treatment of burn wounds was paralleled by antiinflammatory and antibacterial therapy. The patients were divided into 2 groups by the severity of burn injuries. In group 1, the wounds healed without systemic complications; in group 2, healing was paralleled by severe infectious compli-

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0007-4888/09/1476-0753

cations (sepsis, pneumonia, systemic inflammatory response syndrome). Wound discharge for the analysis was collected from deep burn zone by application of sterile paper filters with subsequent extraction of the material with 0.1 M potassium phosphate buffer (pH 7.4). After precipitation of hemoglobin with chloroform-ethanol (3:5) mixture, activities of myeloperoxidase (MPO; marker enzyme of polymorphonuclear leukocytes) [5], catalase (CAT) [4], SOD [8], and glutathione-S-transferase (GST) [1] were measured. The values were standardized by protein content measured after Lowry. In parallel, wound impressions for cytological studies were obtained, stained with eosin and methylene blue after May—Grunwald, and examined under a microscope.

The results were processed using Statistica 6 software. The data are presented as the means and standard deviations. The significance of differences between the groups was evaluated using Mann—Whitney test, between related samples by Wilcoxon test. The differences were considered significant at $p<0.05$.

RESULTS

The two groups of patients did not differ by the total area of burn, but in group 2 the area of deep burn (degree IIIB-IV) was larger ($p<0.01$) and severe infectious complications were detected on days 3-13 (Table 1).

Surgical resection of the crust (necrectomy) was carried out in all patients. However, in group 2 the operation more often included resection of burned tissue reaching the fascia (fascial necrectomy in 10 of 16 operations).

TABLE 1. Comparative Characteristics of Patients

Criteria of comparison	Group 1	Group 2
Number of patients	16	12
Age, years	9.8±3.3	6.3±2.4
Day of hospitalization from the moment of injury	1.4±1.3	6.3±5.7*
Total area of burn, %	26.1±12.0	36/0±12.2
Area of deep burn, %	8.6±6.2	29.5±17.1*
Number of total systems complications		
pneumonia	—	1
sepsis	—	4
sepsis and pneumonia	—	7

Note. *$p<0.01$ compared to group 1 (Mann—Whitney test).

TABLE 2. Comparative Characteristics of Surgical Treatment and Course of Wound Healing in Patients

Parameter	Group 1 (n=12)	Group 2 (n=16)
Day of necrectomy from the moment of injury	5.2±2.5	8.9±5.8*
Volume of necrectomy, %	10.1±4.6	16.9±11.6
Day of autodermoplasty from the moment of trauma	14.8±9.3	25.3±8.9*
Volume of autodermoplasty, %	8.8±4.4	10.3±4.9
Day of marginal epithelialization beginning from the moment of trauma	10.9±5.6	17.8±7.2*
Day of islet epithelialization beginning from the moment of injury	14.9±6.4	22.9±6.6*
Day of autotransplant healing from the day of autodermoplasty	6.5±1.3	7.2±1.3
Day of complete restoration of skin integument from the moment of injury	24.7±10.3	49.3±6.8**
Number of local complications		
secondary crust	3	10
autotransplant lysis	1	4
Presence of microorganisms in wound	7	11

Note. *$p<0.05$, **$p<0.01$ compared to group 1 (Mann—Whitney test).

In group 2 maturation of the granulation tissue, obligatory for subsequent transplantation of skin transplants, and the beginning of marginal and islet epithelialization were significantly delayed in comparison with group 1 (Table 2). These differences could be due to more severe status of patients in group 2 (infectious complications, larger deep burns) and more severe skin lesions (IV degree).

Local complications were more incident in group 2: formation of secondary crust and appearance of bacterial flora.

On the whole, wound healing process in group 2 patients was slower and was associated with many complications.

Comparison of biochemical values of the wound exudation in both groups revealed differences in the dynamics of enzyme activities (Fig. 1).

The concentration of MPO in the wound discharge of group 2 patients was characterized by a more stable increase in activity, maximally pronounced during week 2 after the trauma. This period coincided with the development of local complications (secondary crust, autotransplant lysis). Elevation of GST activity was observed during the same period (group 2).

In both groups SOD activity decreased during week 2, in group 1 this decrease was followed by its gradual increase.

Catalase activity in the discharge increased during week 2 in patients of both groups. In group 2, this parameter continued to increase during week 3, when the difference in the absolute CAT values between the two groups became significant.

Comparison of the biochemical values (Fig. 1) and terms of granulation tissue maturation (evaluated visually) and of marginal and islet epithelialization (Table 2) showed a common regularity: local complications developed during the peak of MPO activity, while the onset of marginal and islet epithelialization in patients

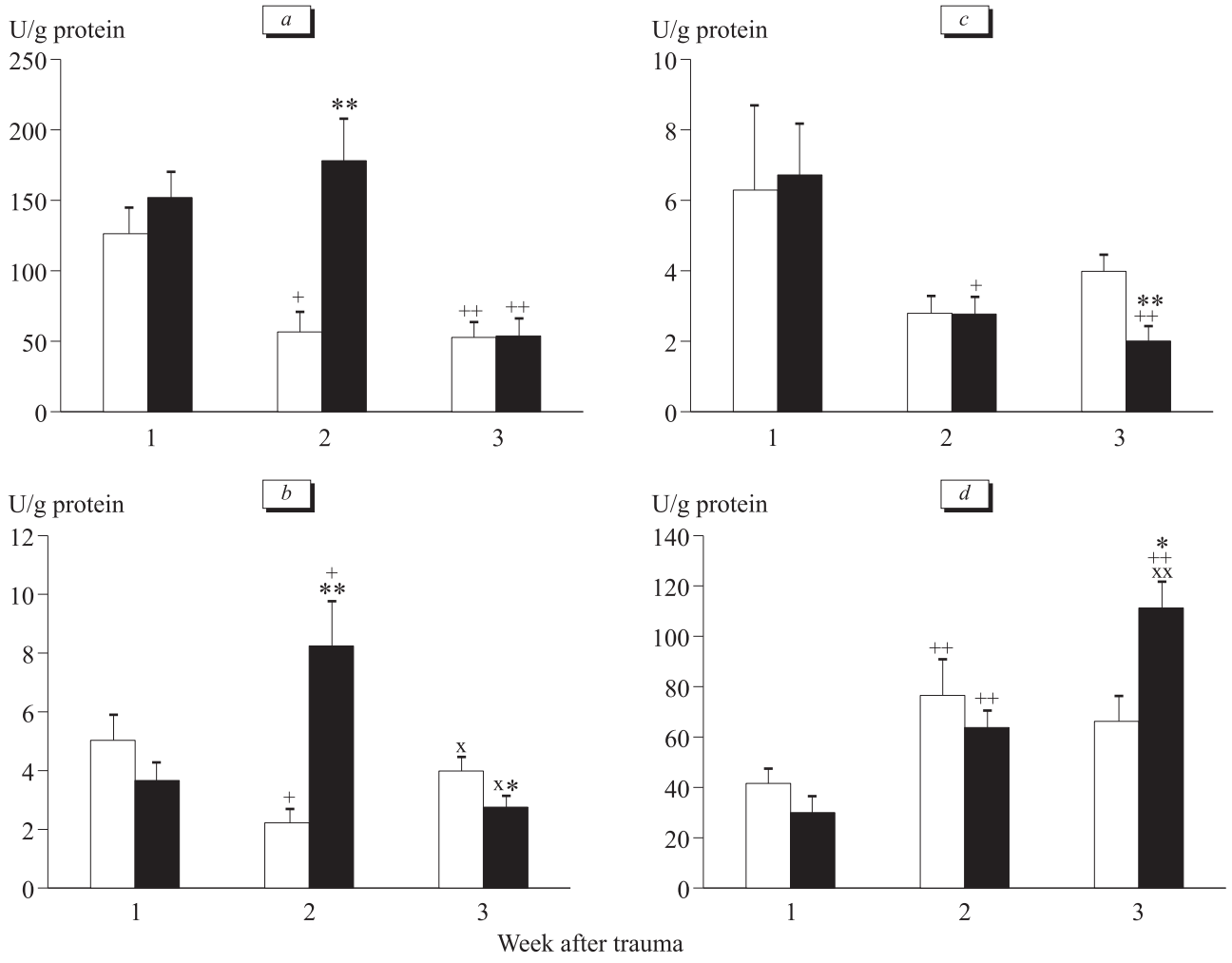

Fig. 1. Time course of enzyme activities in wound exudation of patients without severe infectious complications (group 1, light bars) and with complications (group 2, dark bars). *a*) MPO; *b*) GST; *c*) SOD; *d*) CAT. Significance of differences: $^{*}p<0.05$, $^{**}p<0.05$ (Mann—Whitney) compared to group 1; $^{+}p<0.05$, $^{++}p<0.01$ compared to week 1; $^{x}p<0.01$ compared to week 2 (Wilcoxon test for related samples).

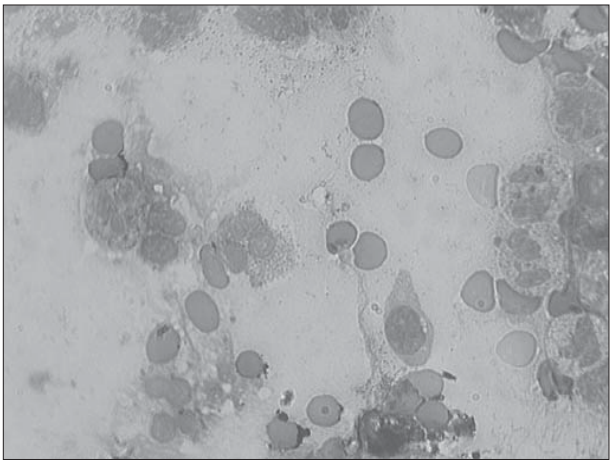

Fig. 2. Patient Zh. group 2: wound impression on day 19 after injury. May—Grunwald staining (×1000).

of both groups was observed during reduction of MPO activity, paralleled by elevation of CAT activity.

Polymorphonuclear leukocyte MPO can be present in their primary granules or directly in exudation (as a result of degranulation). Analysis of wound impressions showed intact polymorphonuclear leukocytes even during the late stages of wound healing process (2-3 weeks after the trauma; Fig. 2). Hence, burn wounds are healed in the presence of neutrophils capable of producing significant amount of active oxygen forms. Long-term infiltration of the wound with neutrophils is fraught with oxidative stress. Increased activity of antioxidant enzymes under these conditions is an adequate defense reaction. Elevation of CAT activity was recorded in the wound discharge of patients of both groups, which is essential for cell protection from H_2O_2 excess.

In the previous study on a model of excision wound and burn injury in rats we found that CAT activity in the wound tissue and in the adjacent skin virtually did not increase in response to trauma [2], which was in good agreement with the data on negative effect of exogenous CAT on the rate of surgical wound healing in mice [9]. It seems, that the complex of antioxidant enzymes in the skin and wound tissue of mice and rats supports optimal concentration of H_2O_2 needed for redox stimulation of wound healing without induction of CAT (for example, at the expense of glutathione peroxidase activation [2,13]). By contrast, analysis of antioxidant enzymes in human skin specimens resected during plastic surgery showed a significant increase of CAT activity by the end of the intervention [2]. According to our data, activity of glutathione peroxidase in human skin is at least 5-fold lower than in the rat skin. Presumably, CAT is more actively involved in the maintenance of H_2O_2 balance in human wound and skin. It is CAT, released by platelets, that rapidly neutralizes H_2O_2 in cytotoxic amounts and protects the bone marrow granulation tissue [6].

Comparison of CAT activities in wound discharge from patients with and without complications detected no difference in the activities of CAT 2 weeks after the trauma; a significant increase of the activity of GST (for which lipoperoxides serve as substrates) was detected in patients with complications. It seems that CAT induction in patients with complications lags behind H_2O_2 production or that the enzyme is partially inactivated by oxygen radicals (superoxide, peroxide), and lipids and lipoproteins are damaged as a result.

Presumably, local application of 3% H_2O_2 solution will inhibit wound healing, similarly as the CAT-inhibiting drugs. High incidence of bacterial contamination of burn wounds, particularly in systemic infectious complications, necessitates the search for methods of its control. The effect of local application of inhibitors of radical-producing activity of neutrophils can be ambiguous. From this viewpoint, antioxidants of plant origin deserve special interest [7,12].

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