Preventive and Therapeutic Effect of Complex Antioxidant Preparation in Rats with Burn Trauma

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The production of blood radicals and activity of superoxide dismutase in erythrocytes increased in rats with contact burn trauma (20%). In animals with burn trauma antioxidant activity of the plasma was much lower, while myeloperoxidase content in the lung tissue and epidermis was higher than in control rats. The complex of antioxidants (Immudzhen) inhibited radical generation at the peak of inflammation (day 4), increased antioxidant activity of the plasma, and normalized myeloperoxidase content in the lung tissue.

Key Words: *burns; oxidative stress; antioxidant vitamins*

High risk of complications during burn trauma is related to the systemic inflammatory reaction [9]. Thermal trauma is followed by the release of cytokines and prostaglandins and intensive interaction of leukocytes and platelets with endothelial cells. Activation of leukocytes (polymorphonuclear leukocytes, PMNL) [6] and platelets intensifies production of reactive oxygen and nitrogen species. These changes and high adhesion of PMNL to the endothelium increase the risk of damage to organs and tissues. Under normal conditions the increased production of reactive oxygen and nitrogen species is compensated by activation of protective antioxidant enzymes, including superoxide dismutase (SOD), catalase, and glutathione peroxidase. The imbalance between activity of the radical-generating and antioxidant systems leads to excessive accumulation of free radicals playing a role of circulating pathological signals. This process underlies the pathogenesis of systemic polyorgan damage [5].

Here we studied local and systemic free radical disorders and assayed antiinflammatory activity of the complex preparation containing antioxidant vitamins and amino acids in rats with burn trauma.

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MATERIALS AND METHODS

Experiments were performed on 20 Wistar rats weighing 350-400 g. Contact burn trauma (IIIA-B, 20% of skin area) was produced under general anesthesia [4]. The animals were divided into experimental and control groups (10 rats per group). Experimental animals perorally received *per os* Immudzhen, a complex preparation of vitamins and amino acids (IDI Farm) starting from the 2nd day after trauma. In our study the dose of Immudzhen 2-fold exceeded that recommended for humans (140 mg/kg). The preparation (140 mg) contains 3 mg ubiquinone, 3 mg α-tocopherol, 12 mg methionine, 3 μg selenium aspartate, and 12 mg soybean phospholipids. The control animals received 0.9% NaCl.

For chemiluminescence study, the blood was sampled from the caudal vein at 24-h intervals for 12 days. Further experiments were conducted with 5 ketamine-narcotized rats of each group on days 4 and 12. Samples of the epidermis were taken at a distance of not less than 10 mm from the zone of injury. The blood was obtained from the vena cava inferior to isolate the plasma and erythrocytes. The animals were euthanized by overdosing barbiturates. Lung tissue was dissected.

The total production of radicals in the blood was determined by luminol-dependent chemiluminescence stimulated with phorbol ester (0.156 μM) [10].

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Activities of glutathione peroxidase, catalase [7], and SOD were measured in erythrocytes [8]. Antioxidant activity (AOA) of the plasma was estimated [1]. We evaluated the ability of the plasma to inhibit lipid peroxidation in the model system. AOA was calculated as follows: $AOA=(A_{WP}-A_P)/A_{WP}\times100\%$, where A_{WP} and A_P are the contents of thiobarbituric acid-reactive products in samples not containing and containing the plasma, respectively.

Lung tissue and epidermis were washed with phosphate buffer, homogenized in a glass homogenizer under cooling, and centrifuged at 1000*g* and 10°C for 15 min. Hemoglobin was removed from the supernatant with a 3:5 chloroform-ethanol mixture. Myeloperoxidase (MPO) activity in supernatants was measured using *o*-dianisidine [7]. Protein content was estimated by the method of Lowry.

The wound area was measured planimetrically after excision of the eschar (day 12).

The results were analyzed by Student's *t* test.

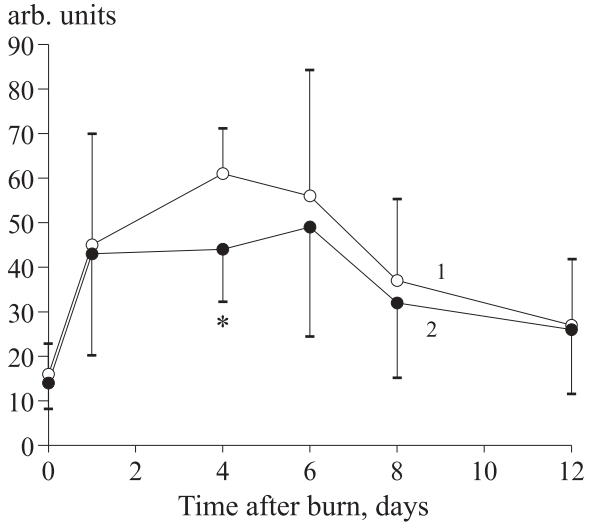

Fig. 1. Entire production of radicals in the blood of control (*1*) and treated animals (*2*). Entire production of radicals was assayed by chemiluminescence of the sample (1 ml) containing 10 µl whole blood, 0.2 mM luminol, 0.156 µM phorbol ester, and Hanks solution. Here and in Fig. 2: **p*<0.05 compared to the control.

RESULTS

Burn trauma induced acute inflammatory reaction in control rats (Table 1). On day 4 we observed activation of radical generation in the whole blood and increase in MPO activity in lung tissue and epidermis (by 1.6 and 2.1 times, respectively, $p<0.05$). SOD activity increased, while the contents of glutathione peroxidase and catalase in erythrocytes remained unchanged on day 4 after trauma. AOA of the plasma in rats with burn trauma decreased more than by 3 times compared to controls. On day 12 the intensity of radical generation in the blood decreased, while glutathione peroxidase activity in erythrocytes increased. It should be emphasized that AOA of the plasma and MPO content in lung tissue significantly differed from normal.

The sharp decrease in plasma AOA reflects uncompensated generation of compounds with high oxidant activity in the blood. The imbalance in the erythrocyte system of antioxidant protection developed on day 4 after injury. Activity of the enzyme converting superoxide anion radicals into H_2O_2 (SOD) increased, while the amount of H_2O_2-utilizing enzymes (glutathione peroxidase and catalase) remained unchanged. The increased production of radicals by leukocytes was probably followed by the formation of excess H_2O_2, lipid peroxides, and products of conversion.

Administration of the test preparation containing α-tocopherol, selenium, and ubiquinone inhibited the production of radicals at the peak of inflammation (by 28%, day 4, Fig. 1), partially prevented the decrease in plasma AOA (Fig. 2), and reduced MPO concentration in the lung tissue (marker of inflammation, Fig. 3). Immudzhen had no effect on MPO content in the epidermis and antioxidant enzyme activity in erythrocytes (SOD, catalase, and glutathione peroxidase).

Planimetry showed that the rate of wound healing tended to increase in rats of the Immudzhen group on day 12 after injury. In control and Immudzhen-receiving rats the wound area was 1718±450 and 1426±385 mm^2, respectively.

TABLE 1. Radical Generation and Blood AOA in Control Animals (*M*±*m*)

Parameter	Before burn	After burn, days	
		4	12
Entire production of radicals, arb. units	16.3±6.6	61.2±9.9*	27.2±14.7
Erythrocyte SOD, U/mg protein	57.2±25.1	142.1±42.3*	92.7±32.7
Erythrocyte glutathione peroxidase, U/mg hemoglobin	15.3±1.2	16.9±2.5	36.8±13.7
Erythrocyte catalase, U/µg protein	39.7±13.8	38.9±4.1	—
Plasma AOA, %	72.2±6.3	21.2±4.7*	27.4±16.7*

Note. **p*<0.05 compared to parameters observed before burn.

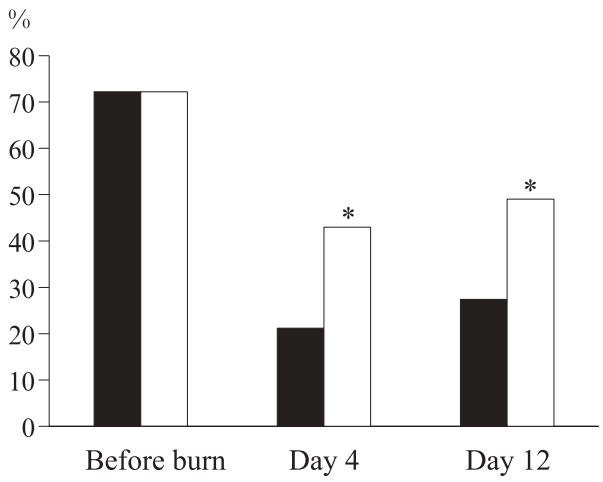

Fig. 2. Total antioxidant activity of the plasma from control (dark bars) and treated animals (light bars).

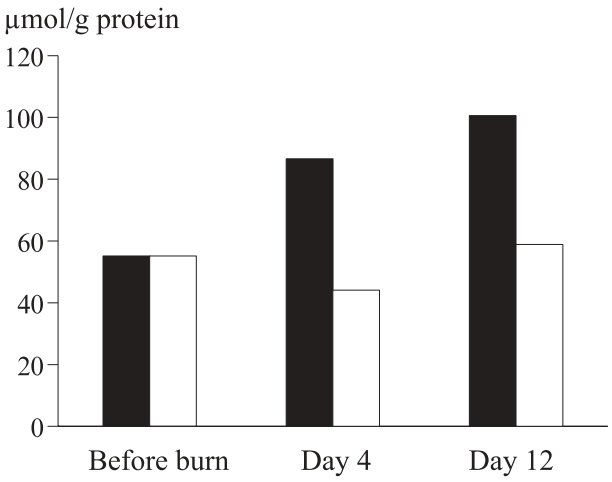

Fig. 3. Myeloperoxidase activity in lung tissue of control (dark bars) and treated animals (light bars).

Previous studies showed that the complex preparation Immudzhen normalizes the reduced content of coenzyme Q and α-tocopherol in human plasma and leukocytes [2] and activity of antioxidant enzymes in neutrophils during acute inflammation [3].

It can be hypothesized that the complex of antioxidant vitamins modulates protective function of the plasma and PMNL in the acute phase of inflammation, decreases the risk of oxidative damage to cells and tissues, and contributes to adaptation of the blood antioxidant system to oxidative overload. Treatment with this preparation in the early stage of the disease decreases the risk of oxidative damage to tissues.

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