Protective Effect of Complex Antioxidant Preparation Containing Vitamins and Amino Acids in Rats with Burn Trauma Complicated by Endotoxemia

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Burn trauma increased blood chemiluminescence, while lipopolysaccharide in a dose of 1 mg/kg potentiated this effect, activated LPO, and decreased plasma antioxidant activity. In erythrocytes, superoxide dismutase activity increased, while activity of peroxide-utilizing enzymes decreased. Myeloperoxidase content increased in the lungs and epidermis. The preparation of α-tocopherol, selenium aspartate, and ubiquinone abolished the effect of lipopolysaccharide, but did not modulate the increase in chemiluminescence under the influence of this agent.

Key Words: *burns; lipopolysaccharide; chemiluminescence; antioxidants*

Burn-related inflammation of the lungs, gastrointestinal tract, and kidneys is the major cause of death and determines the severity of burn disease. Burn-related dysfunction of internal organs probably results from cell oxidative stress [5]. The development of infection aggravates clinical symptoms of disturbances. Ischemic tissues with increased activity of tissue xanthine oxidase, as well as macrophages and prestimulated neutrophils, are the main sources of oxygen radicals. They cause generalized oxidative stress during burn trauma. Endotoxins, including lipopolysaccharide (LPS) of gram-negative bacteria, induce priming of neutrophils, increase cell adhesion, and stimulate production of reactive species of oxygen, and nitrogen (respiratory burst), and other inflammatory mediators (*e.g.*, tumor necrosis factor and interleukin-1) [12]. The presence of LPS in blood flow during severe burn trauma is associated with bacterial translocation from the intestine [13] and burn scab [6].

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Our previous studies showed that complex antioxidant preparation containing vitamin E, ubiquinone, and selenium decreases blood content of oxygen radicals in animals with burn trauma at the peak of inflammation (day 4 after the incidence of burn). This preparation also reduced the risk of neutrophil-mediated damage to the lung tissue [2].

Here we studied the prooxidant and antioxidant response of the blood and migration of neutrophils to the skin and lungs of rats with burn trauma complicated by endotoxemia. We also evaluated the possibility of correcting these changes with complex antioxidant preparation.

MATERIALS AND METHODS

Experiments were performed on 12 male Wistar rats weighing 350-380 g. Six experimental animals perorally received Immudzhen (IDI Farm) 2 times a day. Immudzhen was given over 4 days before and after trauma. Control mice received 0.9% NaCl. The daily dose of Immudzhen (280 mg/kg) corresponded to 0.7 mg/kg coenzyme Q, 0.7 mg/kg α-tocopherol, 0.7 μg/kg selenium as-

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partate, 3 mg/kg methionine, and 8.7 mg/kg soybean phospholipids.

Contact burn IIIA-B (20% of skin area) was induced under general anesthesia [4]. The solution of *E. coli* O111:B4 endotoxin (Sigma) in a dose of 1 mg/kg was injected intraperitoneally on day 4 after burn trauma. Cell chemiluminescence was measured in whole venous blood [2]. The epidermis (10 mm from the zone of injury) was excised before treatment, on day 4 of the postburn period, and 24 h after endotoxin injection. The lung tissue was sampled and treated routinely. The epidermis was homogenized as described elsewhere [2].

Blood plasma was obtained by centrifugation and used to study LPO (concentration of malonic dialdehyde, MDA) and total antioxidant activity (AOA) [1]. Erythrocyte pellet was washed from the plasma. Activities of glutathione peroxidase [7] and glutathione S-transferase (GST) [11] in lysed erythrocytes were measured by the reaction with 1,1-chloro-2,4-dinitrobenzene (CDNB). Activities of superoxide dismutase (SOD) [9] and catalase were measured in the chloroform ethanol extract of lysed erythrocytes [7]. Myeloperoxidase (MPO) activity in the supernatant of epidermal homogenate and lung tissue extract was estimated by *o*-dianisidine content [7]. Protein concentration was measured by the method of Lowry.

The results were analyzed by Student's *t* test. The data were expressed as $M \pm m$.

RESULTS

The increase in blood cell chemiluminescence in control animals reflected the generalized inflammatory response and persisted until the 4th day after treatment (Fig. 1). This parameter decreased after Immudzhen administration, which is consistent with the results of our previous experiments [2].

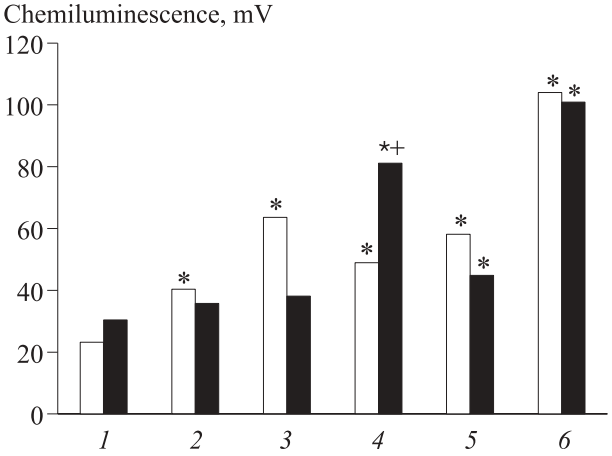

Fig. 1. Blood cell chemiluminescence in animals with burn trauma receiving LPS. Before burn trauma (*1*); 1 day after burn trauma (*2*); 4 days after burn trauma (*3*); 2 h after LPS injection (*4*); 9 h after LPS injection (*5*); 24 h after LPS injection (*6*). Light bras, control group; dark bars, experimental group. $p<0.05$: *compared to the preburn level; +compared to the control.

Blood chemiluminescence in animals of both groups sharply increased 24 h after endotoxin injection (Fig. 1). These changes were not accompanied by an increase in the absolute number of leukocytes in the blood of animals.

The response to endotoxin developed progressively. Two hours after endotoxin injection chemiluminescence slightly decreased in control rats, but temporally increased in treated animals (Fig. 1). The increased production of radicals in the blood was accompanied by a decrease in plasma AOA and activation of LPO (Table 1). It should be emphasized that the intensity of LPO decreased, while AOA increased in treated rats compared to control animals.

Activities of glutathione peroxidase and GST in erythrocytes significantly decreased 24 h after LPS injection. Catalase activity tended to decrease under these conditions. SOD activity increased in control

TABLE 1. Blood Antioxidant Activity in Animals with Burn Trauma 24 h after Intraperitoneal Injection of Endotoxin in a Dose of 1 mg/kg

Parameter	Normal (intact animals)	Group	
		control	treatment
AOA, %	72.2±6.3	7.5±4.6*	19.1±4.8*+
MDA, μmol/liter	1.04±0.34	2.55±0.22*	2.26±0.13*+
Glutathione peroxidase, U/mg hemoglobin	5.4±1.7	3.3±0.2*	3.0±0.66*
GST, μmol CDNB/g hemoglobin/min	5.4±1.1	2.50±0.04*	2.30±0.07*
SOD, U/mg protein	57.2±25.1	98.8±26.7*	68.5±18.7
Catalase, U/μg protein	39.7±13.8	26.4±3.4	24.8±3.9

Note. $p<0.05$: *compared to normal; +compared to the control.

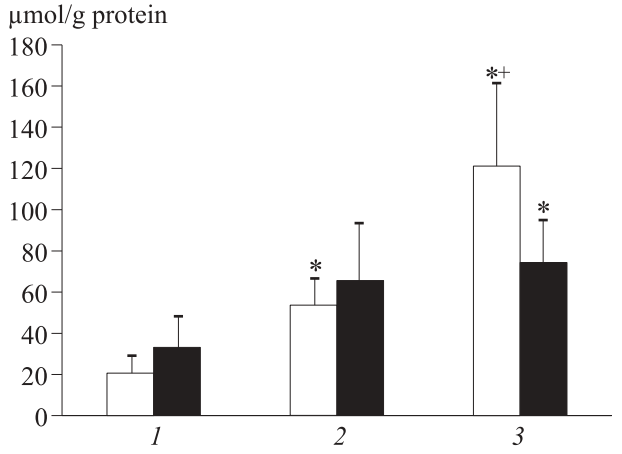

Fig. 2. Myeloperoxidase content in intact epidermis. Before burn trauma (*1*); 4 days after burn trauma (*2*); 24 h after LPS injection (*3*). Light bras, control; dark bars, experiment. $p<0.05$: *compared to the preburn level; +compared to the level observed 4 days after burn trauma.

rats, but remained unchanged in treated animals. Previous studies showed that burn trauma is followed by an imbalance in the antioxidant system of erythrocytes. These changes are related to the increase in SOD activity not accompanied by corresponding activation of peroxide-utilizing enzymes [2]. We showed that LPS increases the degree of this imbalance due to reduction of enzyme activity. The risk of endothelial damage with hydrogen peroxide increases under these conditions.

MPO activity serves as a criterion of neutrophil function. The increase in MPO content reflects migration of neutrophils to the lung tissue. MPO content in healthy animals was 40.5±4.5 μmol/g. On day 5 after burn trauma (24 h after endotoxin injection) MPO content in control and treated rats was 283.5± 147.0 and 121.5±47.8 μmol/g, respectively ($p<0.05$ compared to the control). Injection of endotoxin increased MPO content in the lung tissue of burned animals by 7 times compared to normal. It should be emphasized that MPO content in rats with uncomplicated burn trauma exceeded the normal by 1.6 times [2]. In animals of both groups MPO content in the epidermis adjacent to the burn area increased 4 days after burn trauma (Fig. 2). LPS significantly increased MPO content only in control rats.

Many experimental and clinical studies were performed to evaluate the ability of antioxidant agents α-tocopherol (vitamin E), ascorbate, glutathione, ubiquinone, N-acetylcysteine, and organic compounds of selenium to protect the tissue from oxidative stress. Several compounds had synergistic effects [8]. The complex antioxidant preparation of various compounds in low doses partly compensated for a sharp decrease in plasma AOA and activation of LPO. Moreover, this preparation prevented the increase in erythrocyte SOD activity 24 h after LPS injection. The preparation abolished LPS-induced neutrophil infiltration in intact skin, which reduced the risk for extension of the paranecrotic area. The number of neutrophils in the lung tissue of treated rats was lower than in control animals.

The observed changes in blood chemiluminescence and antioxidant activity indicate that the test preparation acts as a radical-trapping agent and regulates several pathways of reactive oxygen species generation. The protective effect of the complex antioxidant preparation Immudzhen in the blood and organs (lungs and skin) was not accompanied by intensification of radical production by blood neutrophils, which prevented the development of septic complications.

REFERENCES

1. G. I. Klebanov, I. V. Babenkova, Yu. O. Teselkin, *et al*., *Lab. Delo*, No. 5, 59-62 (1988).
2. E. V. Mikhal'chik, A. V. Ivanova, M. V. Anurov, *et al*., *Byull. Eksp. Biol. Med*., **138**, No. 9, 299-301 (2004).
3. H. Baskaran, M. L. Yarmush, and F. Berthiaume, *J. Surg. Res*., **93**, 88-96 (2000).
4. N. A. Busch, E. M. Zanzot, P. M. Loiselle, *et al*., *Infect. Immun*., 3349-3351 (2000).
5. O. Cetinkale, D. Konukoplu, O. Senel, *et al*., *Burns*, **25**, 105-112 (1999).
6. M. Eski, M. Deveci, B. Zelikuz, *et al*., *Ibid*., **25**, 739-746 (2001).
7. R. A. Greenwald, *Handbook of Methods for Oxygen Radical Research*, Florida (1986), pp. 291-302.
8. J. W. Horton, *Toxicology*, **189**, 75-88 (2003).
9. H. P. Mizra and I. Fridovich, *J. Biol. Chem*., **247**, 3170 (1972).
10. H. S. Murphy, R. L. Warner, and N. Bakopoulos, *Shock*, **12**, 111-117 (1999).
11. H. Sies, *Am. J. Med*., **91**, 315-385 (1991).
12. V. M. Victor and M. De La Funte, *Physiol. Res*., **52**, 101-110 (2003).
13. P. Y. Xia, J. Zheng, H. Zhou, *et al*., *World J. Gastroenterol*., **8**, No. 3, 546-550 (2002).