Effect of Erysod (Erythrocyte Superoxide Dismutase) on Blood Concentration of Reactive Oxygen Species in Patients with Severe Burns and Burn Shock

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The dynamics of blood concentrations of reactive oxygen species and LPO products in patients with thermal injuries of different severity was studied. Monitoring of these parameters by chemiluminescent and spectrophotometric techniques helps to predict the course of burn shock and prevent complications. Erysod (0.004% solution, 33-66 μg/min, daily dose 24-32 mg) added to antishock infusion therapy during the early periods after injury suppressed generation of free radicals (by 20% after 15 min and by 30-40% after 24 h), promoted normalization of their contents, and reduced damage to visceral organs during acute period of thermal injury.

Key Words: *thermal injury; infusion therapy; superoxide dismutase; reactive oxygen species; lipid peroxidation*

After thermal injuries, the skin with subcutaneous tissues become a potent source of free radicals. This leads to excessive production of reactive oxygen species (ROS) and sharp intensification of LPO in the viscera as early as during the first hour after injury. Cytolysis and LPO activation in ischemic areas surrounding the necrotic zone in the burn wound cause active release of these products into the plasma [2]. Hyperproduction of ROS and LPO products disturbs functioning of visceral organs, decreases myocardial contractility [7], induces cerebrovascular spasm [4] and irreversible changes in the kidneys [8], intestine [5], liver [9], and vascular endothelium [10], and promotes hemolysis, which causes anemia in patients with burns [6].

SOD (EC 1.15.1.1), a redox enzyme, the main component of the antioxidant defense in all pro- and eukaryotic cells, occupies a special place among drugs normalizing the content of ROS and LPO products [3].

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Here we studied the effects of Erysod, a preparation obtained from human erythrocytes, on the course of burn shock in patients with extensive skin burns.

MATERIALS AND METHODS

We observed 53 patients with burn shock (burns caused by flame or hot water) hospitalized at Clinic for Thermal Injuries, Military Medical Academy, and Burn Center, I. I. Dzhanelidze Institute of Urgent Care. Blood concentration of ROS was measured by the method of luminol-dependent chemiluminescence (LDC) using a Lumina LKB-1251 system (LKB): whole blood in heparin-containing buffer was incubated with luminol in chemiluminometer cuvettes (37°C, 30 min) and the inductor (phorbol myristate acetate) was added [11]. The content of LPO products was evaluated by plasma level of MDA measured spectrophotometrically by the reaction with TBA [1].

The content of ROS (measured by LDC) in normal human blood collected from the ulnar vein was taken for the normal value (2.64 mV, Table 1). The

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content of ROS in patients with burn shock was measured by LDC of venous and (if possible) arterial blood starting from the moment of hospitalization (initial LDC level) for 1-5 days of antishock infusion therapy.

Erysod added to the complex antishock therapy for 11 burned patients was infused intravenously (33-66 μg/min, daily dose 24-32 mg, 0.004% solution in lactosole, Ringer solution with lactate, 0.9% NaCl with glucose). In 5 patients (group 1) infusion of Erysod and standard treatment were started simultaneously (<1 h after injury). In 4 patients infusions of Erysod was started 12-24 h after beginning of antishock therapy, when LDC intensity in venous blood 10-fold and more surpassed the normal (group 2). Two patients had extensive burns combined with thermoinhalation injury (group 3) and extremely high levels of LDC and MDA 42 and 3 times surpassing the normal (110 mV and 11.53 μM, respectively) before the start of Erysod infusion (24 h after beginning of therapy).

The means and standard deviations were calculated for each group and significance of differences from the control was evaluated using Student's *t* test at 0.95 probability.

RESULTS

The dynamics of LDC is determined by the severity of injury and positively correlates with the development of burn disease (Fig. 1).

Extensive burns of the skin lead to a drastic (3-fold) increase in blood content of ROS during the 1st hour after injury (initial LDC, Tables 1 and 2). When the course of burn shock is favorable (Fig. 1, curves *1*, *2*) the intensity of LDC always peaked on days 2-3 (ROS content 8-fold surpassed the normal, point B) and then gradually decreased (days 3-4). During the first hours after injury LDC intensity in venous blood is lower than in arterial blood, but when capillary spasm is resolved, these parameters became equal (point A), and then LDC of venous blood is higher (point B). This can be explained by improvement of microcirculation and release of ROS from peripheral vessels (first affected by hypocirculation during shock of any etiology, including shock caused by severe thermal injury) to central veins. It should be emphasized that the absolute values of LDC and times corresponding to points A and B differed in different patients and depended on the severity of injury and the course of shock, but the dynamics of LDC was the same in all cases (typical values are presented in Fig. 1).

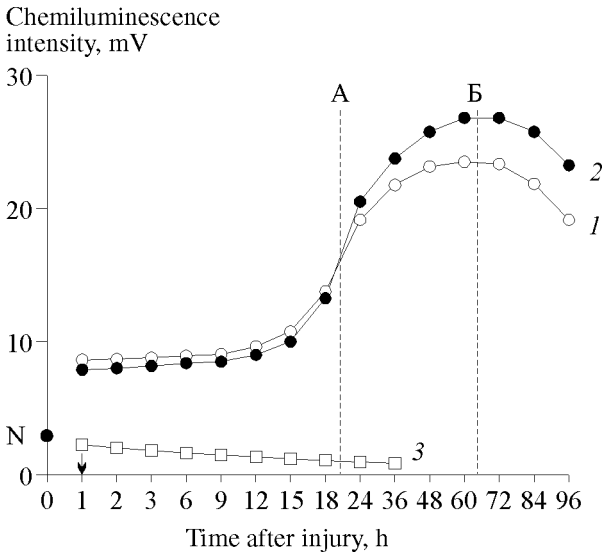

Fig. 1. Typical dynamics of chemiluminescence intensity in patients with severe burns. *1*) arterial blood; *2*) venous blood; *3*) venous blood in cases with lethal outcome. Arrow shows the start of therapy. *N*: normal; *A* and *B*: explanations in the text.

When the course of burn shock was unfavorable and microcirculatory disorders progressed, blood LDC at admission were below normal (about 2.5 mV, Fig. 1, curve *3*). Despite active antishock therapy, homeostasis disorders progressed, while LDC of venous and arterial blood gradually decreased attaining zero directly before death, which occurred within the first 48 h after hospitalization (all patients in this group were elderly individuals with extensive deep burns incompatible with life).

Plasma concentration of MDA in early hours after injury did not differ from that in healthy controls (in contrast to LDC). It is noteworthy that the concentration of TBA-reactive products does not adequately reflect the course of LPO processes, because only lipid peroxides degrading with the formation of MDA react with TBA. Plasma concentration of MDA gradually increased during burn shock (in all 12 patients) from

TABLE 1. Blood Chemiluminescence in Burned Patients with Burn Shock during Antishock Therapy without Erysod (mV, 30 min, *M*±*m*, *n*=12)

Blood vessel	Normal (healthy donors)	Initial (before therapy)	After the start of therapy, h			
			6	12	18	24
Artery	—	8.51±0.47	8.93±0.81	9.18±0.29	12.41±1.37	19.72±2.13
Vein	2.64±0.40	8.14±0.61	8.55±0.37	8.84±0.42	14.66±1.47	20.12±0.98

TABLE 2. Blood Chemiluminescence in Burned Patients with Burn Shock during Antishock Therapy Including Erysod Infusion (Group 1, Artery/Vein, mV, 30 min)

Patient No.	Initial value (before therapy)	Time after start of therapy		
		15 min	12 h	24 h
1	8.1/7.6	5.7/4.4	7.4/6.9	10.5/12.8
2	8.7/8.1	6.4/6.6	11.3/14.4	—/—
3	9.8/9.3	6.7/6.6	8.5/8.2	—/14.9
4	10.1/9.6	8.3/7.8	—/9.4	—/13.6
5	8.2/7.4	7.7/7.3	8.4/8.2	11.9/13.3
M±*m*	8.98±0.78/8.40±0.84	6.96±0.83*/6.54±0.86*	8.90±1.20/9.42±1.99	11.20±0.70^{+}/13.65±0.63^{+}
(Δ, %)		(-22/-22)		(-43/-32)

Note. $p<0.05$: *compared to initial value, $^{+}$ compared to the control (without Erysod, Table 1).

3.91±0.70 μM before therapy to 3.92±0.65, 4.52±0.78, and 5.18±0.87 μM 24, 42, and 72 h after the start of therapy (*vs.* 3.92±0.66 μM in donors [1]). However, this parameter increased significantly (to 6-7 μM) only after the end of shock on days 4-5 of observation, which can be associated with aggravation of burn-induced toxemia.

In case of early (on days 3-4 after injury) complications of burn disease (acute renal failure, respiratory distress syndrome, early burn-induced sepsis) the contents of ROS and MDA increased again (second wave) and this increase 12-24 h preceded clinical manifestations of these complications. Hence, dynamic monitoring of LDC and MDA levels allows evaluation of treatment efficiency and helps to prevent complications.

Erysod was used in the treatment of patients with most severe burns (for this reason the mean initial level of LDC in group 1 surpassed the data presented in Table 1). Erysod significantly decreased the content of ROS in group 1 patients and improved acid-base balance and blood gases as early as 15 min after the start of infusion. After 12 h LDC intensity in patients treated with Erysod did not differ from that in patients receiving no Erysod (Table 1), but then this parameter increased slower. Early start of Erysod infusions promoted stabilization of ROS level even in extremely severe burn shock. In 2 patients of group 2 LDC intensity decreased, but remained very high (more than 20 mV after 5 h) and did not tend to decrease. In other patients of group 2 and in group 3 infusion of Erysod decreased LDC and MDA levels to normal (after 5 h).

Hence, the dynamics of blood content of ROS in burned patients depends on the severity of injury and on the development of burn disease. Severe thermal injury leads to a drastic increase in ROS content, first in arterial and then, after opening of the microcirculatory bed, in venous blood and to intensification of LPO processes. Second wave of ROS and MDA predicts severe complications of burn disease. Blood level of ROS did not increase in patients with irreversible course of burn shock resistant to therapy.

Addition of Erysod to infusion therapy during the early period after injury suppressed generation of free radicals, promoted normalization of their level, and decreased visceral injuries during the acute period of thermal injury.

REFERENCES

1. V. G. Gavrilov, A. R. Gavrilova, and L. M. Mazhul', *Vopr. Med. Khim.*, **33**, 118-122 (1987).
2. E. I. L'vovskaya, *Disorders in Lipid Peroxidation Processes in Thermal Injury and Pathogenetic Validation of Treatment with Plasma Antioxidants*, Abstract of Doct. Med. Sci. Dissertation, Moscow (1998).
3. E. B. Men'shikova and N. K. Zenkov, *Uspekhi Sovrem. Biol.*, **113**, 442-455 (1993).
4. S. A. Mirzoyan, S. L. Mkrtchyan, and A. A. Manukyan, *Eksper. Klin. Farmakol.*, **56**, 18-19 (1993).
5. N. V. Pasechka, *Morphogenesis of Destructive and Regenerative Processes in the Small Intestinal Mucosa in Severe Burns and Use of Antioxidants*, Abstract of Cand. Med. Sci. Dissertation, Kiev (1988).
6. G. Bekyarova, I. Kazarev, and T. Yankova, *Acta Physiol. Pharmacol. Bulg.*, **15**, 68-73 (1989).
7. J. W. Horton, K. P. Burton, and D. J. White, *Trauma*, **39**, 563-569 (1995).
8. D. Saitoh, T. Kadota, and A. Senoh, *Am. J. Emerg. Med.*, **11**, 355-359 (1993).
9. K. Sugito, K. Dohi, K. Yamada, *et al.*, *Surgery*, **101**, 746-752 (1987).
10. G. Till, L. Guilds, and M. Mahrougui, *Am. J. Pathol.*, **135**, 195-202 (1989).
11. T. Toho-Oko, N. Ueno, and T. Matsumoto, *Clin. Immun. Immunopathol.*, **26**, 66-75 (1983).