Antinecrotic and Antioxidant Effects of Superoxide Dismutase during Skin Ischemia

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Antinecrotic activity of SOD was studied in rats with experimental skin ischemia. Treatment with SOD increased activity of endogenous SOD in skin homogenates (by 70 and 26% compared to the ischemic and intact skin, respectively). However, the rate of superoxide anion generation remained unchanged after SOD treatment. Creatine phosphate content and NAD/NADH redox potential increased by 16 and 21%, respectively, on day 3 after SOD administration. The increase in functional activity of the energy supply system and rise in the reserve capacity of the antioxidant protection system contribute to inhibition of lactate dehydrogenase and creatine phosphokinase and decrease in the cytolysis index under the influence of SOD. Our results indicate that SOD produces the antinecrotic effect and holds much promise for the therapy of skin ischemia.

Key Words: *superoxide dismutase; skin ischemia; necrotic changes*

The search for new drugs modulating metabolic processes in the skin and improving skin resistance to oxygen deficiency is an urgent problem in dermatology [4,5]. Impairment of energy generation in the chain reaction of mitochondrial oxidative phosphorylation accompanies ischemia of various organs and skin and is closely related to calcium transport abnormalities, acceleration of apoptosis, changes in membrane potential, and burst activation of reactive oxygen species formation [4-6]. Superoxide anion radical is generated in the mitochondrial respiratory chain. Oxidation of this anion to hydrogen peroxide is catalyzed by superoxide dismutases (SOD) [2]. Much attention is now given to the search for therapeutical methods of tissue transplantation and increase in tissue survival under ischemic conditions. Here we studied the mechanisms underlying the action of SOD, the major enzyme of the antioxidant protection system during skin ischemia.

MATERIALS AND METHODS

Antinecrotic activity of SOD was studied on male outbred rats weighing 170-220 g. The animals were maintained in a vivarium under standard conditions. After 7-day acclimatization, the rats were randomized into 3 groups (7 rats per group): intact animals, group 1; control animals, group 2; treated animals, group 3. Skin ischemia was modeled in rats of groups 2 and 3. A skin fold on the back (length 4.5-5.0 cm, height 1 cm) was sutured with a silk thread. Group 3 rats received intraperitoneal injection of SOD (0.02 mg/kg) 1 h before skin ischemia modeling. Group 2 rats received an equivalent volume of physiological saline.

Modeling of skin ischemia, isolation of skin homogenates, and measurement of the contents of

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adenyl and pyridine nucleotides, creatine phosphate, cytochrome *c*, lactate, pyruvate, and malonic dialdehyde (MDA), activities of catalase and SOD, concentration of ubiquinone, and enzyme activities of the succinate-ubiquinone reductase and NADH-ubiquinone reductase system were performed as described elsewhere [1]. Creatine phosphokinase (CPK) activity was estimated using LaRoche kit. Activity of glutathione peroxidase, rate of superoxide anion generation [3], and contents of alanine transaminase (ALT) and aspartate transaminase (AST) were measured with Bio-La-Test kit (Lachema). Lactate dehydrogenase (LDH) activity was determined using Diakhim test system.

Statistical treatment involved Student's *t* test for small samples of dependent and independent groups (STAT-Soft software). The differences were significant at $p<0.05$.

RESULTS

Blood activities of CPK and LDH in group 3 rats decreased by 38 and 19%, respectively. Activities of ALT and ASP in these animals decreased by 10 and 11%, respectively (Table 1). Exogenous SOD decreased the cytolysis index by 38%. These data suggest that SOD not only prevents necrotic processes during skin ischemia, but also reduces the damaging effect of endotoxins on the liver and heart under conditions of skin necrosis.

Treatment with SOD was followed by a significant increase in activity of endogenous SOD in skin homogenates (by 70 and 26% compared to the ischemic and intact skin, respectively; Table 2). Despite the increase in endogenous SOD activity, the rate of superoxide anion generation did not decrease after SOD administration. The rate of O_2^- generation in treated rats did not differ from that in control animals. It should be emphasized that the intensity of lipid peroxidation decreased under these conditions. MDA concentration decreased by 11% and did not differ from the normal. Administration of SOD was followed by an increase in glutathione peroxidase activity. Our results indicate that SOD increases the reserve capacity of antioxidant protection enzymes during skin ischemia.

Creatine phosphate content increased by 16% on day 3 after SOD administration. The content of adenyl nucleotides, concentration of cytochrome *c* (major enzyme of the mitochondrial respiratory chain), and activity of succinate dehydrogenase remained unchanged under these conditions (Table 3). SOD had a stronger effect on the pool of pyridine nucleotides. NAD concentration, NAD/

TABLE 1. Antinecrotic Effect of SOD during Skin Ischemia (*M*±*m*)

Parameter	Intact group	Ischemia	
		control group	treated group
CPK, IU/ml	7.7±0.4	29.9±2.2***	18.4±0.8***++
ALT, μmol	1.96±0.10	2.96±0.19**	2.69±0.11**+
AST, μmol	1.19±0.05	1.70±0.04**	1.52±0.07**+
Cytolysis index, CPK/AST	6.6±0.4	16.3±2.6***	12.8±1.5**+
LDH, mmol NAD/mg protein/min	0.19±0.01	0.32±0.02***	0.26±0.02**+

Note. Here and in Tables 2 and 3: *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared to normal; +$p<0.05$, ++$p<0.01$, +$p<0.001$ compared to the control.

TABLE 2. Effect of SOD on the Antioxidant Protection System in Ischemic Skin (*M*±*m*)

Parameter	Intact group	Ischemia	
		control group	treated group
Rate of O_2^- generation, μmol/mg protein/min	33.0±5.0	64.0±6.0	66.0±6.0
SOD, U/mg protein/min	0.27±0.01	0.20±0.01*	0.34±0.02*+
Glutathione peroxidase, nmol NADP/mg protein	2.4±0.1	2.5±0.1*	3.1±0.2**++
Catalase, nmol H_2O_2/mg protein/min	71.0±2.0	64.0±4.0	72.0±4.0*+
MDA, μmol/mg protein	0.87±0.01	0.94±0.03	0.84±0.02

TABLE 3. Effect of SOD on the Energy Supply System during Skin Ischemia (*M*±*m*)

Parameter	Intact group	Ischemia	
		control group	treated group
Cytochrome *c*, μmol/mg	0.99±0.04	0.92±0.01	0.92±0.01
ATP, μmol/g wet tissue	3.51±0.04	2.54±0.10**	2.71±0.11*
ADP, μmol/g wet tissue	1.44±0.03	1.64±0.03**	1.60±0.02**
AMP, μmol/g wet tissue	0.53±0.04	0.65±0.04*	0.69±0.04*
CP, μmol/g	4.2±0.1	3.1±0.2**	3.6±0.2*+
NAD, μmol/g	2.75±0.05	2.0±0.1**	2.31±0.06*+
NADH, μmol/g	2.72±0.06	2.75±0.10	2.60±0.08
NAD/NADH	0.99±0.02	0.73±0.05*	0.88±0.02*+
Coenzyme Q_{10}, μg/g wet tissue	1612±68	927±54***	1463±67+
Lactate, μg/g wet tissue	351.0±24.0	518.0±21.0**	437.0±22.0*+
Pyruvate, μg/g wet tissue	5.4±0.3	6.7±0.6*	6.3±0.4*
Lactate/pyruvate	66.0±6.0	82.0±9.0	75.0±6.0
SDH, μg formazan/mg protein/min	5.2±0.2	4.7±0.1*	4.7±0.1*
LDH, nmol NAD/mg protein/min	0.19±0.01	0.32±0.02***	0.26±0.02*+

Note. CP, creatine phosphate.

NADH redox potential, and coenzyme Q_{10} content increased by 15.5, 21, and 58%, respectively. SOD administration was followed by a 16% decrease in lactate concentration, which became high after skin ischemia. However, SOD had little effect on pyruvate concentration. Hence, the lactate/pyruvate ratio decreased to a normal level.

Our findings suggest that the antinecrotic effect of SOD is realized via an increase in the reserve capacity of the antioxidant protection system. SOD holds much promise as an antioxidant agent under conditions of ischemic and hypoxic damage to the skin.

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