Effectiveness of Combined Treatment with Superoxide Dismutase and Reamberin during Skin Ischemia

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Experimental skin ischemia in rats was induced by suturing a skin fold on the back with a silk thread. Combined pretreatment with superoxide dismutase (intraperitoneally) and Reamberin (intravenously) in doses of 0.01 and 6.25 mg/kg (by succinate concentration), respectively, produced a strong protective effect on the skin. The index of cytolysis decreased by 39%. The more pronounced antinecrotic effect of combined treatment with superoxide dismutase and Reamberin compared to the effect of Reamberin alone was related to a sharp increase in the reserve capacity of the antioxidant system. After combined therapy, activity of antioxidant defense enzymes not only increased, but even exceeded the normal level. The increase in activity of endogenous superoxide dismutase under the influence of combined therapy was accompanied by suppression of superoxide anion production.

Key Words: *superoxide dismutase; Reamberin; skin ischemia; antinecrotic effect*

Much evidence exists that succinate produces a antihypoxic effect [2,3]. Succinate serves as an energy substrate under conditions of energy deficiency. The succinate-containing drug Reamberin exhibits antioxidant properties, which potentiates the antihypoxic effect of succinate. Antioxidant activity of succinate is similar to that of a synthetic antioxidant ionol [4]. Succinic acid is not the product of glucose oxidation. Activation of succinate dehydrogenase (SDH) and increase in oxidation of succinic acid contribute to stimulation of the reserve pathway for energy production under ischemic conditions. Activation of the reserve pathway for energy production prevents the increase in the degree of acidosis, which is related to a decrease in the concentration of end products of glycolysis. These changes contribute to removal of reducing equivalents from the cytoplasm via the mitochondrial respiratory chain and normalization of calcium exchange in mitochondria [3-5,7]. Superoxide dismutase (SOD) is a major enzyme for neutralization of superoxide anion formed in the mitochondrial respiratory chain. Hence, SOD activation could increase the reserve capacity of the antioxidant defense system.

Here we studied the effectiveness of combined treatment with Reamberin and SOD during skin ischemia.

MATERIALS AND METHODS

Experiments were performed on 32 male rats (body weight 170-220 g). The animals were maintained

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in a vivarium under standard conditions. The rats were randomized into 4 groups (8 animals per group): intact animals (group 1), controls (group 2), and experimental groups 3 and 4. A skin fold on the back (length 4.5-5.0 cm, height 1 cm) was sutured with a silk thread to cause ischemia. Reamberin in a dose of 25 mg/kg was injected intravenously to group 3 rats 10 min before ischemia modeling. Group 4 rats received SOD (0.01 mg/kg intraperitoneally, 1 h before ischemia) in combination with Reamberin (6.25 mg/kg intravenously, 10 min before ischemia). Skin ischemia was produced as described elsewhere [1]. We measured the concentration of coenzyme Q_{10}, enzyme activity of SDH and succinate-ubiquinone reductase and NADH-ubiquinone reductase systems, content of ubiquinone, and activities of creatine phosphokinase (CPK) and antioxidant defense enzymes [1]. Activities of alanine transaminase (ALT) and aspartate transaminase (AST) were estimated with Bio-La-Test kits (Lachema). Lactate dehydrogenase activity was measured using a Diakhim test system. The rate of superoxide anion generation was measured [6].

The results were analyzed by Student's *t* test (STAT-Soft software). The differences were significant at $p<0.05$.

TABLE 1. Antinecrotic Effect of Combined Treatment with SOD and Reamberin during Skin Ischemia (*M*±*m*)

Parameter	Intact (group 1)	Ischemia		
		control (group 2)	Reamberin (group 3)	Reamberin and SOD (group 4)
CPK, IU/ml	8.0±0.6	32.0±3.9***	21.6±2.0***++	17.5±1.3**+++x
ALT, μmol/ml	1.95±0.10	3.0±0.2**	2.88±0.08*	2.60±0.04**++x
AST, μmol/ml	1.25±0.03	1.72±0.05*	1.40±0.06*+	1.44±0.06*+x
Cytolysis index, CPK/AST	6.6±0.4	18.4±1.6***	15.3±1.0**+	11.3±1.1**+++x
Lactate dehydrogenase, mmol NAD/mg protein/min	0.16±0.01	0.29±0.02**	0.24±0.02**+	0.20±0.01*++x
ATP, μmol/g wet tissue	3.65±0.06	2.57±0.07**	2.90±0.07**+	3.20±0.03**+++x
ADP, μmol/g wet tissue	1.24±0.07	1.61±0.09*	1.40±0.04+	1.54±0.05*x
AMP, μmol/g wet tissue	0.53±0.05	0.69±0.05*	0.61±0.04*	0.63±0.03*
Creatine phosphate, μmol/g	4.2±0.2	3.0±0.1**	3.4±0.1*+	3.9±0.2*+x
Lactate, μg/g wet tissue	359.0±7.0	519.0±36.0***	718.0±32.0**++	437.0±22.0**+xx
Pyruvate, μg/g wet tissue	5.5±0.2	6.8±0.1**	7.7±0.3**+	6.7±0.3**
Lactate/pyruvate	66.0±5.0	80.0±8.0*	94.0±5.0*	75.0±6.0*x
SDH, μg formazan/mg protein/min	6.95±0.36	5.8±0.2*	6.75±0.22*x	6.2±0.2*+x
NADH-ubiquinone reductase, μmol/mg protein/min	20.9±1.4	17.5±0.9*	18.0±1.0*	19.0±1.0
Succinate-ubiquinone reductase, μmol/mg protein/min	1.22±0.08	0.80±0.05**	0.95±0.06*+	1.05±0.06*++

Note. Here and in Table 2: *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared to intact animals; +$p<0.05$, ++$p<0.01$, +++$p<0.001$ compared to the control; x$p<0.05$ and xx$p<0.01$ compared to the Reamberin group.

TABLE 2. Effect of Combined Treatment with SOD and Reamberin on the Antioxidant Defense System in Ischemic Skin (*M*±*m*)

Parameter	Intact (group 1)	Ischemia		
		control (group 2)	Reamberin (group 3)	Reamberin and SOD (group 4)
SOD, U/mg protein/min	0.25±0.01	0.22±0.01*	0.26±0.01+	0.36±0.02*++x
Rate of O_2 generation, μmol/mg protein/min	0.26±0.01	0.65±0.01***	0.42±0.03*+++	0.19±0.01*
Glutathione peroxidase, nmol NADP/mg protein	2.4±0.1	2.7±0.1*	3.2±0.3**+	3.8±0.2**++x
Catalase, nM H_2O_2/mg protein/min	71.0±2.0	68.0±4.0	79.0±4.0*+	80.0±4.0*+
MDA, μmol/mg protein	0.88±0.01	0.95±0.03	0.88±0.02	0.86±0.02+x

RESULTS

ATP content in the ischemic skin decreased by 30% on day 3. These changes were accompanied by a 30% increase in ADP concentration. AMP content remained unchanged under these conditions. The ATP/ADP ratio decreased by 44% (Table 1). Creatine phosphate content decreased by 28.5%. Monotherapy with Reamberin increased ATP content (by 9%) and decreasd ADP concentration (by 13%), which reflected a shift toward ATP production. The ATP/ADP ratio increased by 30%. Combined treatment with Reamberin and SOD (group 4) resulted in a more significant increase in the concentration of adenyl nucleotides. The contents of ATP and creatine phosphate increased by 24.5 and 30%, respectively. These changes were accompanied by a 15% decrease in lactate concentration. The lactate/pyruvate ratio decreased and did not differ from the normal. The increase in SDH activity after combined treatment with Reamberin and SOD was less significant compared to that observed in group 3 animals receiving Reamberin monotherapy. No differences were revealed in activities of succinate-ubiquinone reductase and NADH-ubiquinone reductase during treatment with Reamberin alone and in combination with SOD (Table 1).

Functional improvement of the energy supply system in the ischemic skin underlies the suppression of necrotic processes. As compared to group 3 (Reamberin monotherapy) and group 2 (control), combined treatment with SOD and Reamberin (group 4) decreased activities of CPK (by 19 and 45%, respectively), ALT (by 9.7 and 13.3%, respectively), and lactate dehydrogenase (by 16.7 and 31%, respectively); cytolysis index decreased by 26 and 39%, respectively (Table 1). The more pronounced antinecrotic effect of SOD and Reamberin is probably associated with a sharp increase in the reserve capacity of the antioxidant defense system. These changes were not observed during Reamberin monotherapy (Table 2). After combined therapy activity of antioxidant defense enzymes not only increased (as observed during Reamberin monotherapy), but even exceeded the normal level. Activities of SOD, glutathione peroxidase, and catalase exceeded the normal level by 44, 58, and 13%, respectively. The increase in endogenous SOD activity under the influence of combined therapy was accompanied by a decrease in superoxide anion production by 15 and 55% compared to Reamberin-treated rats (group 3) and control animals (group 2), respectively (Table 2). SOD and Reamberin (group 4) increased activity of protective enzymes, which contributed to the maintenance of lipid peroxidation at a safe level. Under these conditions malonic dialdehyde (MDA) concentration decreases to normal.

Our results are important to develop new methods for rational therapy of hypoxic and ischemic skin injury.

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