Protective Effect of Reamberin on Functional Activity of Mitochondria during Skin Ischemia

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Reamberin in a dose of 25 mg/kg (succinate concentration) was injected intravenously for 3 days starting from the 1st hour after skin ischemia modeling. This treatment decreased activities of lactate dehydrogenase, aspartate transaminase, and creatine phosphokinase in skin homogenates by 1.6 times, 19%, and 51.3%, respectively. The index of cytolysis decreased by 18%. Reamberin had an energotropic effect, which manifested in an increase in the total ATP content and concentration of creatine phosphate (by 16 and 10%, respectively). After administration of Reamberin, activity of the succinate-ubiquinone reductase system increased by 17%. Under these conditions succinate dehydrogenase activity exceeded the normal by 21%. Reamberin had no effect on the mitochondrial NADH-ubiquinone reductase system in dermal cells during skin ischemia. Superoxide dismutase activity in the area of necrosis increased to the control level on day 3 of treatment with Reamberin. Activities of catalase and glutathione peroxidase increased by 13 and 19%, respectively. Our results indicate that the course of intravenous treatment with Reamberin for 3 days contributes to an increase in reserve capacities of the antioxidant protection system and produces a protective effect during skin ischemia.

Key Words: *skin ischemia; mitochondria; energotropic effect; Reamberin; antioxidant protection system*

Ischemia and hypoxia of the skin are accompanied by functional and metabolic disorders mediated by a decrease in the concentrations of macroergic compounds, ATP, and creatine phosphate (CP) [1]. Functional changes in the mitochondrial respiratory chain are initiated at the substrate level. The intensity of NADH-dependent oxidation initially increases, but then decreases [5]. These changes result in reduction of electron transport in the NADH-ubiquinone system and inhibition of oxidative phosphorylation. Despite the impairment of NAD-dependent oxidation in the early state of ischemia, the concentration of intracellular macroergic compounds is maintained at a normal level due to activation of compensatory metabolic pathways; an important role is played by oxidation catalyzed by superoxide dismutase (SOD). Progressive hypoxia is accompanied by blockade of a terminal cytochrome oxidase complex in the mitochondrial respiratory chain [2,5].

Here we studied whether complex substrate antihypoxant Reamberin (generation IV infusion drug for intensive care and resuscitation) possessing antioxidant activity [3] can maintain reserve capacities of the mitochondrial system during skin ischemia.

MATERIALS AND METHODS

Experiments were performed on 24 male albino rats weighing 165-210 g. The animals were maintained in a vivarium under standard conditions and fed a standard diet. The study was performed in compliance with

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the Declaration of Helsinki on the welfare of animals used for research. After quarantine, the rats were randomly divided into 3 groups of 8 specimens each. Intact animals received intravenously 0.64-0.81 ml physiological saline, which depended on body weight and volume of Reamberin solution containing the specified dose of succinate. A 3-day course of injections was performed after removal of hair on the back under hexenal anesthesia. Control rats with skin ischemia received intravenous injections of physiological saline in an equivalent volume 1 h and over 3 days after surgery. Reamberin in a dose of 25 mg/kg (succinate concentration in the corresponding control volume of solution) was injected intravenously to animals of the main group 1 h and over 3 days after the incidence of skin ischemia. Modeling of skin ischemia and measurement of the contents of adenyl and pyridine nucleotides, CP, cytochrome *c*, lactate, pyruvate, malonic dialdehyde (MDA), enzyme activity of the succinate-ubiquinone reductase and NADH-ubiquinone reductase system, activities of antioxidant protection enzymes catalase, glutathione peroxidase, and SOD, and activities of creatine phosphokinase (CPK), lactate dehydrogenase (LDH), alanine transaminase (ALT), and aspartate transaminase (AST) were performed as described elsewhere [1].

Statistical treatment of data involved the methods for small and independent samples (STAT-Soft software). The significance of differences was estimated by means of Student's *t* test.

RESULTS

LDH activity in skin homogenates increased by 1.6 times on day 3 after the incidence of skin ischemia. These changes were accompanied by an increase in plasma ALT and ASP activities by 71 and 38%, respectively (Table 1). CPK activity and index of cytolysis (CPK/AST ratio) increased by 4 and 3 times, respectively, which reflect the development and progression of destructive changes in the area of skin ischemia (Table 1). The course of Reamberin treatment started 2 h after the incidence of ischemia. Reamberin decreased activities of AST and CPK. On day 3 enzymes activities were 19.0 and 51.3%, respectively. In this period the index of cytolysis decreased by 18%.

The energotropic effect was observed in the early period of treatment with Reamberin. The total concentration of ATP increased by 16%, while ADP content decreased to normal (Table 1). Reamberin significantly increased the ATP/ADP ratio, which reflects changes in regulatory processes toward ATP synthesis. CP content decreased by 20% on day 3 after the incidence of skin ischemia, but increased by 10% after administration of Reamberin. The model of skin ischemia used in our experiment (moderate ischemia) is characterized by an increase in the concentrations of lactate and pyruvate by 70 and 29%, respectively, compared to normal. Therefore, we did not found a significant increase in the lactate/pyruvate ratio. Lactoacidosis did not develop under these conditions. These data show that Reamberin had no effect on the contents of lactate and pyruvate and lactate/pyruvate ratio under conditions of experimental skin ischemia (Table 1). NAD concentration decreased by 24%, while NADH content remained unchanged. The NAD/NADH ratio and redox potential of the energy supply system decreased by 17%. The contents of oxidized and reduced NAD and redox potential of the energy supply system remained practically unchanged after the course of Reamberin treatment.

The energotropic effect of Reamberin was manifested in an increase in reserve capacities of the succinate-dependent pathway for electron transport in the mitochondrial respiratory chain. Activity of the succinate-ubiquinone reductase system in mitochondria decreased by 32% during skin ischemia, but increased by 17% after administration of Reamberin. Under these conditions succinate dehydrogenase (SDH) activity exceeded the normal by 21%. It can be hypothesized that SDH and succinate ubiquinone reductase serve as the targets for Reamberin. Activation of succinate-dependent oxidation under the influence of Reamberin is probably a key pathogenetic compensatory mechanism for the maintenance of mitochondrial function during skin ischemia. Reamberin had no effect on the mitochondrial NADH-ubiquinone reductase system in dermal cells during skin ischemia.

Destructive changes in mitochondria during skin ischemia induce overproduction of reactive oxygen species, which results in impairment of detoxification function of the skin, increase in the severity of endotoxemia, formation of secondary damage to membranes, and progression of postischemic edema [5]. The physiological system for antioxidant protection of the skin is a well-organized multilevel protective mechanism, which protects the cell from oxidative stress. The regulatory mechanism to maintain equilibrium between antioxidant protection enzymes is impaired during skin ischemia. SOD activity did not increase (compensatory reaction to oxidative stress), but even decreased by 14% in the ischemic area. Catalase activity was maintained at a pseudonormal level. Only glutathione peroxidase activity increased by 13%. SOD activity in the ischemic area increased to a normal level after 3-day treatment with Reamberin. Catalase activity exceeded the level observed not only during skin ischemia, but also under normal conditions (by 13 and 11%, respectively). Glutathione peroxidase activity increased by 19 and 42%, respectively (Table

TABLE 1. Antinecrotic and Energotropic Effects of Reamberin during Skin Ischemia

Parameter	Intact skin (normal)	Skin ischemia	
		control	Reamberin
CPK, U/ml	8.6±0.9	32.7±3.9***	21.6±2.0*+
ALT, mmol/ml	1.75±0.09	3.0±0.2**	2.88±0.08*
AST, mmol/ml	1.25±0.03	1.72±0.09*	1.40±0.06*+
Index of cytolysis, CPK/AST	6.43±0.78	18.4±1.6***	15.3±1.0**+
LDH, mmol NAD/mg protein/min	0.17±0.01	0.27±0.02**	0.240±0.002*+
ATP, μmol/g wet tissue wet	3.70±0.15	2.58±0.09*	2.90±0.07**+
ADP, μmol/g wet tissue wet	1.24±0.07	1.61±0.09*	1.40±0.04+
AMP, μmol/g wet tissue wet	0.53±0.05	0.69±0.05*	0.61±0.04*
ATP/ADP	4.00±0.04	3.39±0.13	3.67±0.05
CP, μmol/g	4.1±0.2	3.2±0.1	3.35±0.18
NAD, μmol/g wet tissue wet	2.9±0.1	2.2±0.1**	2.2±0.1*
NADH, μmol/g wet tissue wet	3.0±0.1	3.2±0.1*	3.0±0.1*
NAD/NADH	0.98±0.04	0.81±0.07*	0.74±0.06*
NAD+NADH, μmol/g wet tissue wet	5.9±0.1	5.6±0.1*	5.2±0.1*+
Lactate, μg/g wet tissue wet	413±7	704±36***	718±32**
Pyruvate, μg/g wet tissue wet	5.8±0.2	7.5±0.2**	7.7±0.3**
Lactate/pyruvate	72±2	98±5*	94±5*
SDH, μg formazan/mg protein/min	6.95±0.36	5.8±0.2*	6.75±0.22*+
Cytochrome *c*, nmol/g wet tissue wet	0.93±0.03	0.91±0.02	0.91±0.03
NADH-ubiquinone reductase, μmol/mg protein/min	20.9±0.8	17.0±1.3*	16.4±0.8**
Succinate-ubiquinone reductase, μmol/mg protein/min	1.19±0.03	0.81±0.04**	0.95±0.06*+

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

TABLE 2. Effect of Reamberin on the Antioxidant Protection System and LPO in Ischemic Skin

Parameter	Intact skin (normal)	Skin ischemia	
		control	Reamberin
SOD, U/mg protein/min	0.25±0.01	0.22±0.01*	0.26±0.01+
Glutathione peroxidase, nmol NADP/mg protein	2.4±0.1	2.7±0.1*	3.2±0.3*++
Catalase, nmol H_2O_2/mg protein/min	71±2	68±4	79±4*+
MDA, μmol/mg protein	0.88±0.01	0.91±0.03	0.88

Note. ++$p<0.01$ compared to the control.

2). Our results seem to support the hypothesis that recovery of the glutathione system plays a major role in detoxification and suppression of lipid peroxidation (LPO) in phospholipid structures of biological membranes [4], prevention of destructive changes in the skin, and inhibition of cytolysis.

Our findings show that 3-day treatment with Reamberin corrects the alternative pathway of succinate oxidase-catalyzed oxidation. This effect is mediated by the increase in SDH activity, improvement of influx of exogenous and endogenous succinate in mitochondria, and activation of succinate-ubiquinone reductase. These changes improve reserve capacities of the antioxidant protection system, which counteracts LPO activation in the skin (constant level of MDA). Therefore, Reamberin produces energotropic, antioxidant, and protective effects during skin ischemia.

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