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Antihypoxic and Antinecrotic Effect of Mexidol in Skin Ischemia

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Course treatment with mexidol in a dose of 25 mg/kg for 3 days decreased activities of aspartate transaminase and creatine phosphokinase in the plasma on day 3 after the incidence of skin ischemia (by 1.3 and 1.66 times, respectively). Under these conditions the index of cytolysis decreased by 1.3 times. Therefore, mexidol prevented progression of necrotic processes in the skin. Mexidol therapy of animals with skin ischemia restored the reserve capacity of systems for energy supply and antioxidant defense. The systems of NADH-ubiquinone reductase and succinate-ubiquinone reductase served as the targets for the action of mexidol. Mexidol significantly decreased the damaging effect of reactive oxygen species. Dermatoprotective properties of mexidol were associated with its influence on the energy supply system (regulation of enzyme activity in the electron transport chain, ubiquinone metabolism) and antioxidant defense.

Key Words: *skin ischemia; mexidol; energy supply system; antioxidant defense system*

The development of ischemic disturbances in energy metabolism constitutes the major pathogenetic stage of skin ischemia. Adaptive increase in the intensity of glycolysis and reserve capacity of the antioxidant defense system in the ischemic tissue plays an important role in the development of metabolic disorders during oxygen deficiency [2]. Pharmacological preparations producing direct antioxidant and antiischemic effects are of particular interest in this respect. Here we studied the therapeutic effectiveness and intracellular targets for the action of mexidol during skin ischemia. The presence of 3-hydroxypyridine derivatives in mexidol determines its direct interaction with lipophilic and water-soluble radicals of phospholipid membranes in the site of radical generation [5].

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MATERIALS AND METHODS

Experiments were performed on male albino rats weighing 170-190 g. The animals were kept in a vivarium under standard conditions. The study was performed according to the Helsinki Regulation for Experiments on Laboratory Animals. The rats were adapted for 4 days and then randomly divided into 3 groups (7 rats per group). Group 1 rats (control) subcutaneously received 0.2 ml physiological saline for 3 days after removal of hair on the back under hexenal anesthesia. In group 2 animals (intact rats) mild ischemia of the skin was modeled and physiological saline in a dose of 0.2 ml was injected subcutaneously 1 h after surgery. These rats received injections for 3 days. Group 3 animals received mexidol in a dose of 25 mg/kg 1 h after modeling of skin ischemia. The preparation was injected into the anterior abdominal wall for 3 days. The rats received intraperitoneal injection of hexenal

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in a dose of 60 mg/kg and were fixed on a surgical table (by the limbs and tail). The skin on the upper third of the back was treated with 70% ethyl alcohol. Ischemia was produced by suturing the skin fold on the back (length 4.5-5.0 cm, height 1.0 cm) with a silk thread. The base of the fold was sutured with a single interrupted suture (no spaces between stitches). The material for biochemical study was taken from the standard area (top middle point of the fold) on day 4.

Preparation of cell-free homogenates from the skin and measurement of the contents of adenyl and pyridine nucleotides, creatine phosphate (CP), cytochrome *c*, lactate, pyruvate, and malonic dialdehyde (MDA), glycolytic activity, and activities of superoxide dismutase (SOD) and catalase were performed as described elsewhere [1,4]. Creatine phosphokinase (CPK) activity was estimated using LaRoche kit. Glutathione peroxidase activity was measured as described previously [7]. Lactate dehydrogenase (LDG) activity was determined in the reaction with 2,4-dinitrophenylhydrazine using Diakhim test systems. Activities of alanine transaminase (ALT) and aspartate transaminase (AST) were estimated using Bio-La-Test kits (Lachema). The index of cytolysis was calculated as the CPK/AST ratio. Enzyme activity of the succinate-ubiquinone reductase and NADH-ubiquinone reductase system was measured without exogenous ubiquinone (A_1) and after *in vitro* administration of this compound (A_2). The increase in activity after ubiquinone administration reflects a deficiency of endogenous ubiquinone in enzyme systems and characterizes binding of the enzyme to ubiquinone [8]. Ubiquinone deficiency in tissues and coefficient of activation of the ubiquinone-dependent enzyme system were calculated as follows:

$$\text{enzyme activation coefficient}=(A_2-A_1)/A_1\times 100\% \text{ and}$$
$$\text{ubiquinone deficiency}=(A_2-A_1)/A_2\times 100\%,$$

where A_1 is activity of the succinate-ubiquinone reductase or NADH-ubiquinone reductase system without exogenous ubiquinone; and A_2 is activity of the enzyme system after administration of ubiquinone into the reaction mixture. Enzyme activity was expressed in μmol per 1 mg mitochondrial protein over 1 min. Ubiquinone content in liver mitochondria was measured as described elsewhere [6].

The data were processed using teats for small and independent samples. The results were analyzed by Student's *t* test.

RESULTS

Plasma activities of ALT and AST in control animals increased by 50 and 36%, respectively, on day 3 after modeling skin ischemia. In this period CPK activity increased by more than 3 times (Table 1). LDG activity in the skin increased by 1.5 times (Table 2). These changes are typical of persistent necrotic processes on day 3 of the postischemic period. The course treatment with 25 mg/kg mexidol beginning from the 2nd hour of ischemia produced a general therapeutic and antinecrotic effect. On day 3 activities of AST and CPK and index of cytolysis decreased by 1.3, 1.66, and 1.3 times, respectively. These results indicate that mexidol prevented progression of necrotic processes in the skin.

Cytochrome *c* content in the mitochondrial respiratory chain remained practically unchanged during skin ischemia (Table 2). NAD content decreased by 24%, while NADH concentration remained unchanged. Table 2 shows that the NAD/NADH ratio (redox potential of the energy supply system) decreased by 1.36 times. The decrease in the redox potential of the energy supply system was followed by impairment of adenyl nucleotide synthesis. We revealed a decrease in the amount of ATP by 26%. The content of degradation products ADP and AMP increased by 14 and 15%, respectively. The ATP/ADP and ADP/AMP ratios were shifted toward an increase in the concentration of degradation products. The content of CP decreased by 15%. We observed a significant decrease in glycolytic activity judging from lactate content (by 11%). Pyruvate content increased despite the increase in lactate concentration (by 23%). It was followed by a change in the lactate/pyruvate ratio. The compensa-

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

Parameter	Intact rats	Ischemia	
		control	mexidol
CPK, IU/ml	8.3±1.1	29.9±2.2	18.4±0.8
ALT, μmol/ml	1.99±0.07	2.99±0.13*	2.93±0.13*
AST, μmol/ml	1.25±0.03	1.70±0.04*	1.33±0.03+
Cytolytic index, CPK/AST	5.93±0.15	15.1±2.6*	11.5±1.9*+

Note. Here and in Table 2: $p<0.05$: *compared to intact rats; +compared to the control.

tory reaction and activation of aerobic glycolysis probably occur under ischemic conditions.

The course treatment with mexidol in a dose of 25 mg/kg significantly increased NAD concentration (by 14.3% compared to ischemia). However, NAD concentration remained below the normal level. The increase in the content of oxidized NAD after mexidol administration was not accompanied by accumulation of reduced NAD. Therefore, the redox potential of the energy supply system returned to normal (NAD/NADH, Table 2). The increase in the redox potential was followed by a shift in the energy supply system toward synthesis of ATP and CP. We observed the increase in ATP concentration increase by 32%. CP concentration increased by 8.3%, but did not reach the normal. Glycolytic activity returned to normal. Mexidol increased SDG activity by 7.5% (Table 2). Skin ischemia was accompanied by a 1.5-fold decrease in the concentration of coenzyme Q_{10}. After administration of mexidol coenzyme Q_{10} concentration significantly increased, but did not reach the normal. The reduction of ubiquinone content underlay the decrease in activity of the succinate-ubiquinone reductase system in skin mitochondria in the absence and presence of exogenous ubiquinone (by 32 and 27%, respectively). Activity of the succinate-ubiquinone reductase system completely recovered after mexidol administration. Moreover, activity of the enzyme system 1.7-fold exceeded normal under the influence of exogenous ubiquinone (Fig. 1, *a*). It can be hypothesized that the succinate-ubiquinone reductase system serves as a target for the action of mexidol. The increase in activity of this system is probably the major pathogenetic compensatory mechanism of activation of the energy supply system in the skin after mexidol administration.

The NADH-ubiquinone reductase system can be also considered as a target for the action of mexidol (Fig. 1, *b*). Treatment with mexidol completely re-

TABLE 2. Effect of Mexidol on the Energy Supply System during Skin Ischemia (*M*±*m*)

Parameter	Intact rats	Ischemia	
		control	mexidol
Cytochrome *c*, μmol/mg	0.93±0.03	0.91±0.02	0.91±0.03
ATP, μmol/g	3.50±0.14	2.52±0.29*	3.31±0.05*$^{+}$
ADP, μmol/g	1.39±0.05	1.58±0.03*	1.32±0.30^{+}
AMP, μmol/g	0.66±0.04	0.76±0.03*	0.77±0.05*
CP, μmol/g	4.00±0.04	3.39±0.13	3.67±0.05
NAD, μmol/g	2.75±0.04	2.1±0.1*$^{+}$	2.40±0.05**
NADH, μmol/g	2.74±0.05	2.69±0.12*	2.60±0.08*$^{+}$
NAD/NADH	0.99±0.02	0.73±0.07*$^{+}$	0.93±0.04*$^{+}$
Coenzyme Q_{10}, μg/g wet tissue	1607±93	1099±41	1430±72
Lactate, μg/g wet tissue	416±7	511±17*	469±10*$^{+}$
Pyruvate, μg/g wet tissue	5.7±0.4	7.6±0.4*	7.9±1.0*
Lactate/pyruvate	75±5	68±4	55±10
Glycolytic activity	2.36±0.09	2.1±0.1*	2.21±0.03
SDG, μg formazan/mg protein/min	5.4±0.1	4.9±0.1*	5.8±0.1*$^{+}$
LDG, mmol NAD/mg protein/min	0.18±0.01	0.27±0.11*	0.19±0.01^{+}

TABLE 3. Effect of Mexidol on the Antioxidant Defense System in Ischemic Skin Tissue (*M*±*m*)

Parameter	Intact rats	Ischemia	
		control	mexidol
SOD, U/mg protein/min	0.25±0.01	0.22±0.01*	0.26±0.01^{+}
Glutathione peroxidase, nmol NADPH/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±0.02

Note. *$p<0.05$ compared to intact rats; $^{+}p<0.05$ and $^{++}p<0.01$ compared to the control.

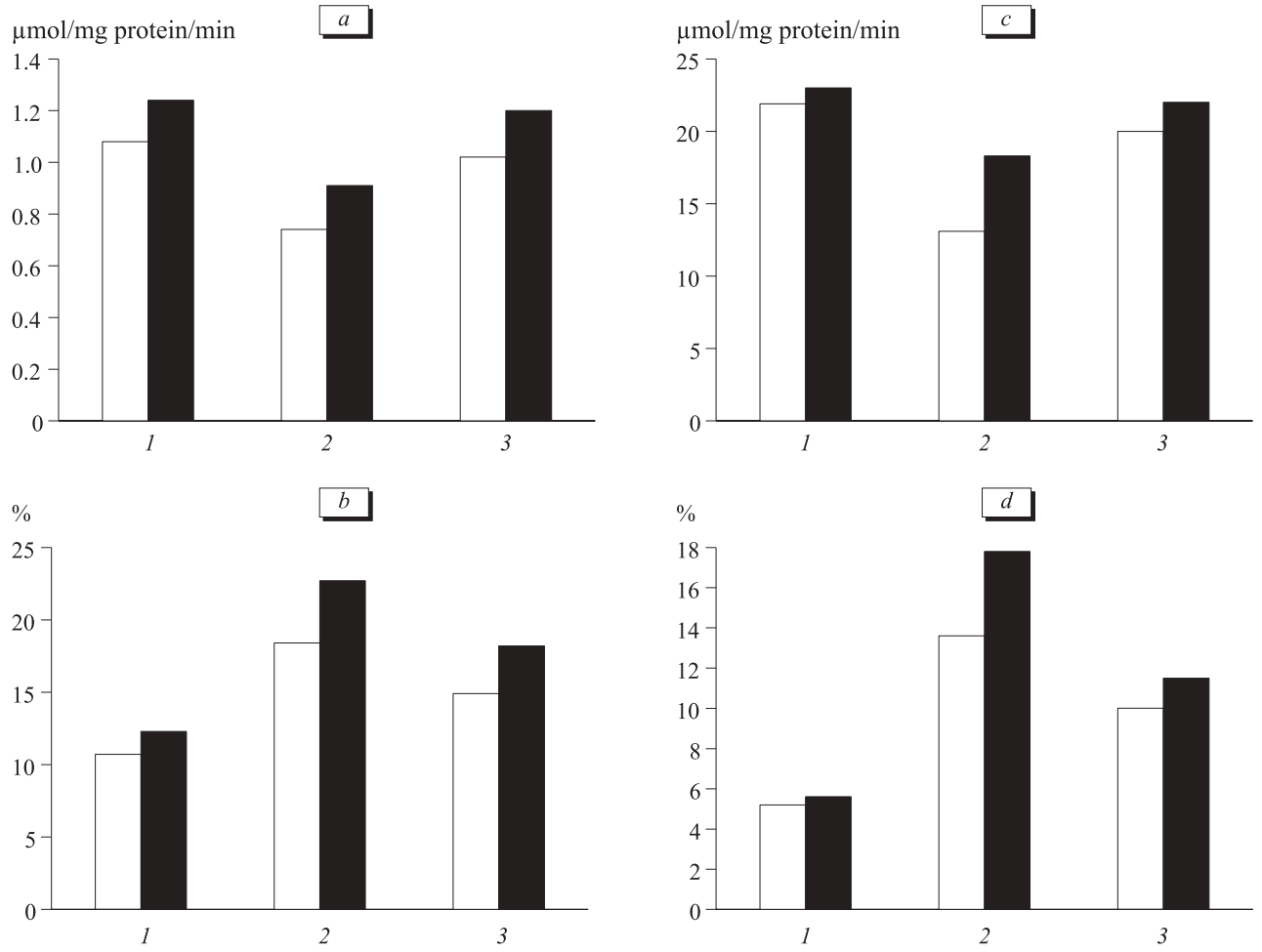

Fig. 1. Enzyme activity of the succinate-ubiquinone reductase (*a*, *b*) and NADH-ubiquinone reductase system (*c*, *d*) in mitochondria of rat ischemic skin after mexidol administration: normal (*1*), skin ischemia (*2*), skin ischemia+mexidol (25 mg/kg, *3*). Light bars: without exogenous ubiquinone. Dark bars: addition of exogenous ubiquinone. Deficiency of endogenous ubiquinone, percent (*a*, *c*). Coefficient of activation of mitochondrial systems after *in vitro* addition of ubiquinone (*b*, *d*).

stored activity of this system even in the absence of exogenous ubiquinone. After administration of ubiquinone activity of the NADH-ubiquinone reductase system surpassed normal.

Activity of the antioxidant defense system increases under the influence of stressogenic factors. This system maintains the balance between enzyme activity and free radical generation, which is required to prevent tissue injury. The mitochondrial respiratory chain is a major source of reactive oxygen species (ROS). Long-term adaptation to reduced blood flow is followed by structural disorganization of the antioxidant defense system in the skin flap. These changes manifested in the absence of a compensatory activation of SOD and catalase. We observed an increase only in activity of glutathione peroxidase (by 12.5%).

SOD is responsible for elimination of the superoxide anion. Enzyme activity in the area of necrosis increased to the control level on day 3 after treatment with mexidol. During ischemia of skin catalase activity surpassed SOD activity by 13%. Catalase activity in treated rats was 11% higher than in intact animals. Activity of glutathione peroxidase neutralizing hydrogen peroxide increased most significantly (by 19 and 50% in control and treated rats, respectively). The course treatment with mexidol in a dose of 25 mg/kg for 3 days modulated activity of the antioxidant defense system in the skin. These data reflect recovery of the balance not only in the energy supply system, but also in the antioxidant defense system. Mexidol probably neutralized ROS and relived the secondary damaging effect. It should be emphasized that we did not reveal the increase in MDA content. Our findings suggest that impairment of membrane permeability, degree of destructive peroxidation in the skin, and intensity of cytolysis were less pronounced under these conditions.

Our results indicate that dermatoprotective activity of mexidol is associated with its influence on the system for energy supply (regulation of enzyme activity in the electron transport chain, ubiquinone meta-

bolism) and antioxidant defense. Mexidol produces antihypoxic and antioxidant effect not only in the myocardium [3,5] and brain [2], but also in the skin. The pharmacological effect of mexidol during skin ischemia concerns general pathogenetic manifestations of the disorder.

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