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Effect of Ubiquinone-10 and Succinic Acid on Functional Characteristics of Erythrocytes in Rats with Epinephrine Toxemia

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Prophylactic dietary intake of synthetic ubiquinone-10, succinic acid, or mixture of these substances prevented disturbances in aggregation and electrophoretic mobility of erythrocytes and inhibited lipid peroxidation in cells of rats with experimental epinephrine-induced toxemia.

Key Words: *hypoxia; erythrocytes; ubiquinone-10; succinic acid*

Our previous studies showed that synthetic ubiquinone-10 produces strong cardioprotective and radioprotective effects in rats with experimental epinephrine-induced myocardial injury and radiation damage. It was associated with antihypoxic activity of ubiquinone-10 [5,7]. The effect of ubiquinone can be potentiated by activation of the substrate site in the respiratory chain via the alternative NADP oxidase pathway (succinate oxidase oxidation) [8,9]. Here we studied the effect of combined treatment with synthetic ubiquinone-10 and succinic acid in rats with experimental epinephrine-induced toxemia. The form of succinate was selected taking into account that permeability of blood-tissue barriers for this compound increases under conditions of hypoxia and ischemia [10]. The effectiveness of preparations was determined by changes in functional characteristics of erythrocytes, including the charge, aggregation, and lipid peroxidation (LPO). Microcirculatory disturbances (*e.g.*, impairment of blood rheological characteristics) are an obligatory stage in all pathological processes. Function of the microcirculatory bed depends on these properties of erythrocytes. Ubiquinone-10 was synthesized at the BVK factory (Kstovo). The method for ubiquinone-10 synthesis was developed at the Sintezbelok Institute (Russian Academy of Sciences). We also used commercial preparation of succinic acid.

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MATERIALS AND METHODS

Experiments were performed on female rats weighing 180-200 g. The animals were maintained in a vivarium under standard conditions. Each group included 8-10 rats. Epinephrine (0.5 ml, 0.1%) was injected intramuscularly for 3 days to cause toxemia (epinephrine-induced myocarditis) [1]. Succinic acid (0.5 g/kg), ubiquinone (10 mg/kg) in olive oil, or a mixture of succinic acid and ubiquinone-10 was administered via a gastric tube for 7 days before epinephrine treatment. Control animals received an equivalent volume of vegetable oil (0.5 ml). The blood was taken from the sublingual vein on the next day after the end of epinephrine treatment. Erythrocyte aggregation was assayed as described elsewhere [2]. Erythrocytes were washed by 3-fold

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centrifugation in 0.85% NaCl at 1000 rpm. Aggregation was stimulated with 20% dextran blue. The surface charge of erythrocytes was estimated by the method of microelectrophoresis [4]. Electrophoretic mobility of erythrocytes (EPME) was determined by incubation of 0.1% erythrocyte suspension in Tris-HCl buffer (pH 7.4) at 10 mA. LPO in erythrocytes was studied by measuring the concentration of MDA in a color reaction with thiobarbituric acid [3]. The results were analyzed by Student's *t* test.

RESULTS

Epinephrine-induced toxemia (control) was accompanied by dysfunction of erythrocytes (increased aggregation, decreased EPME, and stimulation of LPO). Pretreatment of animals with the test substances prevented these changes in erythrocytes (Table 1).

TABLE 1. Aggregation (Percentage of Aggregated Cells), EPME (m×cm/V/sec), and LPO (MDA concentration, nmol/ml) in Rat Erythrocytes after Administration of Substances ($M \pm m$)

Group	Aggregation	EPME	MDA concentration
Intact	30.0±4.7	1.58±0.16	1.4±0.5
Control (epinephrine)	54.9±4.9*	0.89±0.13*	3.0±0.6*
Succinic acid	48.2±4.2*	0.98±0.23*	1.6±0.7+
Ubiquinone	36.2±4.0+	0.92±0.24*	1.6±1.0+
Succinic acid+ uiquinone	32.0±4.1+	1.19±0.15*+	1.5±0.9+

Note. $p<0.05$: *compared to intact animals; +compared to the control.

The most important rheological characteristic of the blood (aggregation constant) remained unchanged after pretreatment with ubiquinone-10, succinic acid, or mixture of these substances. Administration of ubiquinone alone or in combination with succinic acid was most potent in this respect. Parameters of aggregation in animals with experimental epinephrine-induced toxemia practically did not differ from those in intact rats.

The increase in the negative charge of erythrocytes is one of the major mechanisms reducing their aggregation. The study of EPME showed that the test substances increase the negative charge of erythrocytes, which was low in animals with epinephrine-induced toxemia. EPME of erythrocytes from rats receiving the mixture of the test substances did not differ from that in intact animals.

The charge of erythrocytes did not differ in treated and intact specimens. It can be hypothesized that the test substances increase structural stability of the membrane, which mainly depends on the intensity of LPO. Antioxidant activity of substances was determined by measuring the concentration of MDA in erythrocytes. Epinephrine-induced toxemia was accompanied by a sharp increase in MDA concentration in erythrocytes from control rats. As differentiated from control rats, MDA concentration in the blood did not increase in rats exposed to individual or combined treatment with the test substances (Table 1).

Oxygen deficiency and energy deficiency in the cell are accompanied by progressive dysfunction of mitochondrial enzymes. Ubiquinone and succinic acid have a corrective effect on the cytochrome and substrate sites, respectively. Our results show that combined treatment with ubiquinone and succinic acid more significantly prevents hypoxic alteration of erythrocytes compared to individual administration of these substances. We believe that prophylactic dietary intake of these energy-generating components of mitochondrial complexes contributes to the formation of high energy potential of erythroblasts during the early stage of erythropoiesis. These changes are followed by migration of mature and hypoxia-resistant erythrocytes into the vascular bed. The value of EPME depends on LPO and determines aggregation. Variations in EPME are a typical response to alteration [6], which reflects the pathogenesis of various diseases. The maintenance of energy stability of erythrocytes is important to correct hypoxia of the organism.

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