Neuroprotective Activity of Mexidole and 3-Hydroxypyridine Acetylcysteinate against the Background of Cerebral Ischemia in the Complex of Experimental Diabetes Mellitus and Exogenous Hypercholesterolemia

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We studied the interaction of mexidol and 3-hydroxypyridine acetylcysteinate on the model of experimental ischemic stroke in rats. The preparations were effective in a dose 50 mg/kg (10-day treatment): they reduced the incidence of neurological disturbances (pareses, sensitivity disturbances) and improved antioxidant defense of the plasma.

Key Words: *mexidol; 3-hydroxypyridine acetylcysteinate; brain ischemia; neurological abnormalities*

Therapy of stroke is aimed at restoration of the patency of the artery (tissue activator) and prevention of thrombus formation (fibrinolytics, anticoagulants, antiaggregants) and neuronal death. To this end, either single neuroprotective preparation, or their combinations can be used. Some vasoactive preparations (vinpocetine, nicergoline, cinnarizine, *etc*.) prescribed for improvement of circulation in the ischemic tissue also exhibit cerebroprotective activity. However, steal phenomenon cannot be excluded, which manifests in reduction of the blood flow in the ischemic zone due to its activation in normal tissues [3,6].

Antioxidants are perspective drugs in the therapy in acute damage to CNS. Experimental data suggest that free radicals play an important role in the development of tissue damage. They act via different mechanisms: LPO in cell membranes, microvascular damage, edema, *etc*. [7].

Mexidol is a donor of protons [1,5]. The preparation directly modulates activity of membrane-bound enzymes SOD and catalase [4]. Mexidol is an antihypoxiant exhibiting direct energizing effect [2]; it inhibits platelet aggregation and produces a cholesterol-lowering effect (reduces the level of total cholesterol, LDL, and cholesterol/phospholipid ratio). The effects of mexidol are determined by its membrane-stabilizing and antioxidant properties, modulating effects on receptors, ionic channels, and energy metabolism. The preparation produces antiischemic, antistress, nootropic, cardioprotective, and immunomodulating effects [9].

3-Hydroxypyridine acetylcysteinate (3-HPC) is a water-soluble antioxidant, a structural analog of vitamin B_6-like compounds; it possesses antiradical and antioxidant activity. Moreover, it includes cysteine, a low-molecular-weight (thiol) antioxidant. 3-HPC reduces manifestations of cytolytic syndrome and improves protein-synthesizing function of the liver against the background of experimental diabetes mellitus.

Here we analyzed the therapeutic effects of mexidol and 3-HPC in rats with diabetes mellitus in complex with exogenous hypercholesterolemia

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complicated by ischemic stroke and evaluated pharmacological effects of these preparations.

MATERIALS AND METHODS

Experiments were carried out on outbred albino mature male and female rats (n=100) weighing 180-220 g maintained under standard vivarium conditions with free access to food and water. After preliminary 24-h food deprivation (with free access to water), the animals received single intraperitoneal injection of alloxan in a dose of 30 mg/kg for modeling diabetes mellitus [8]. Diabetes mellitus developed over 2 weeks. Then, the animals received oil suspension of cholesterol (40 mg/kg in 0.5 ml vegetable oil *per os* daily for 14 days). Vitamin D (12,500 U/kg body weight) was added to the emulsion for increasing its peroxidation status. Brain ischemia was modeled by ligation of the left common carotid artery in animals narcotized with sodium ethaminal (40 mg/kg intramuscularly). Neurological deficit and movements were recorded 24 h after surgery.

The animals were divided into 6 groups (7 rats per group). Group 1 rats (intact) were maintained on vivarium ration throughout the experiment. In group 2 rats (controls), cerebral ischemia was modeled and physiological saline was injected intramuscularly (50 mg/kg) 30 min after surgery and then daily for 10 days. Other groups comprised animals with experimental diabetes mellitus, exogenous hypercholesterolemic, and cerebral ischemia, in whom correction of these damages was performed with the test antioxidant preparations: mexidol in doses of 5 and 50 mg/kg (groups 3 and 4) and 3-HPC in the corresponding doses (groups 5 and 6).

The dynamics of disturbances induced by brain ischemia in the complex with experimental diabetes mellitus and exogenous hypercholesterolemia and the effect of mexidol and 3-HPC on rat behavior were studied for 12 days. Neurological status was analyzed by neurological deficit score (6-point scale) on day 3 of ischemia [10]; the rate of recovery of surface sensitivity was evaluated. The effect of the test drugs on LPO was studied.

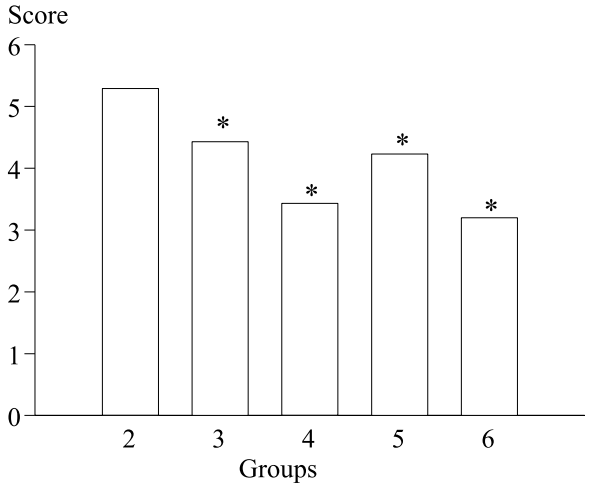

Fig. 1. Severity of neurological disturbances in rats with cerebral ischemia treated with antioxidants. *p<0.05 compared to group 2.

The results were analyzed by method of variation statistics; the arithmetic mean and error of the mean were calculated. Significance of differences was evaluated using Students' t test.

RESULTS

In rats with modeled ischemia, ptosis at the side of damage, hemiparesis, hypodynamia, pain sensitivity disturbances, extrapyramidal disorders (tremor), ataxia, and behavioral changes (suppressed or paradoxical defense reactions) were observed. In grave cases, adynamia, atonia, and absence of reflexes and sensitivity were observed. In animals with ischemic cerebral damage against the background of experimental diabetes mellitus and exogenous hypercholesterolemia, the mean neurological score was 5.29±0.18. In rats treated with mexidol in a dose of 5 mg/kg, ptosis at the side of damage was observed, neurological deficit manifested by spastic hemiparesis and pronounced gait abnormalities; the mean neurological score was 4.43±0.18.

TABLE 1. Effect of Mexidol and 3-HPC on Parameters of LPO in Blood Serum in Rats with Experimental Cerebral Ischemia (M±m)

Parameter	Group					
	1	2	3	4	5	6
MDA, μmol/liter	5.50±0.03	8.84±0.13*	4.61±0.06*	4.12±0.04*	4.58±0.04*	3.52±0.09*
Catalase, μcat/sec/liter	0.420±0.006	0.170±0.001*	0.200±0.003*	0.320±0.004*	0.230±0.005*	0.420±0.003*

Note. *p<0.001 compared to group 1.

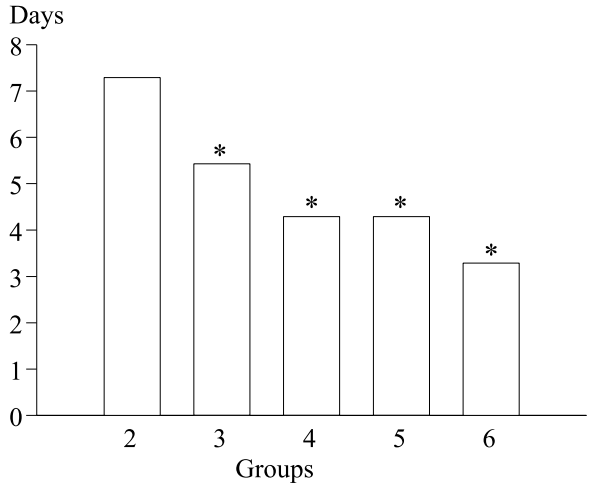

Fig. 2. Effect of mexidol and 3-HPC on the rate of recovery of surface sensitivity in rats with experimental cerebral ischemia. *p<0.0001 compared to group 2.

In animals receiving mexidol in a dose of 50 mg/kg, the mean neurological score was 3.43±0.20. Rats receiving 3-HPC in a dose of 5 mg/kg had ptosis on the side of damage, moderate hemiparesis, hypodynamia, ataxia, and inhibition of defense reactions; the mean neurological score was 4.23±0.20. In animals receiving 3-HPC in a dose of 50 mg/kg, the mean neurological score was 3.20±0.18 (Fig. 1).

In animals with ischemic cerebral damage against the background of experimental diabetes mellitus and exogenous hypercholesterolemia, surface sensitivity was restored on days 7.29±0.18. In rats receiving mexidol in doses of 5 and 50 mg/kg, surface sensitivity recovered on days 5.43±0.2 and 4.29±0.18, respectively. In animals receiving 3-HPC in doses of 5 and 50 mg/kg, this recovery was observed on days 4.29±0.18 and 3.29±0.18, respectively (Fig. 2).

In animals receiving physiological saline, plasma content of MDA increased by 160% compared to group 1; in groups 3, 4, 5, and 6, this parameter decreased by 84, 75, 83, and 64%, respectively.

Plasma catalase activity decreased by 40% in animals receiving saline (compared to group 1), by 48 and 76% in groups 3 and 4, and by 55 and 95% in groups 5 and 6 (Table 1).

By the end of the experiment, the content MDA considerably decreased and plasma catalase activity increased in all experimental groups compared to intact animals, which reflected inhibition of LPO and activation of antioxidant defense system.

Thus, our experiments demonstrated neuroprotective effects of 3-hydroxypyridine derivatives on the model of cerebral ischemia caused by occlusion of the left common cerebral artery. 3-HPC and mexidol were most effective in a dose of 50 mg/kg. The preparations considerably reduced the severity of disturbances caused by stroke, improved neurological status, and produced an anxiolytic effect. These findings substantiate the use of mexidol and 3-HPC in clinical practice in patients with ischemic stroke and explain the peculiarities of their therapeutic effects.

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