Antiischemic Activity of New Domestic Antioxidant 3-Hydroxypyridine Etoxidol Derivative

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Experiments on rats with myocardium infarction showed that intravenous administration of etoxidol in a dose of 14.2 mg/kg restricted the size of necrosis area and reduced the ratio of necrosis/ischemia zones. The test compound reduced the risk of rhythm and conduction disturbances induced by administration of toxic epinephrine doses to the mice, and increased mouse survival. Etoxidol administered intravenously to cats with acute myocardial ischemia reduced epinephrine concentration and intensity of free radical oxidation in zones of myocardial lesions against the background of the increase in norepinephrine concentration and antioxidant activity in the myocardium. By antiischemic and antioxidant activity, etoxidol was superior to its structural analogue mexidol.

Key Words: *etoxidol; antiischemic effect; ischemia and necrosis zones; catecholamines; lipid peroxidation*

The development of pharmacology in the second half of XX and beginning of XXI centuries is based upon principles of reasonable combination of clinical efficiency and safety in newly created medicinal products [4]. This ideology stimulated the development and clinical application of medicinal products of natural and synthetic origin, conditionally named "metabolic action compounds" [4,10]. These drugs are capable to regulating energy metabolism in cells, acid-base balance, and lipid peroxidation, what eventually stipulates their cytoprotective and organoprotective effects.

A representative of this drug group, 3-hydroxy-6-methyl-2-ethylpyridine succinate known under trade names mexidol and mexicor, is widely used in clinical practice [9]. At the same time, some 3-hydroxy pyridine derivatives are potentially not inferior to known drugs by pharmacological activity, *e.g.* 3-hydroxy-6-methyl-2-ethylpyridine malate, a new domestic antioxidant (patented trade name etoxidol). Here we studied antiischemic activity of this drug.

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MATERIALS AND METHODS

Antiischemic activity of etoxidol (substance of OOO, All-Russian Research Center for Safety of Biologically Active Compounds) was studied in experiments on adult male Wistar rats (n=7) anesthetized with sodium thiopental(40 mg/kg intraperitoneally [i.p.]) and transferred to controlled ventilation (Ugo Basile) using discriminative indicator method for measuring the size of ischemia and necrosis zones under conditions of developing myocardium infarction as described elsewhere [8]. Control experimental series was carried out on 17 animals. Evans blue and triphenyltetrazolium chloride (Sigma) were used in this study.

The efficiency of etoxidol in preventing rhythm and conduction disturbances and mortality in albino mice of both sexes with catecholamine micronecro-

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ses was studied by the method described elsewhere [12]. Epinephrine hydrochloride was injected in a dose of 200 μg/kg (0.1% solution in 1 ml ampules; AO Moscow Endocrine Manufacture) i.p. 10 minutes after single i.p. administration of the test compound. Two-three minutes before catechoamine injection, the animals were subjected to intratracheal instillation of ether in a volume of 0.1 ml/kg using a micropipette. ECG was recorded in experimental animals in standard lead II (EKT-02).

The effects of etoxidol on catecholamine level in the myocardium of outbred cats of both sexes (n=7) with myocardium infarction were assessed fluorometrically [7] using an FM-3MA fluorometer.

The effects of etoxidol on lipid peroxidation intensity and antioxidant system in the myocardium (n=7) of cats with experimental myocardium infarction were studied by the method of induced chemoluminescence [3] on an Emilite-EL 1105 device.

Etoxidol was administered intravenously in a dose proportional to 5% of LD_{50} determined after i.p. administration to mice and with consideration for interspecies dose conversion [11]. In experiments on mice, rats, and cats, the doses were 22, 14.2, and 6.7 mg/kg, respectively. In all experimental models, the effects of etoxidol were compared with those of mexidol (mexidol OOO PF Pharmsoft; solution in 2 ml ampules) and propranolol (Obzidan, officinal 0.1% solution in 5 ml ampules; Isis Pharma) administered in equitoxic doses in experiments on mice and rats.

Results were statistically processed using standard Excel, Statistics 5.5 for Windows XP software. Significance of differences was assessed using parametric Student's t test and nonparametric χ^2-test for dependent and independent samples [5].

RESULTS

At the 1 stage we studied intrinsic antiischemic activity of etoxidol substance in experiments on rats (Table 1). Control animals received 1 ml 0.9% NaCl physiological saline 10 min before coronary artery ligation. Intravenous administration of the reference drug mexidol (14.1 mg/kg) did not significantly reduced ischemia and necrosis zones, but significantly reduced their ratio. Etoxidol administration restricted necrosis zone and decreased the necrosis/ischemia zone ratio and this effect was not inferior to that of $\beta_{1,2}$-adrenoblocker propranolol (0.5 mg/kg).

Considering numerous investigations focused on antiischemic effects of 3-hydroxypyridine derivatives [1,2], at the next stage we compared the efficiency of etoxidol with that of propranolol and mexidol on mouse model of epinephrine toxicity. In control series (Table 2), epinephrine hydrochloride administration was associated with the development of ventricular arrhythmias and blocks in the majority of animals. Mortality in the control group was 100% and was due to conduction disturbances and asystole. Propranolol (1.0 mg/kg) significantly reduced frequency of rhythm and conduction disturbances, but did not reduce animal mortality. Etoxidol and mexidol possessed no antiarrhythmic effect in a dose of 22.0 mg/kg, but reduced animal mortality. After doubling etoxidol dose, its efficiency markedly increased: alongside with minimal mortality, the number of mice with ectopic rhythm disturbances decreased and a trend toward reduction of conduction blocks was observed.

Since hypercatecholemia and hyperactivation of lipid peroxidation play the key roles in the pathogenesis of acute ischemic myocardium lesions [6], we studied the effects of etoxidol on epinephrine and norepinephrine concentrations and lipid peroxidation system in the heart of cats with acute myocardium infarction. In animals with experimental myocardial infarction, epinephrine concentration in lesion area of the left ventricle significantly increased from 2.81±0.31 to 7.36±0.31 nmol/g (Table 3), while norepinephrine concentration in the lesion area tended to decrease from 2.24±0.27 to 1.17±0.14 nmol/g. Similar pattern of catecholamine levels in ischemic myocardium was

TABLE 1. Effects Etoxidol and Reference Drugs on the Size of Ischemia and Necrosis Zones 4 h after Coronary Artery Occlusion (M±m)

Drug	Dose, mg/kg	n	Ratio of necrosis area to total myocardium weight, %	Ratio of ischemia area to total myocardium weight, %	Ratio of ischemia area to necrosis area, %
Control	-	17	34.0±2.6	22.0±2.0	64.7±4.3
Mexidol	14.1	7	34.0±3.6	15.0±4.3	44.1±5.4*
Propranolol	0.5	7	30.0±3.5	11.0±1.6*	39.0±5.5*
Etoxidol	14.2	7	32.3±2.7	12.0±3.7*	40.0±4.1*

Note. Here and in Table 2: *p<0.05 in comparison with the control (χ^2 test).

TABLE 2. Efficiency of Etoxidol in Epinephrine Toxicity in Mice

Drug	Dose mg/kg	Number of animals			Mortality, %
		in experiment	with AV-blocks	with arrhythias	
Control	-	14	11 (79)	13 (93)	14 (100)
Mexidol	22.0	13	10 (77)	8 (62)	1 (8)*
Propranolol	1.0	14	4 (29)*	7 (50)*	11 (79)
Etoxidol	22.0	14	10 (71)	10 (71)	2 (14)*
	44.0	14	7 (50)	8 (57)*	1 (7)*

Note. AV: atrioventricular; percentage is shown in parentheses.

reported previously [2]. Intravenous administration of etoxidol and mexidol significantly increased epinephrine concentration in myocardium areas distant from the ischemic zone with simultaneous decrease in hormone concentration in the infarction area. Conversely, the content of sympathetic neurotransmitter norepinephrine significantly increased after etoxidol administration.

Analysis of antioxidant properties of etoxidol under conditions of regional heart ischemia demonstrated its capacity to limit free radical processes in the lesion area. The intensity of lipid peroxidation decreased more than twice under the effect of etoxidol in myocardium areas with limited blood flow. At the same time it should be noted that the effects of etoxidol were more pronounced than those of mexidol. Antioxidant activity in the lesion area against the background of etoxidol increased by 3.7 times, which also significantly surpassed the effect of mexidol.

In intact myocardium area of animals receiving etoxidol, the intensity of free radical lipid peroxidation did no significantly differ from the control and was 8.90±1.02 cpm. It can be hypothesized that there is no need in activation of alternative oxidation pathways in the uninjured area, because the main energy production pathway is preserved. In this connection, maintenance of lipid peroxidation at the control level may additionally indicate that lipid peroxidation pattern is adaptive and controlled.

Thus, the new 3-hydroxypypiridine derivative etoxidol possesses protective properties in ischemic heart lesions. Antiischemic action is realized due to inhibition of free radical processes in cardiomyocyte membrane and limitation of humoral adrenergic influences on the myocardium. At the same time, one may assume that the increase in norepinephrine concentration after etoxidol administration is associated with increased survival of sympathic terminals in the myocardium when lesion area is limited. We also demonstrated some differences in the pharmacodynamics of etoxidol and its structural analogue mexidol. In particular, etoxidol had higher antiischemic potential and antioxidant activity. Higher activity of the substance with chemically modified 3-hydroxypyridine base can be determined by properties of malic acid residue in etoxidol molecule.

TABLE 3. Catecholamine Levels in Cat Myocardium in Experimental Infarction and Etoxidol Treatment ($M \pm m$)

Hormones		Experimental conditions		
		control, nmol/g	mexidol (6.7 mg/kg), nmol/g	etoxidol (6.7 mg/kg), nmol/g
Epinephrine	ventricle, distant area	2.81±0.31	4.43±0.16*	3.54±0.09*
	ventricle, ischemia area	7.36±0.31	3.38±0.19*	2.88±0.28*
	atrium	0.56±0.17	1.03±0.36	0.69±0.17
Norepinephrine	ventricle, distant area	2.24±0.27	1.86±0.29	1.31±0.11*
	ventricle, ischemia area	1.17±0.14	4.1±0.15*	3.39±0.13*
	atrium	0.79±0.36	0.82±0.19	0.77±0.19

Note. *$p<0.05$ in comparison with the corresponding control (Student's t test).

TABLE 4. Effects of Etoxidol (6.7 mg/kg) and Mexidol (6.7 mg/kg) on Free Radical Processes and Antioxidant System in Different Compartments of Cat Heart in Experimental Myocardium Infarction (*M*±*m*)

Subject	Lipid peroxidation, cpm			AOA, cpm		
	control	mexidol	etoxidol	control	mexidol	etoxidol
Atrium	4.98±1.36	4.45±0.99	4.39±1.79	4.78±1.99	20.88±2.26*	22.37±9.58*
Ventricle, distant area	10.50±1.69	9.30±1.49	8.90±1.02	3.20±0.34	17.50±2.35*	19.27±3.54*
Ventricle, ischemic area	42.90±4.88	23.90±2.12*	16.20±1.26*+	13.60±2.92	43.50±3.32*	52.50±2.02*+

Note. $p<0.05$ in comparison with: *control, +mexidol. AOA: antioxidant activity.

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