Efficiency of Structurally Different Antioxidants in Combined Myocardial Damage in Mice

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Cardioprotective effects of derivatives of 3-hydroxypyridine, benzimidazole, and a peptide substance were studied on the model of combined damage to the myocardium (catecholamines and physical exercises) in mice. The most pronounced antioxidant and cardioprotective effects were produced by 3-hydroxypyridine acetylcysteinate (50 mg/kg), benzimidazole derivative Be-2m (50 mg/kg), and peptide substance γ-glutamyl histamine (1 mg/kg).

Key Words: *antioxidants; cardioprotection; myocardial damage*

Cardiovascular diseases are still the leading causes of disability and related economical losses [7]. Standard therapy does not compensate metabolic disturbances developing in patients with coronary stenosis under conditions of increased load to the cardiovascular system [1]. In light of this, the search for new drugs improving quality of life of CHD patients, including recovery of their physical activity, is an urgent problem [4]. Combined use of antioxidants and other antianginal drugs seems to be a promising approach [6]. Since the targets of antioxidant action is determined by chemical structure of these drugs, these peculiarities should be taken into account during the development of new antioxidant preparations [2].

Here we studied cardioprotective properties of derivatives of 3-hydroxypyridine, benzimidazole, and a peptide substance in mice with combined myocardial damage.

MATERIALS AND METHODS

Experiments were performed on 155 outbred mature albino mice. The animals were divided into 11 groups (14 rats per group). Group 1 comprised intact animals. In group 2 mice, catecholamine-induced myocardial damage was modeled by intraperitoneal injection of 0.1% adrenaline hydrochloride (1.5 mg/kg) and oxytocin (7.5 U/kg). After 48 h, the animals were subjected to intensive physical exercise (swimming over 40 min). The test compounds were intramuscularly injected to group 3-6 mice 30 min before modeling catecholamine damage and then after 24 and 48 h (30 min before forced swimming). Animals of groups 3, 4, 5, and 6 received mexidol (50 mg/kg, reference preparation), 3-hydroxyproline acetylcysteinate (3HPC, 50 mg/kg), benzimidazole derivative (laboratory code Be-2m, 50 mg/kg, and γ-glutamyl histidine (γ-GH, 1 mg/kg), respectively. In groups 7-11 including animals with catecholamine-induced damage to the myocardium subjected to physical exercise and receiving mexidol, 3-HPC, Be-2m, and γ-GH, endurance was evaluated by determining the mean duration of swimming until the end of active movements. In all mice, ECG was recorded using EK1T-04 electrocardiograph and an ASK-3107 digital memory oscillograph at a tape rate of 500 mm/sec. At the end of the experiment, MDA content in heart homogenates was measured by the method of S. G. Konyukhova based on the reaction between MDA and 2-TBA at high temperature and acid pH yielding a stained product (trimethine complex) with absorption maximum at 532 nm. Catalase activity in myocardial tissue was measured by the method of M. O. Korolyuk based on recording of changes in optical density due to reaction between hydrogen peroxide with molybdenum salts at 410 nm. Serum AST was assayed using a unified method by optimized optical test

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based on the differences in absorption of reduced and oxidized nicotinamide adenine nucleotide at 340 nm. Serum creatine phosphokinase (CPK) was assayed using a unified clinical test. This method is based on the fact that total CPK activity is proportional to the concentration of inorganic phosphorus formed during acid hydrolysis of creatine phosphate catalyzed by the enzyme. Serum calcium concentration was measured using a colorimetric test with Arsenazo III (the dye forms a blue-colored complex with calcium ions at neutral pH). Potassium content in blood serum was measured by nephelometric assay without deproteinization (potassium ions added to the reaction mixture form a stable suspension; turbidity of this suspension is proportional to concentration of potassium ions). Biochemical assays were performed on a Bioscience analyzer.

The data were processed statistically using Excel software, the arithmetic means, errors of the means, and significance of the differences between the means were evaluated using Student's *t* test at 5% significance level.

RESULTS

Intensive physical exercise can be a catecholamine whip for the myocardium [8] sharply increasing oxygen demands and intensifying LPO processes [5]. In control series, MDA content significantly increased by 112% (p_i<0.001), while catalase activity sharply decreased by 73% (p_i<0.001) in comparison with the corresponding parameters in intact animals. Comparative analysis showed that all studied compounds reduced LPO intensity in the myocardium and improved antioxidant defense (Table 1). γ-GH and Be-2m were most effective. MDA content in animals receiving γ-GH and Be-2m decreased by 57 and 78% (p_c<0.001) in comparison with the control. Catalase activity in the corresponding groups increased by 1766 and 1496% (p_c<0.001) in comparison with the control, which attests to a strain in the antioxidant defense system.

In control mice, plasma CPK activity considerably increased by 43% in comparison with intact animals (p_i<0.005), which attests to the influence of physical exercise on both skeletal muscles and myocardium. 3HPC produced no appreciable effect. Mexidol produced a moderate cardioprotective effect, which can be explained by not only its antioxidant properties, but also the presence of succinate in this preparation restoring the disturbed oxidative phosphorylation process. γ-GH produced a moderate protective effect: CPK decreased by 37% in comparison with the control (p_c<0.001) and approximated the level of intact control. Be-2m was the most effective compound: CPK decreased by 47% in comparison with the control (p_c<0.001) and approximated the level of intact control.

Plasma AST activity in control animals also increased by 10% in comparison with intact mice. 3HPC produced a pronounced protective effect. The efficiency of mexidol and Be-2m was lower. γ-GH had maximum cardioprotective effect and reduced AST activity by 30% in comparison with that in the control group (p_c<0.001).

Combination of physical exercise and catecholamine damage to the myocardium led to pronounced hyperkalemia. Potassium content increased by 24% in comparison with that in intact animals (p_i<0.05). After administration of γ-GH, 3HPC, and Be-2m, potassium content decreased in comparison with the control, but did not attain the initial level. Mexidol was more effective: potassium content decreased by 17% in comparison with the control (p_c<0.05).

It is known that hyperkalemia against the background of acidosis is also accompanied by calcium deficiency [3]. Pronounced hypocalcemia was observed after physical exercise combined with catecholamine damage to the myocardium. Calcium content decreased by 26% in comparison with that in intact mice (p_i<0.001). Mexidol and γ-GH increased calcium content in comparison with its level in the control and caused hypercalcemia. Be-2m increased calcium content to moderate hypercalcemia. Against the background of 3HPC treatment, calcium level increased by 35% in comparison with the control (p_c<0.001) and returned to the initial level.

In controls, HR increased by 24% in comparison with that in intact animals (p_i<0.01). Mexidol did not abolish tachycardia, while 3HPC had a normalizing effect on this parameter. This can be explained by more effective inhibition of LPO due to antioxidant properties of cystein (thiol antioxidant) [2]. 3HPC reduced HP by 19% in comparison with the control (p_c<0.005) and this parameter approximated the initial level. The treatment with γ-GH was also effective: HR decreased by 17.5% in comparison with the control (p_c<0.005), *i.e.* attain the level of intact control. Be-2m effectively corrected tachycardia; HR decreased by 22% in comparison with the control (p_c<0.005).

Analysis of ECG showed that physical exercise against the background of catecholamine damage to the myocardium led to deceleration of ventricular conduction. *QRS* complex duration increased by 18% in comparison with that in intact animals (p_i<0.005). Treatment with 3HPC moderately improved ventricular conduction in comparison with the control. Mexidol more effectively corrected ventricular conduction: the duration of *QRS* complex approximated the control. The use of γ-GH restored ventricular conduction: the duration of *QRS* complex decreased by 27% in comparison with the control (p_c<0.001). Be-2m effectively restored ventricular conduction: the duration of

TABLE 1. Biochemical and ECG Parameters in Mice with Combined Damage to the Myocardium and after Correction with the Test Compounds (*M*±*m*)

Parameter	Intact (*n*=14)	Control (*n*=14)	Mexidol, 50 mg/kg (*n*=14)	3HPC, 50 mg/kg (*n*=14)	Be-2m, 50 mg/kg (*n*=14)	γ-GH, 1 mg/kg (*n*=14)
Duration of swimming, min	—	78.3±9.9	101.3±8.1 p_c<0.001	123.4±9.4 p_c<0.001	120.6±11.0 p_c<0.001	116.7±11.0 p_c<0.001
MDA in the myocardium, mmol/liter	15.04±1.50	31.91±4.80 p_i<0.001	26.16±3.49 p_i<0.001 p_c<0.05	22.66±4.42 p_i<0.001 p_c<0.005	7.10±1.74 p_i<0.001 p_c<0.001	13.43±1.50 p_c<0.001
Catalase in myocardium, μcat/sec/liter	1.67±0.25	0.45±0.12 p_i<0.001	0.270±0.078 p_i<0.001 p_c<0.05	7.10±1.74 p_i<0.001 p_c<0.001	8.40±1.22 p_i<0.001 p_c<0.001	7.18±1.01 p_i<0.001 p_c<0.001
Plasma CPK, U/liter	1331.6± 209.0	1899.9± 392.0 p_i<0.005	1257.7± 209.0 p_c<0.001	1683.89± 238.00 p_i<0.001	1000±281 p_i<0.05 p_c<0.001	1213±192 p_c<0.001
Plasma AST, U/liter	128.2±25.2	140.7±16.9	121.3±14.4 p_c<0.001	105.7±18.68 p_c<0.001	125.4±18.4	99.9±14.4 p_i<0.005 p_c<0.001
Potassium in blood plasma, mmol/liter	6.800±0.067	8.40±1.85 p_i<0.05	6.95±0.94 p_c<0.05	7.46±0.78	7.79±0.98 p_i<0.05	7.95±1.04
Calcium in blood plasma, mmol/liter	1.85±0.24	1.37±0.09 p_i<0.001	2.18±0.15 p_i<0.001 p_c<0.001	1.84±0.05 p_c<0.001	2.03±0.07 p_i<0.05 p_c<0.001	2.31±0.20 p_i<0.001 p_c<0.001
HR	419±26.57	519.3±81.5 p_i<0.001	501.9±62.4 p_i<0.005	417.9±59.7 p_c<0.05	406.56±80 p_c<0.001	427.5±58 p_c<0.001
QRS duration, msec	15.7±1.0	18.5±1.9 p_i<0.005	16.2±0.9 p_c<0.005	17.8±1.8 p_i<0.005	15.5±3.8	13.6±1.8 p_i<0.001 p_c<0.001

Note. p_i: significance of differences from intact animals; p_c: significance of differences from the control.

QRS complex decreased by 17% in comparison with the control and approximated the initial level.

The worst endurance was noted in catecholamine damage to the myocardium. Treatment with the test compounds improved tolerance to physical exercise. Mexidol considerably increased this parameter: the duration of swimming increased by 29% in comparison with the control (p_c<0.001). 3HPC, γ-GH, and Be-2m most effectively improved tolerance to physical load in comparison with the control group and increased the duration of swimming by 58% (p_c<0.001), 49% (p_c<0.001), and 54% (p_c<0.001), respectively.

Thus, 3HPC (50 mg/kg), Be-2m (50 mg/kg), and γ-GH (1 mg/kg) produce more pronounced antioxidant and cardioprotective effect in combined damage to the myocardium than mexidol (50 mg/kg); hence, the search for effective antioxidant in different classes of chemical compounds remains a promising approach.

REFERENCES

1. S. N. Vaslyaeva, O. V. Tsigankova, I. G. Gordeev, and N. A. Volov, *Ros. Med. Zh*., No. 3, 50-54 (2004).
2. V. G. Zaitsev, O. V. Ostrovskii, and V. I. Zakrevskii, *Eksp. Klin. Farmakol*., **66**, No. 4, 66-70 (2003).
3. A. I. Karpishchenko, *Medical Laboratory Technologies and Diagnostics, Handbook* [in Russian], St. Petersburg (1999).
4. O. V. Korkina, A. N. Hatkevich, V. I. Kapel'ko, and E. K. Ruge, *Kardiologiya*, No. 6, 41-45 (2001).
5. V. Z. Lankin, A. K. Tikhaze, and Yu. N. Belenkov, *Ibid.,* No. 7, 48-61 (2000).
6. L. N. Sernov, L. D. Smirnov, G. I. Shaposhnikova, and N. N. Guranova, *Klinicheskie Issledovaniya Lekarstvennyh Sredstv v Rossii*, No. 1, 24-28 (2004).
7. G. I. Sidorenko, S. M. Komisarova, and Yu. P. Ostrovskii, *Kardiologiya*, No. 3, 19-24 (2006).
8. G. A. Chumakova, E.V. Kiseleva, V. I. Chursina, and V. G. Lychev, *Ibid.,* No. 2, 71-72 (2003).