MOLECULAR-BIOLOGICAL PROBLEMS OF DRUG DESIGN AND MECHANISM OF DRUG ACTION

ANTIOXIDANT AND HYPOLIPIDEMIC PROPERTIES OF MEXIDOL AND EMOXYPINE DURING PROLONGED IMMOBILIZATION STRESS

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Increased level of the products of lipid peroxidation (LPO) is considered a factor leading to the risk of atherosclerosis development [1 – 3]. At the same time, experimental investigations confirm the ability of antioxidants to produce positive effects with respect to appearance and development of the atherosclerosis process [4 – 5].

As is known, a significant role in pathogenesis of a number of diseases involving increased LPO level as a general pathogenic factor may belong to chronic stress. The results of some investigations showed that antioxidants such as emoxypine and mexidol may offer protection against acute stressor damage [6, 7]. The purpose of this study was to characterize the antioxidant and hypolipidemic properties of mexidol and emoxypine on the model of prolonged immobilization-induced stress.

EXPERIMENTAL PART

Experiments were performed on a group of 33 sexualy matore male Chinchilla rabbits weighing 2 – 3 kg. The immobilization-induced stress in the test animals was achieved by holding them in wire-framework cages, which strongly restricted the mobility but did not hinder access to food and water.

There were three series of experiments. The control group of 12 rabbits was kept under the strong hypodynamia conditions for 30 days. Animals in the second (12 rabbits) and third (9 rabbits) groups were kept during the same period under identical conditions and daily injected in their edge auricular (otic) veins with 1 mg / kg of emoxypine or mexidol, respectively. Both before the mobility limitation and in the course of the experiment (7th, 14th, and 30th days) blood samples were taken and the plasma and erythrocytes were analyzed for malonic dialdehyde (MDA, the final LPO product) using a thiobarbituric acid test. Erythrocytes were also analyzed for glutathione peroxidase (GP) [8], catalase [9], and superoxide dismutase (SOD) [10]. The total blood serum cholesterol was determined according to [11], the content of low-density lipoproteins (LDLs) was studied turbodimetrically [12], the total lipid and triglyceride levels were determined using the standard reagent kits (Sigma, USA), high-density lipoproteins (HDLs) were analyzed after heparin-manganese precipitation and the deposition of apoprotein-β-containing lipoproteins (apo-β-Ls) according to [11], and the blood serum albumin was determined by electrophoresis in agar. After the immobilization period, the animals were killed by intravenous injection of a lethal dose of hexenal (100 mg / kg). The results of investigation were processed by methods of variational statistics with determination of the Student *t*-criterion.

RESULTS AND DISCUSSION

Prolonged immobilization-induced stress resulted in a high level of lethality (24 %) in the control group of animals.

The immobilization leads to activation of the LPO process, as indicated by increasing MDA level in the blood plasma (by 99, 144, and 128 % on the 7th, 14th, and 30th day, respectively) and, albeit to a lower extent and within a

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TABLE 1. Effects of Emoxypine and Mexidol on the Malonic Dialdehyde Level and the Enzymatic Activity of the Antioxidant System During Prolonged Immobilization Stress

Test day	MDA, μM		GP, mmole/(min · liter)		Catalase, mcat/(ml · sec)	
	plasma	erythrocytes	plasma	erythrocytes	plasma	erythrocytes
Initial level	1.8 ± 0.1	2.4 ± 0.3	16.02 ± 3.09	7.0 ± 0.5	0.63 ± 0.09	3.48 ± 0.37
			Hypodynamia (control)			
7	3.6 ± 0.3	3.6 ± 0.4	34.07 ± 1.27	27.3 ± 2.1	0.47 ± 0.15	2.62 ± 0.73
	$p < 0.001$	< 0.001	< 0.001	< 0.001	> 0.05	> 0.05
14	4.4 ± 0.2	4.6 ± 0.3	32.3 ± 2.9	28.3 ± 3.6	0.27 ± 0.04	2.45 ± 0.65
	$p < 0.001$	< 0.001	< 0.001	< 0.001	< 0.001	> 0.05
30	4.1 ± 0.35	2.8 ± 0.4	23.17 ± 0.28	18.3 ± 3.2	0.39 ± 0.06	1.35 ± 0.39
	$p < 0.001$	> 0.05	< 0.05	< 0.05	< 0.05	< 0.001
			Hypodynamia + emoxypine			
7	2.8 ± 0.2	1.9 ± 0.3	14.6 ± 2.2	13.9 ± 1.7	0.62 ± 0.02	5.06 ± 0.33
	$p_1 < 0.03$	< 0.05	< 0.001	< 0.001	> 0.05	< 0.001
14	2.8 ± 0.3	1.3 ± 0.2	28.7 ± 1.5	20.1 ± 2.8	0.36 ± 0.01	3.62 ± 0.35
	$p_1 < 0.001$	< 0.001	> 0.05	< 0.05	> 0.05	> 0.05
30	2.0 ± 0.4	1.2 ± 0.2	14.6 ± 1.4	20.3 ± 2.4	0.36 ± 0.04	3.4 ± 0.6
	$p_1 < 0.001$	< 0.001	< 0.001	> 0.05	> 0.05	< 0.001
			Hypodynamia + mexidol			
7	3.2 ± 0.7	2.2 ± 0.4	33.0 ± 2.0*	27.2 ± 1.6*	0.4 ± 0.1*	2.48 ± 0.30*
	$p_1 > 0.05$	< 0.05	> 0.05	> 0.05	> 0.05	> 0.05
14	2.0 ± 0.3	1.4 ± 0.2	28.8 ± 1.4	21.9 ± 4.7	0.24 ± 0.01*	2.39 ± 0.60
	$p_1 < 0.001$	< 0.001	> 0.05	> 0.05	> 0.05	> 0.05
30	1.6 ± 0.1	1.26 ± 0.10	28.3 ± 0.7*	33.1 ± 0.4*	0.24 ± 0.09*	1.80 ± 0.68
	$p_1 < 0.001$	> 0.05	> 0.05	< 0.001	< 0.05	> 0.05

Note. Here and in Table 2: p is the reliability of differences from the initial level, p_1 is the reliability of differences from control; * $p < 0.05$ for the difference from emoxypine.

shorter period of time, in erythrocytes (by 51 and 93 %, on the 7th and 14th day respectively, Table 1). The maximum MDA content is observed on the 14th day of the immobilization test, that is, in the stage of a maximum lethality level in the control group of animals.

Reaction of the antioxidant system (AOS) to the regime of hypodynamia is characterized by an increase in the GP activity in the blood plasma (by 112, 105, and 45 % on the 7th, 14th, and 30th day, respectively) and especially in erythrocytes (by 287, 298, and 157 % on the 7th, 14th, and 30th day, respectively). The catalase activity in both plasma and erythrocytes remains suppressed within the entire period of observation, while the SOD activity in erythrocytes shows a reliable increase (from 1.05 ± 0.19 to 4.93 ± 1.52 AU, $p < 0.001$) only after the first week of the immobilization test.

The immobilization-induced stress produces a proatherogenic effect, as evidenced by an increase in the total cholesterol in the blood serum (by 166 % on the 30th day), LDLs (47 %), triglycerides and total lipids (50%), and a decrease in HDLs (31 %), and by a 21-fold growth of the atherogenic index (Table 2).

As is seen from the data presented in Table 1, the use of both emoxypine and mexidol markedly reduces the degree of LPO activation caused by the the immobilization-induced stress.

On the emoxypine background, the MDA level decreases both in the blood plasma (by 22, 36, and 59 % on the 7th, 14th, and 30th day, respectively) and, to even greater extent, in erythrocytes (by 48, 65, and 58 %, respectively). At the same time, mexidol exhibits a trend of more intensively suppressing the formation of LPO products in the plasma.

The difference between the AOS reactions on the introduction of the antioxidants studied is manifested by the fact that emoxypine, unlike mexidol, reduces the hypodynamia-related increase in the GP level in the blood plasma and erythrocytes and lowers the degree of suppression of the catalase reaction (the level of this reaction in the test erythrocytes exceeds that in the control by 82, 43, and 156 % on the 7th, 14th, and 30th day, respectively). At the same time, neither of the two drugs studied produce any significant effect on the SOD activity in erythrocytes.

Both emoxypine and mexidol exhibit a hypocholesterolemic action (Table 2). Emoxypine inhibits the immobilization-stress induced increase in the total cholesterol and triglyceride (by 56 and 75 %, respectively), but does not lift up a decrease in the HDL and an increase in the LDL levels. The atherogenic index decreases under the action of emoxypine by 78 %. Unlike this, mexidol increases the HDL level (by 83 %), thus further reducing the atherogenic index,

TABLE 2. Effects of Emoxypine and Mexidol on the Lipid and Protein Spectra in the Blood Serum During Prolonged Immobilization Stress

Parameter	Initial level	Hypodynamia		
		Control	Emoxypine	Mexidol
Total cholesterol, mM	1.2 ± 0.1	3.2 ± 0.2 $p < 0.001$	1.4 ± 0.2 $p_1 < 0.001$	2.12 ± 0.24* < 0.001
HDL, mM	1.0 ± 0.1	0.7 ± 0.07 $p < 0.001$	0.78 ± 0.23 $p_1 > 0.05$	1.28 ± 0.27* < 0.01
LDL, o.d. units	13.9 ± 1.5	20.44 ± 3.27 $p < 0.05$	19.19 ± 2.26 $p_1 > 0.05$	15.71 ± 1.37 > 0.05
Atherogenic index, units	0.17 ± 0.03	3.6 ± 0.3 $p < 0.001$	0.79 ± 0.01 $p_1 < 0.001$	0.65 ± 0.04* < 0.001
Triglycerides, mM	0.27 ± 0.02	0.86 ± 0.10 $p < 0.01$	0.22 ± 0.02 $p_1 < 0.001$	0.23 ± 0.02 < 0.001
Total lipids, g/liter	2.7 ± 0.3	4.03 ± 1.37 $p > 0.05$	6.38 ± 0.69 $p_1 > 0.05$	5.89 ± 1.23 > 0.05
Albumins, g/liter	36.01 ± 1.29	33.0 ± 3.1 $p > 0.05$	36.35 ± 0.74 $p_1 > 0.05$	39.02 ± 1.92* < 0.05

and leads to growth in the content of albumins in the blood plasma.

Thus, both emoxypine and mexidol produce a protective antistressor action when administered under the prolonged immobilization conditions. The effect is apparently due to the LPO inhibition by activation of the antioxidant system (for the most part, of the glutathione peroxidase chain) and normalization of the lipid spectrum in the blood plasma.

It is known that oxygen plays an active role in the LDL oxidation. The oxidation products accumulate in the monocytes and convert them into "foamy" cells, thus leading to the formation and development of atherosclerotic disorders [13]. The antioxidants emoxypine or mexidol inhibit the accumulation of LPO products and decrease the probability of LDL conversion into oxidized forms, thus producing the antiatherogenic effect. An additional advantage of the mexidol administration consists in retaining a high albumin level in the blood serum, which inhibits the formation of free radicals, thus significantly reducing the cell damage [14].

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