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EFFECT OF AN ANTIOXIDANT OF THE 3-HYDROXYPYRIDINE CLASS ON MICROCIRCULATORY DISTURBANCES ASSOCIATED WITH EXPERIMENTAL DYSLIPOPROTEINEMIA AND ITS ALIMENTARY CORRECTION

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UDC 616.153.915-092.9-085.874.2.015.2:615.272.014.425]036.8-07:616.16-008.1

KEY WORDS: dyslipoproteinemia, regression of experimental atherosclerosis, lipid peroxidation, antioxidant.

The important role of dyslipoproteinemia (DLP) in microcirculatory disturbances (MCD) has now been established. The response of the microcirculatory system to DLP is generalized in character and is accompanied by the development of pathological changes in the target organs [9, 4, 10]. During prolonged spontaneous regression of experimental atherosclerosis complete restoration of lipid homeostasis and of MCD is not observed [5]. In clinical investigations MCD have been found in association with acute disturbance of the coronary circulation and an atypical course of myocardial infarction [2, 12]. It has been shown that during remission of coronary heart disease changes in the terminal vascular bed undergo virtually no degree of regression, and this promotes progression of the disease [11]. An important stage of atherogenesis and, in particular, of membrane pathology in DLP, is activation of lipid peroxidation (LPO). Recent investigations [1, 6-8, 9] have demonstrated the vasoconstrictor properties of LPO products and their ability to accelerate aging of erythrocytes and to slow the blood flow at the level of small and medium-sized vessels. This accounts for the importance of elucidating the role of LPO in the MCD associated with DLP.

In the investigation described below the effect of the antioxidant mexidol was studied on parameters of lipid metabolism, LPO, and the state of the microcirculatory system in the early stages of atherogenesis and during alimentary correction of DLP.

EXPERIMENTAL METHOD

Experiments were carried out on 50 male chinchilla rabbits weighing 2-2.5 kg. The animals of group 1 received cholesterol in a dose of 0.3 g/kg body weight with vegetables for 2 months. Animals of group 2, also on an atherogenic diet (AGD) additionally received the antioxidant (AO) mexidol from the second month in a dose of 30 mg (for 1 month). It was shown previously that a marked rise of the level of atherogenic lipoproteins (ALP), MCD, and initial atherosclerotic changes in the aorta are observed in animals kept on an AGD for 1 month [4, 5]. After 2 months on the AGD, animals of groups 1 and 2 were transferred to a standard diet (SD) for 9 months (alimentary correction of DLP). Intact animals receiving SD throughout the same period served as the control. The animals were withdrawn from the experiment by air embolism. The index of atherosclerotic damage of the aorta (IDA) was determined by Avtandilov's method. The state of the microcirculatory bed was studied in total film preparations of the mesentery of the small intestine (Kupriyanov's method). The area of cross section of the microvessels was determined with the aid of a semiautomatic image analysis system (Leitz "ASM," West Germany) and their adrenergic innervation was studied by the method of Falck and Ovman. The intensity of specific fluorescence of catecholamines in the

Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR. Research Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 3, pp. 234-237, March, 1990. Original article submitted October 7, 1989.

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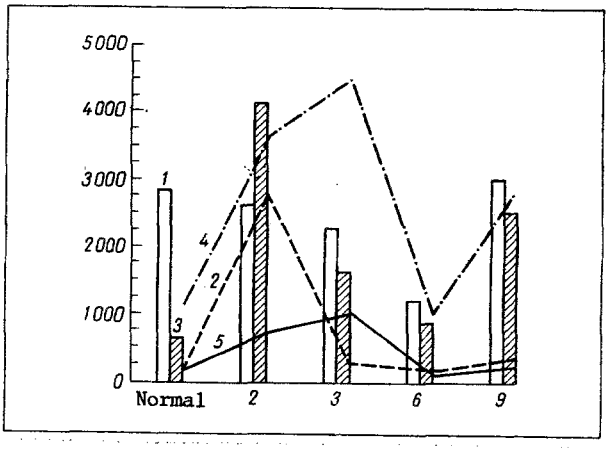

Fig. 1. Content of total ALP fraction, MDA, various forms of erythrocytes, and SA in rabbits with DLP and during its alimentary correction. Abscissa: times on AGD and correction of DLP (in months); ordinate: 1) number of normocytes (in %), 2) ALP concentration (in mg%), 3) number of echinocytes (in %), 4) MDA concentration (in nmoles/ml), 5) SA. Scale: 1, 3, 4, 5) 1:100, 2) 1:1.

terminal portion of the vascular bed was estimated photometrically on the LYUMAM microscope. Erythrocytes treated by the standard method were studied in an S-500 scanning electron microscope ("Hitachi," Japan). Different fractions of lipoproteins and the malonic dialdehyde (MDA) concentration in the blood serum were determined [13].

EXPERIMENTAL RESULTS

After 2 months on an AGD the ALP level in animals of group 1 was more than 20 times higher than initially, but at the same time the concentration of the fraction of antiatherogenic high-density lipoproteins (HDL) fell, and the ratio of ALP to HDL increased to 7, evidence of an increase in the atherogenic potential of the blood; the MDA level rose sharply (Fig. 1).

On the addition of mexidol to the AGD (group 2) the blood ALP level was 9-10 times higher than initially, the HDL concentration was higher than in group 1, and activation of LPO was less pronounced (Figs. 1 and 2). The increase in the ALP fraction in the blood and in the MDA concentration was accompanied in both groups 1 and 2 by an increase in the number of echinocyte forms of erythrocytes and by the formation of complex and stable aggregates (SA), with a simultaneous decrease in the number of normocytes (Figs. 1 and 2). In the animals of group 2 erythrocytic aggregates were found mainly in the venules of the microcirculatory bed and were local in character. Comparison of IAD in the animals of the two groups revealed significant differences in the degree of involvement of the main vessels in atherosclerosis (22 and 1.5%, respectively) and showed that the value of this parameter depends on the severity of MCD. Less marked changes in lipid metabolism and inhibition of LPO after addition of mexidol to the AGD were accompanied by disappearance of the changes in the various components of the microcirculatory bed (MCB). Microvessels had smoother contours, the constriction of arterioles and precapillaries found in the animals of group 1 was not observed, the lumen of the capillaries differed only a little from that in the control, and dilatation was still found only in the venules (Table 1).

Substantial differences also were found in the adrenergic innervation of the microvessels of animals kept on an AGD and after the addition of mexidol to the AGD. Whereas in group 1 axons of the periarterial plexuses were greatly thickened, large varicose expansions were observed, the intensity of specific luminescence of catecholamines was 6.5 ± 0.37 conventional units (compared with 4.67 ± 0.05 conventional units in the control), in group 2 the adrenergic plexuses were almost indistinguishable in their structure from the control, and the intensity of luminescence in them was 3.9 ± 0.12 conventional units.

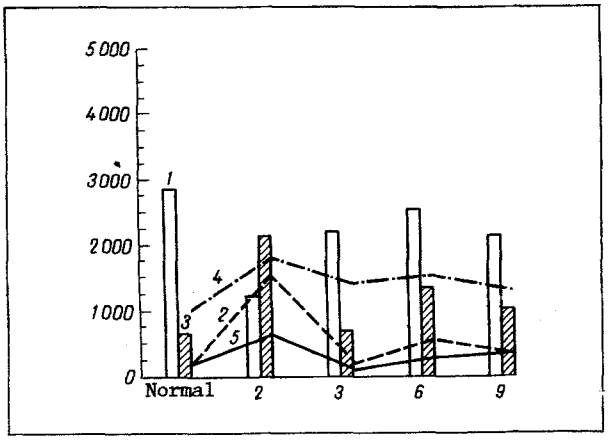

Fig. 2. Effect of mexidol on content of total ALP fraction, MDA, numbers of different forms of erythrocytes, and of SA in rabbits with DLP and during its alimentary correction. Legend and scale the same as Fig. 1.

TABLE 1. Effect of Mexidol on State of Various Components of MCB during DLP and its Correction ($M \pm m$)

Experimental conditions	Area of cross section of microvessels, μ				
	arterioles	venules	precapillaries	capillaries	postcapillaries
Intact (control)					
At beginning of experiment (5)	205,60±7,20	310,30±13,62	40,36±2,32	35,95±1,20	85,63±4,95
At end of experiment (5)	196,15±18,54	443,15±41,20*	35,49±4,78	39,71±2,53	124,17±7,64*
2 months on AGD					
1- (10)	108,21±5,24*	459,24±18,50*	28,50±2,43*	29,95±1,60*	114,74±5,23*
2- (10)	229,25±13,6	445,43±7,80*	48,21±5,70	39,52±1,10	101,20±7,20*
Correction of DLP (9 months)					
1- (10)	142,36±6,31*	428,08±16,89*	37,07±1,71	31,75±1,91	88,18±3,86
2- (10)	215,63±9,27	399,73±14,09*	43,77±2,23	40,39±5,18	140,15±10,13*

Legend. *$p < 0.05$ Compared with value in animals of control group at beginning of experiment. Number of animals in group shown in parentheses.

In the animals of group 1, after 6 months on SD the ALP/HDL ratio, the MDA concentration, and the degree of echinocytosis came close to their initial level. In group 2 during this period a tendency was observed for ALP, the ALP/HDL ratio and the MDA concentration to rise, but not significantly, and the degree of echinocytosis also rose a little, and was accompanied by an increase in the number of normocytes in the blood (Figs. 1 and 2).

After 9 months the parameters of lipid metabolism and of LPO in the animals of group 2 regained their initial values (Fig. 2), the changes in MCB disappeared, leaving only a minor degree of dilatation in the venules (Table 1). The adrenergic innervation of the microvessels, just as in the control, was represented by thin axons with small varicose expansions. The intensity of specific luminescence of catecholamines was 4.6 ± 0.3 conventional units, the same as its value in intact animals at the beginning of the experiment. IAD did not exceed 1%.

The dynamics of these parameters was opposite in character in animals of group 1 after 9 months on SD. Besides an increase in ALP and, in particular, in the MDA level, significant changes also were found in MCB: constriction of arterioles, reduction of the capillary lumen, dilatation of venules, and intra- and extravascular disturbances. The adrenergic perivascular plexuses consisted of thickened axons with large varices, the intensity of luminescence of which was significantly higher than normal, at 5.3 ± 0.4 conventional units. An increase in the number of echinocytic forms of erythrocytes and an increase in their aggregating powers were found in the animals' blood (Figs. 1 and 2).

It is noteworthy that when parameters of the state of MCB in control animals of the same age were studied a tendency was found for the lumen of the arterioles to be reduced, for the venules of MCB to be dilated (Table 1), and for a decrease in the intensity of specific luminescence of catecholamines in the adrenergic axons innervating the microvessels.

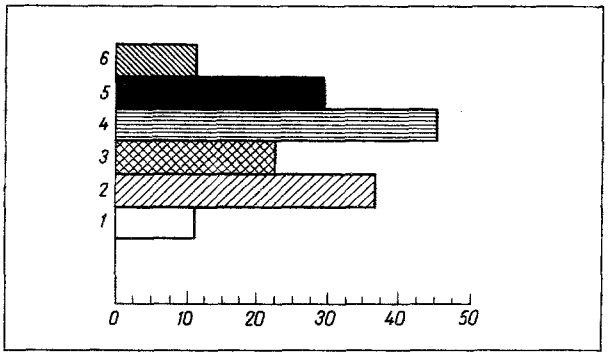

Fig. 3. MDA concentration (in nmoles/ml) in peripheral blood of rabbits with DLP and during its correction. 1) Control, 2) 2 months on AGD, 3) 2 months on AGD + AO, 4) 3 months of spontaneous regression after 2 months on AGD, 5) 9 months on SD after 2 months on AGD, 6) 9 months on SD after 2 months of a combination of AGD with AO.

Thus the addition of mexidol to the AGD prevented disturbances of lipid homeostasis in the microcirculation due to DLP, and was accompanied by regression of atherosclerotic changes in the aorta. A more marked effect of AO on the state of lipid metabolism of the microcirculatory system and on the degree of atheromatosis in the aorta was found when LPO was inhibited in conjunction with correction of DLP. Consequently, starting with the early stages of atherogenesis, definite correlation was found between the severity of MCD and of the atherosclerotic process in the aorta.

Abolition of the structural changes in the adrenergic apparatus of the microvessels and the lowering of tone of the arterioles and precapillaries in animals receiving mexidol are evidently connected with the beneficial action of AO on the sympathicoadrenal system. Investigations [8] have shown that AO belonging to the 3-hydroxypyridine class possess high psychotrophic activity and that their antistress action is linked with involvement of neurotransmitter, including catecholaminergic, systems.

The results as a whole are evidence of the important role of LPO activation in the development of MCD associated with DLP and on the possibility of using AO of the 3-hydroxypyridine class to abolish these disturbances, especially if accompanied by correction of DLP. A combined attack on the different stages of atherogenesis may be more effective in the prevention and treatment of atherosclerosis.

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