Correction of Hyperlipoproteinogenic Microangiopathy and Organ Pathology with Thymalin and Leu-Enkephalin in the Early Stages of Atherogenesis

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The effects of thymalin and leu-enkephalin on lipid peroxidation and microcirculatory disorders in the early stages of atherogenesis are compared. Correction of the generalized microcirculatory response to hyperlipoproteinemia with the peptides manifested itself in the regression of atherosclerotic lesions in the aorta and restoration of the morphofunctional state of the myocardium and liver.

Key Words: *thymalin; leu-enkephalin; hyperlipidemia; microangiopathy; myocardium; liver*

The universal nature of the primary microcirculatory response to hyperlipidemia (HLP) indicates that this reaction is a hyperlipoproteinogenic microangiopathy and an obligatory factor in the genesis of organ pathology, including the progression of atherosclerotic changes in organ and major arteries [3-5,12]. Previously, HLP correction and inhibition of lipid peroxidation (LPO) was shown to have a positive effect and a relationship was established between the degree of these disorders, on the one hand, and atherosclerotic lesions in organ and major vessels and organ pathology, on the other [8,9,15].

Impairment of the immunological status of the organism [6] and of the integral neurotransmitter regulation play an important role in atherogenesis. A polypeptide isolated from the thymus has been used for the correction of neuroimmune processes [1,11]. Endogenous regulatory opioid peptides are involved in the maintenance of homeostasis in different pathologies, specifically, enkephalins have been found to possess a hypolipidemic activity [7,10,14].

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We examined the effect of thymalin and leu-enkephalin on lipid metabolism disorders, LPO, microcirculatory disorders, and structural shifts in the target organs in the early stages of atherogenesis.

MATERIALS AND METHODS

Experiments were performed on 43 male Chinchilla rabbits weighing 2-2.5 kg. Group 1 rabbits (control) were maintained on the standard diet during a 2-month period. Group 2 rabbits were given 0.3 g/kg body weight cholesterol with vegetables (atherogenic diet, ATD) during the same period. Rabbits of groups 3 and 4 received ATD for 1 month and then were injected with thymalin (25 mg/kg) and leu-enkephalin (40 μg/kg) intramuscularly every day for 10 days of the second month. The animals were sacrificed by air embolism.

The state of the microcirculatory bed (MCB) was examined using total film preparations of the intestinal mesentery (the method of V. V. Kupriyanov). The cross-section area of microvessels was determined with an ASM semiautomatic image analyzer (Leitz), and the adrenergic innervation was studied by the method of Falk-Owmen. The intensity of catechola-

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mine fluorescence in the vascular bed terminals was assessed in a LYuMAM microscope. Routine erythrocyte preparations were studied in an S-500 scanning electron microscope (Hitachi). The index of atherosclerotic damage to the aorta was determined by the method of G. G. Avtandilov. Vascular alterations in the myocardium and liver were studied under a light microscope (Goldman and Van Gieson staining). For electron microscopy pieces of the organs were fixed in 1% buffered osmium tetroxide solution, embedded in Araldite, contrasted with uranyl acetate and lead citrate after Reynolds, and viewed in a JEM-7A electron microscope. Lipoprotein fractions and the malonic dialdehyde content were determined in the serum using conventional techniques.

RESULTS

Disorders of lipid homeostasis and activation of LPO were observed in group 2 rabbits two months after the start of the study. The concentration of atherogenic lipoproteins increased more than 10-fold compared with the control, the malonic dialdehyde content increased 3-fold, and the index of aortic damage was 20% (Figs. 1 and 2). In the MCB, pronounced vascular, intra-, and extravascular alterations - arteriolar constriction, venular dilatation, and massive intravascular aggregates - were seen. The intensity of catecholamine fluorescence in the adrenergic component of the microvascular innervation increased (Table 1). The number of echinocytes in the venous blood increased and the number of normocytes decreased. The microcirculatory disorders developed by rabbits fed ATD for 2 months were paralleled by dystrophic and destructive alterations of the myocardium and liver.

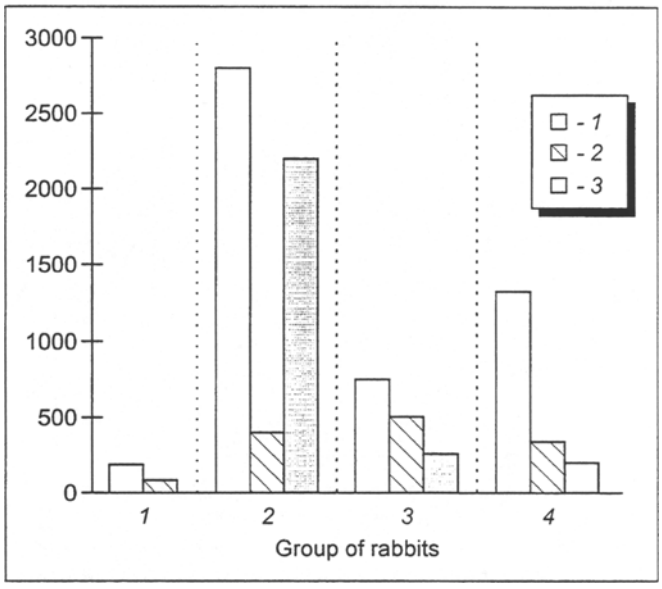

Fig. 1. Changes in the lipoprotein fraction (mg/100 ml) and the index of atherosclerotic damage to the aorta (×100%) in HLP and HLP corrected with thymalin and leu-enkephalin. *1*) atherogenic lipoproteins; *2*) high density lipoproteins; *3*) index of atherosclerotic damage to the aorta.

Administration of the peptides against the background of ATD (groups 3 and 4) led to a restoration of lipid homeostasis, inhibition of LPO, and termination of changes in the MCB. The content of atherogenic lipids was increased 3- and 5.5-fold and the malonic dialdehyde level was raised 2- and 2.5-fold, respectively. The index of atherosclerotic damage to the aorta was 2.6 and 3.5%, respectively (Figs. 1 and 2). Arteriolar and capillary constriction was much less pronounced, the diameter of capillaries was the same as in the control, venular dilatation was preserved, and the intensity of catecholamine fluores-

TABLE 1. Effect of Thymalin and Leu-Enkephalin on the State of Different MCB Components and Adrenergic Innervation of Microvessels (*M*±*m*)

Rabbits	Cross-section area, μ^2					Intensity of catecholamine fluorescence, arb. units
	arterioles	venules	precapillaries	capillaries	postcapillaries	
Group 1: control (*n*=13)	205.60±7.2	310.30±13.02	40.36±2.32	35.95±1.20	85.63±4.95	4.6±0.05
Group 2: 2 months on ATD (*n*=10)	108.21±5.24*	459.24±18.50*	28.50±2.49*	29.95±1.60*	114.74±5.23*	6.3±0.4*
Group 3: 2 months on ATD+thymalin (*n*=10)	178.37±3.10*	379.22±16.51*	36.20±1.42	31.60±2.17	113.11±4.29*	5.1±0.4*
Group 4: 2 months on ATD+leu-enkephalin (*n*=10)	192.24±12.75	356.18±16.84*	42.60±3.21	38.20±2.61	105.29±6.84*	5.5±0.28*

Note. Asterisk indicates significant differences compared with the control animals.

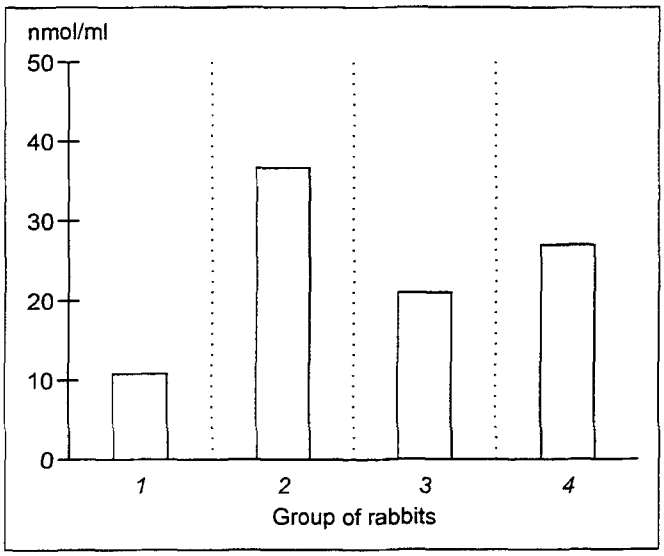

Fig. 2. Dynamics of the blood malonic dialdehyde content in HLP and HLP corrected with thymalin and leu-enkephalin.

cence diminished (Table 1). We did not find any positive effect on the erythrocyte membranes or on erythrocyte transformation.

It was important to find out whether there is a relationship between the intensity of microcirculatory disorders and the morphofunctional state of internal organs. It is known that the liver plays a key role in the regulation of lipid metabolism and that the myocardium is the main target organ in HLP.

Signs of vacuolar, granular, and fatty dystrophy were found in the liver 2 months after the start of the study. In hepatocytes, the organelles were vacuolized, glycogen was absent, focal chromatolysis occurred in the nuclei, the cytoplasmic reticulum was fragmented, its membranes were degranulated, and the number of ribosomes and polysomes was decreased. Lipemic plasma and erythrocyte aggregates in the microvessels of the portal tracts, plasmarrhagia, and massive perivascular mononuclear infiltrates were seen. These changes can be interpreted as different stages of nonspecific reactive hepatitis. Thus, alterations of tissue metabolism and hepatocyte function develop in the early stages of atherogenesis.

Hepatocytes of peptide-treated rabbits contained predominantly small lipid inclusions, their organelles were less damaged, mitochondria were accumulated around lipid droplets, and the number of secondary lysosomes was increased. In the microvessels, the plasma was not lipemic and erythrocyte aggregates were not seen. The macrophagal reaction and resorption of lipids were more intensive, the structure of hepatic trabeculae was restored.

Pronounced vascular alterations - capillary stasis, plethora and stagnation in postcapillary and collecting venules and veins, dilatation of small arteries, lipemic plasma in microvessels, and erythrocyte aggregates - were seen in the myocardium of rabbits given ATD for 2 months. Against the background of arteriolar constriction, small intramural arteries formed closed circuits, where lipids were deposited. Damage and transformation of the myocardial MCB coincided with the development of dystrophic and destructive changes in the myocardial contractile apparatus (mitochondria, myofilaments, myofibrils, intercalated and Z disks, etc.) and an increase in the number of collagen fibrils in the pericapillary space and between cardiomyocytes. On the whole, the changes occurring in the myocardium of rabbits fed ATD for 2 months were similar to those observed in myocardiodystrophy.

There were no manifestations of fatty dystrophy in the cardiomyocytes of rabbits treated with the peptides. The state of the mitochondria and nuclei testified to stepped-up protein synthesis apparently aimed at triggering intracellular reparative processes and restoring the structure and function of cardiomyocytes. Lipemic plasma and erythrocyte aggregates were not found in myocardial microvessels. On the basis of these findings, we have concluded that the structural and functional alterations occurring in the myocardium in the early stages of atherogenesis can be reversed by the correction of microcirculatory disorders. Thymalin was more effective in the correction of HLP-related structural shifts in both the myocardium and liver.

Thus, a generalized reaction of the microcirculatory system to HLP was observed during early disorders of lipid metabolism. This reaction was accompanied by the development of organ pathology without pronounced atheromatous alterations in organ and major arteries. New concepts on the pathogenesis of chronic noninfectious diseases developing on the basis of hyperlipoproteinogenic microangiopathy allow us to regard atherosclerosis as a systemic disease and to conclude that HLP, being a risk factor of atherosclerosis, causes an "ascending" type of alterations in the vascular bed.

The peptides thymalin and leu-enkephalin produce a positive effect on lipid metabolism, LPO, and circulatory disorders and put an end to atheromatous changes in major arteries and pathological alterations in internal organs in HLP. The superior effect of thymalin on the HLP-related changes in the myocardium and liver points to the importance of correcting immune status disorders in the early stages of atherogenesis. The beneficial effect of leu-enkephalin on microcirculatory disorders may be associated with a decrease in the sensitivity of the microvessels' adrenoreceptors to the sympathetic neurotransmitter and with the lymph-stimulating effect of this peptide [2,13].

REFERENCES

1. G. A. Vilkov, E. M. Stepanenko, and G. N. Kryzhanovskii, *Byull. Eksp. Biol. Med.*, **104**, № 9, 288 (1987).
2. A. S. Gavrish, *Arkh. Pat.*, № 5, 11 (1990).
3. E. D. Klimenko, A. K. Kranchev, and O. M. Pozdnyakov, *Byull. Eksp. Biol. Med.*, **92**, № 11, 530 (1981).
4. E. D. Klimenko and O. M. Pozdnyakov, *Ibid.*, **114**, № 10, 437 (1992).
5. E. D. Klimenko, *Hyperlipoproteinogenic Microangiopathy in the Genesis of Organ Pathology and Pathogenetic Approaches to its Correction*, Doctoral Dissertation [in Russian], Moscow (1994).
6. A. N. Klimov, V. A. Nagornov, and Yu. N. Zubzhitskii, *Kardiologiya*, № 12, 22 (1982).
7. E. Ya. Nasonov and V. A. Vinogradov, *Byull. Vsesoyuzn., Kardiol. Nauch. Tsentra*, № 1, 3 (1987).
8. O. M. Pozdnyakov and E. D. Klimenko, *Vestn. Akad. Med. Nauk SSSR*, № 2, 35 (1988).
9. O. M. Pozdnyakov, E. D. Klimenko, L. P. Kobozeva, *et al.*, *Byull. Eksp. Biol. Med.*, **115**, № 3, 242 (1993).
10. V. D. Slepushkin, Yu. B. Lishmanov, G. K. Zoloev, *et al.*, *Usp. Fiziol. Nauk*, № 4, 106 (1985).
11. V. Kh. Khavinson, *Vopr. Med. Khimiii*, № 3, 41 (1990).
12. V. K. Khugaeva, P. N. Aleksandrov, E. D. Klimenko, *et al.*, *Byull. Eksp. Biol. Med.*, **100**, № 7, 15 (1985).
13. V. K. Khugaeva, *Fiziol. Zh. SSSR*, **76**, № 1, 92-99 (1990).
14. F. Bloom and I. Herd, in: *Behaviour and Arteriosclerosis*, New York (1983), p. 33.
15. O. M. Pozdnyakov and E. D. Klimenko, *Microcirculatory Studies: Clinical and Experimental*, Linkoping (1991), p. 3.