Effects of Thymalin and α-Tocopherol on Morphofunctional State of Neuroendocrine System at the Early Stages of Atherogenesis

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The effects of thymalin and α-tocopherol on the morphofunctional state of hypothalamo-hypophyseal neurosecretory system, thyroid gland, and adrenals at the early stages of atherogenesis are described. Correction of pathological changes in the neuroendocrine organs was accompanied by restoration of lipid homeostasis, decrease in intensity of lipid peroxidation, prevention of progressive atherosclerotic alterations in major arteries.

Key Words: *thymalin; α-tocopherol; atherosclerosis; neuroendocrine system; thyroid gland; adrenals*

The hypothalamohypophyseal neurosecretory system (HHNS) is a key component of neuroendocrine regulation of lipid homeostasis [2,10]. A correlation has been established between functional state of HHNS during hyperlipoproteinemia and the degree of pathological changes in the microcirculatory bed and major arteries [3,4,6]. The HHNS-produced hormone vasopressin has immunomodulating properties and participates in formation of immunological status [9]. An important role in pathogenesis of atherosclerosis is played by disorders in the function of endocrine glands [5,8]. The state of neuroendocrine system during hyperlipoproteinemia is most certainly affected by activation of lipid peroxidation and decreased antioxidant activity.

We compared the effects of a thymus hormone thymalin and α-tocopherol on the morphofunctional state of HHNS, thyroid gland, and the adrenals at the early stages of atherogenesis.

MATERIALS AND METHODS

Experiments were performed on 45 male Chinchilla rabbits weighing 2.5-3.0 kg. Group 1 rabbits (control) were maintained on the standard diet. Group 2 rabbits were given 0.3 g/kg body weight cholesterol during a 2-month period (atherogenic diet model according to N. N. Anichkov). Group 3 rabbits were injected with intramuscular thymalin (0.25 mg/kg) during 10 days on month 2 of the diet. Similarly, group 4 rabbits were injected with α-tocopherol (50 mg/kg).

In serial brain slices the hypothalamic supraoptic (SO) nuclei and neurohypophysis were studied by the methods of Gomori—Maiorova, Nissl, and Milenkov. The functional state of HHNS was accessed according to [7], taking into account the amount of gomori-positive substance in the neurosecretory cells, hypothalamohypophyseal tract and neurohypophysis. The percentage of "bright" and "dark" cells was counted in the SO nuclei, as well as the amount of pyknomorphous cellular elements. The value of neuronal nucleus and soma areas were determined a Leitz-ASM semiautomatic image analyzer. Structural changes in endocrine glands were revealed by staining with hematoxylin and eosin according to Goldman, Mallory, and van Gieson. The mass indices of thyroid and adrenals were determined, and the index of atherosclerotic damage (IAD) to the aorta [1] was used to access the severity of atherosclerotic process. Lipoprotein (LP) fractions and malonic dialdehyde were determined in serum using conventional techniques.

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RESULTS

After two months on atherogenic diet (group 2 rabbits), the content of atherogenic LP increased more than 20-fold compared with the control, the antiatherogenic LP (HDL) content increased 5-fold, that of malonic dialdehyde 3-fold, and IAD to the aorta was 20% (Table 1).

Pronounced structural alterations were found in HHNS, thyroid gland, and adrenals. The number of "bright" cells in the hypothalamic neurosecretory nuclei decreased by 25% in comparison with intact animals (Fig. 1). In most neurons the cytoplasm was filled with neurosecretory granules, penetrating into cellular projections. Accumulation of neurosecretory substance was also observed in other parts of HHNS (hypothalamohypophyseal tract, and neurohypophysis). The nuclei of the "dark" cells were pyknotic and diminished in size (Fig. 1). Numerous neurosecretory neurons were in the stage of "red pyknosis". Combination of these signs makes it possible to diagnose functional activity of HHNS after a 2-month diet as decreased with a tendency of breakdown.

The mass of thyroid glands was decreased 1.5-fold (Table 2). Small follicles with insignificant content of colloid predominated in the parenchyma. Fatty degeneration was observed in the thyroid epithelium, where the height of cells and volume of their nuclei were drastically decreased. Diffuse and interstitial scleroses were observed in the glandular stroma.

In group 2 rabbits, the adrenal mass increased 2-fold in comparison with the control (Table 2). The thickness of fascicular and reticular zones increased, with a simultaneous decrease in the thickness of glomerular zone. Similar to HHNS, in this period the initial signs of functional degeneration appear in the adrenals (necrobiosis and necrosis of some cells predominantly in the fascicular and reticular zones).

Addition of thymalin to the ration (group 3 rabbits) resulted in a 3.7-fold decrease in blood atherogenic LP compared with group 2 rabbits. At the same time, blood HDL was slightly increased,

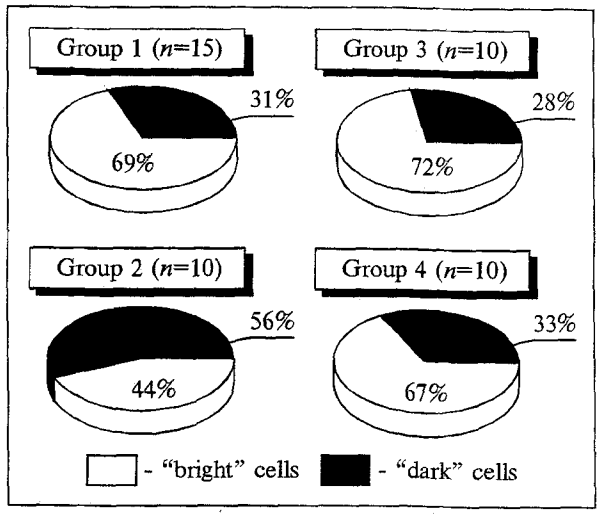

Fig. 1. Effect of thymalin and α-tocopherol on the ratio of "dark" and "bright" cells in the hypothalamic SO nuclei.

while malonic dialdehyde was decreased (Table 1). Aortal IAD was 2.6%.

The sings of activation were marked in group 3 rabbits. The "bright" cells were predominant in SO nuclei, which were oval or polygonal, large bulla-like nuclei, and a small number of neurosecretory fine-grained granules arranged in a narrow band around the nuclei. Morphometric indices characterizing the size of nuclei and neurosecretory neurons did not differ from that of the intact rabbits (Table 2).

Thyroid mass increased 1.3-fold against the control (Table 2). In the thyroid gland, the mean diameter of folliculi decreased, the cell height and nuclear volume of thyroid epithelium increased, the signs of enhanced colloid resorption were observed, the number of parafollicular cells increased, and microfolliculi were formed. Thus, functional activity of the thyroid gland was maintained at a rather high level.

Adrenal mass in group 3 rabbits changed slightly compared with the control (Table 2). Microscopy revealed just negligible alterations in the cortex.

In group 4 rabbits (α-tocopherol against the background of atherogenic diet), the level of athero-

TABLE 1. Effect of Thymalin and α-Tocopherol on Blood Biochemical Indices and the Index of Atherosclerotic Damage (IAD) to the Aorta (*M*±*m*)

Group	ALP, mg/100 ml	HDL, mg/100 ml	Malonic dialdehyde, nmol	Aortal IAD, %
Croup 1, control (*n*=15)	131.5±8.2	81.2±6.3	11.4±3.2	0
Croup 2, 2-month diet (*n*=10)	2805.2±16.4	406.1±10.5	36.3±6.1	20
Group 3, 2-month diet+thymalin (*n*=10)	754.4±9.0	511.4±11.0	22.6±4.3	2.6
Group 4, 2-month diet+α-tocopherol (*n*=10)	1366.4±11.1	506.2±9.6	28.5±6.3	7.1

Note. ALP — atherogenic lipoproteins, HDL — high density lipoproteins.

TABLE 2. Effect of Thymalin and α-Tocopherol on Morphometric Indices of Neurosecretory Neurons and Mass Indices of Some Endocrine Organs (*M±m*)

Group	Hypothalamic SO nuclei		Mass, mg			
	area of neuron's nucleus, μ^2	area of neuron's soma, μ^2	thyroid		adrenals	
			left	right	left	right
Croup 1, control (*n*=15)	39.3±1.7	113.5±2.7	222.3±21.7	206.6±18.3	292.9±27.0	255.5±28.2
Croup 2, 2-month diet (*n*=10)	34.5±0.8	121.3±7.3	145.6±11.3	137.4±9.6	496.3±11.2	477.3±16.2
Group 3, 2-month diet+thymalin (*n*=10)	40.8±1.2	105.9±8.5	281.5±11.1	272.4±6.6	343.5±10.4	308.3±4.5
Group 4, 2-month diet+ α-tocopherol (*n*=10)	37.8±1.9	118.2±4.1	308.2±12.5	269.1±11.2	444.2±9.6	448.6±8.2

genic LP decreased approximately 2-fold compared with group 2 rabbits (2-month cholesterol diet), HDL and malonic dialdehyde were almost the same as in group 2 rabbits (Table 1). Aortal IAD was 7.1%.

In hypothalamic SO nuclei the ratio of "bright" and "dark" cells was close to the control value (Fig. 1). Morphometric indices characterizing the size of neuronal soma and nuclei were also close to the control values (Table 2). However, in the axons of hypothalamohypophyseal tract the granules of neurosecrete were found, which was also revealed in neurohypophysis as Hering bodies of small and middle size, which is known to indicate delayed neurosecretion.

The thyroid mass was 1.4-fold larger than the control value (Table 2). In contrast to variability of morphological indices characterizing functional state of the thyroid gland, it demonstrated a tendency to normalization of functional activity.

The adrenal mass in group 4 rabbits was increased to a lesser degree than in animals receiving atherogenic diet (Table 2). The cytoplasm in some cells of glomerular and reticular zones was vacuolized, the nuclei were decreased and hyperchromic, while some cells were in the necrobiotic state. In the whole, pathological alterations in the adrenals of group 4 rabbits were less expressed than in group 2.

Thus, thymalin and α-tocopherol produce positive effect on the state of neuroendocrine system at the early stages of atherogenesis. Correction of disorders in this system was accompanied by inhibition of lipid peroxidation, restoration of lipid homeostasis, and by less severe atherosclerotic changes in major arteries. A better correction of structural alterations in HHNS and endocrine glands by thymalin may be due to immunocorrecting properties of this peptide. Our findings indicate that effective prevention and therapy of atherosclerosis require modification of the different pathological stages of this disease.

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