GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Interrelation between Different Stages of Atherogenesis in Rabbits with High and Low Resistance to Dyslipidemia

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Changes in lipid metabolism and morphofunctional state of neuroregulatory and circulatory systems during dyslipidemia were studied in rabbits with high and low resistance to this risk factor. It was found that impairment of neuroregulatory systems plays an important role in initiation and progression of microcirculatory disorders and atherosclerosis. Atherogenic index is the most informative parameter for evaluation of pathological changes in the vascular system during dyslipidemia.

Key Words: *dyslipidemia*; *individual resistance*; *neuroregulatory system*; *microcirculation*; *aorta*

Numerous studied showed that dyslipidemia is associated with damage to the endothelium, disturbances in permeability of the microcirculatory bed (MCB) in various organs, spasm of arterioles and small arteries, leukocyte adhesion, microthrombosis and circulatory hypoxia even at the early stages of dyslipidemia. Disturbances in MCB determine the development and progression of polyorganic disorders [1,3, 4,6,8].

Detailed analysis of structural changes in MCB, organ and major arteries during experimental atherogenesis in rabbits revealed some peculiarities in the reaction to dyslipidemia in different animals. Correction of different factors of atherogenesis with various bioactive substances demonstrated a correlation between impairment in regulatory mechanism and the severity of vascular and organic pathologies [2,5,9,10].

Here we studied interrelations between some parameters of lipid metabolism, activity of the hypothalamic-pituitary neurosecretory and sympathoadrenal systems, microcirculatory disturbances, and the severity of atherosclerosis in rabbits with high and low resistance to dyslipidemia.

MATERIALS AND METHODS

Experiments were carried out on 52 male Chinchilla rabbits (2.5-3.0 kg). The animals received standard (control) or atherogenic diet (ATD), which included cholesterol in a daily dose of 0.3 g/kg body weight (Anichkov's model) for 1, 2, or 3 months. Plasma lipoprotein fractions were analyzed [7]. Index of atherosclerotic lesion in the aorta (ALI) was measured by the method proposed by G. G. Avtandilov. The state of MCB was evaluated morphometrically on total mesenteric preparations impregnated with $AgNO_3$ by Kupriyanov using a Leitz ASM image analysis system. Erythrocytes were fixed and stained using conventional technique and examined under an S-500 Hitachi scanning microscope. Adrenergic innervation of the terminal vascular bed was studied by Falck—Owman histochemical method. Intensity of catecholamine fluorescencence was measured photometrically using a Lumam fluorescencence microscope. Supraoptic nuclei of the hypothalamus and neurohypophysis were examined on serial brain sections stained after Gomory,

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Nissl, and Milenkov. The functional state of the hypothalamic-pituitary neurosecretory system was assessed using Polenov's scheme. The content of Gomory-positive substances in neurosecretory cells, hypothalamic-pituitary tract, and neurohypophysis, the percentage of light, dark, and pyknomorphic cells in the supraoptic nuclei, and the areas of neuronal nuclei and bodies were determined.

RESULTS

The animals were divided into two groups, low and high resistant, depending on their ALI (Table 1).

The content of both atherogenic and antiatherogenic lipoproteins considerably increased during ATD in both groups, but to a different extent. The coefficient of atherogenicity in highly resistant animals was significantly lower than in animals with low resistance.

The number of normocytes in highly resistant rabbits after a month of ATD remained unchanged, while the number of echinocytes sharply increased. At later terms, both these indices in low resistant rabbits more markedly differed from control.

Intravascular and perivascular pathological changes were seen in capillaries, arterioles, and venules of animals receiving ADT. Pronounced tortuosity of venules, microaneurysms and abnormal arteriole-to-venule diameter ratio were found. In both groups the number of functioning capillaries increased, but their lumen area decreased, especially in low resistant animals. After 1-2-month ATD, spasm of arterioles was more pronounced in low resistant animals (Table 2).

Disturbances in the sympathoadrenal system play an important role in the pathogenesis of atherosclerosis. Catecholamines injected to experimental animals can promote and accelerate atherogenesis. Arterioles and precapillaries are innervated predominantly by adrenergic nerve fibers releasing norepinephrine, which not only controls the tone of microvessels, but also participates in the regulation of cholesterol metabolism. Our results demonstrated dramatic structural and functional changes in adrenergic innervation of microvessels during dyslipidemia, which were probably responsible for spasm and enhanced aggregation in microvessels.

It is now accepted that dyslipidemia and atherosclerosis are associated with disturbances in endothelium-dependent vascular relaxation due to NO deficiency [11]. Higher intensities of catecholamine fluorescence during ATD, especially, in low resistant animals (Table 2) suggest that sympathetic hyperactivation also contribute to arteriolar spasm. It should be noted that varicose structure of adrenergic fibers was preserved in highly resistant rabbits, while in low resis-

TABLE 1. Effects of Dyslipidemia on Lipid Metabolism, Index Atherosclerotic Lesion (ALI) of the Aorta, and Peripheral Blood Erythrocyte Transformations in Rabbits Receiving Atherogenic Diet (*M±m*)

Parameters	Control	ATD, months					
		1		2		3	
		highly resistant	low resistant	highly resistant	low resistant	highly resistant	low resistant
Content of LDL+VLDL, mg/100 g	131.5±8.2	620.8±40.2	809.0±51.6	1173.4±101.2	2320.7±152.4	2640.0±169.7	3166.0±204.5
Content of HDL, mg/100 g	81.2±6.3	353.0±28.4	375.0±19.8	453.2±33.2	752.4±42.5	263.0±18.9	220.0±21.6
Atherogenicity index	1.62	1.75	2.16	2.58	3.08	10.03	14.39
ALI, %	0	0	1.5	13.7	28.9	29.4	56.2
Types of erythrocytes, %							
normocytes	24.5±2.9	22.5±6.1	13.6±1.2	17.2±4.0	13.8±1.9	18.2±3.7	6.3±1.2
echinocytes	6.8±1.5	24.2±3.3	38.1±3.4	33.1±2.6	41.6±4.4	43.4±3.2	58.1±6.8

TABLE 2. Changes in Microcirculatory Bed at Various Terms of Atherogenic Diet (*M*±*m*)

Parameters	Control	ATD, months					
		1		2		3	
		highly resistant	low resistant	highly resistant	low resistant	highly resistant	low resistant
Cross-section area, μ²							
arterioles	205.6±7.0	134.0±8.3	114.5±6.8	126.4±4.6	93.6±8.2	120.4±7.8	233.5±19.5
precapillaries	40.4±2.3	35.3±1.9	32.6±2.5	22.7±2.4	33.2±1.7	20.4±1.8	53.1±4.2
capillaries	36.0±1.2	28.6±3.2	30.5±2.4	33.1±2.7	20.9±18.6	19.34±2.40	39.8±21.9
postcapillaries	85.6±5.0	92.8±3.2	87.5±2.8	88.9±7.3	132.6±10.5	121.3±7.5	143.5±9.7
venules	310.0±13.6	421.6±23.9	428.3±18.6	345.8±31.9	572.5±37.4	420.0±32.8	593.7±20.3
Intensity of catecholamine fluorescence, arb. units	4.4±0.2	6.5±0.3	7.2±0.6	5.8±0.4	7.0±0.5	6.9±0.8	3.7±0.1

TABLE 3. Changes in Hypothalamic-Pituitary Neurosecretory System at Various Periods of Atherogenic Diet (*M*±*m*)

Parameters	Control	ATD, months					
		1		2		3	
		highly resistant	low resistant	highly resistant	low resistant	highly resistant	low resistant
Light-dark cell ratio	69/31	72/28	79/21	60/40	54/56	45/55	40/60
Area, μ²							
cell body	39.3±1.7	43.4±1.8	47.0±0.5	37.2±3.0	31.8±2.7	27.8±2.6	21.3±2.2
nucleus	113.5±2.7	126.8±6.8	130.9±9.1	118.4±12.4	101.7±9.6	400.6±9.4	100.9±7.5

tant animals we observed uniform bright lines along microvessels.

As atherosclerosis progressed (ATD for 3 months), arteriolar and venular dystonia developed in low resistant animals. Arterioles and venules were filled with blood cell aggregates of various sizes; clusters consisting of leukocytes and other blood cells appeared in the perivascular space, which attested to progressive disturbances in microvessel permeability. The intensity of catecholamine fluorescence decreased. In highly resistant animals parameters of MCB did not differ from those at the previous term (Table 2).

The number of light cells in the supraoptic hypothalamic nuclei increased during the first month of atherogenic diet and decreased by the end of the second month. These changes were most pronounced in low resistant rabbits (Table 3). Together with increased number of dark cells and other signs, this fact attested to impairment of the hypothalamic-pituitary neurosecretory function. Increased number (20%) of red pyknotic neurons in low resistant rabbits compared to the control (9%) and highly resistant rabbits (13%) also attested to exhaustion of the hypothalamic-pituitary system.

Our findings indicate that dysfunction of regulatory systems plays an important role in the development and progression of microcirculatory disorders and atherosclerosis during dyslipidemia. The interrelation between parameters characterizing rabbit tolerance to atherogenic diet was most evident at the early stages of atherogenesis. This can be attributed to peculiarities of the experimental model. Comparison between groups with low and high resistance to dyslipidemia showed that index of atherogenicity, which closely correlated with aortic ALI, was the most informative parameter for assessment of vascular disorders. Our results confirmed adequacy of Anichkov's model for experimental study of atherogenesis.

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