GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Dyslipidogenic Microangiopathy in Guinea Pigs at Early Stages of Atherogenesis

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We studied the effect of dyslipidemia on lipid metabolism, state of microcirculatory system, and morphological alterations in the aorta and liver of guinea pigs at the early stages of experimental atherogenesis. The important role of microcirculatory disorders in the development of regional pathology and atherosclerosis is confirmed. The proposed alimentary model can be used in the development of novel methods for prevention and treatment of atherosclerosis.

Key Words: *dyslipidemia; atherosclerosis; microcirculation; guinea pigs*

Dyslipidogenic microangiopathy is a trigger process, which determines the development and progression of chronic nonspecific regional pathology and atherosclerotic alterations in regional and major arteries in Anichkov's rabbit model [1,3,5,7]. Recent studied demonstrated the leading role of microcirculatory disorders in triggering coronary heart disease and other forms of vascular pathology in humans [6,12,13]. Disorders in lipid metabolism are directly related to disturbances in cholesterol (CH) clearance in the liver during the development of fatty degeneration and chronic nonspecific hepatitis [2].

In our experiments dyslipidemia (DLP) and atherosclerosis in guinea pigs were induced using a dietary model. In contrast to rabbit model, this model is more precise in simulating the state developing during atherosclerosis in humans, because guinea pigs did not develop extreme rise in CH during exogenous hypercholesterolemia [10]. In guinea pigs, the morphological signs of atherosclerosis are revealed when the animals are kept on atherogenic diet (ATD) no less than for 4 months [11]. However, the state of microcirculatory bed (MCB) at the early stages was not examined in this model.

The search for new approaches to correct disturbances in lipid metabolism, microcirculation, and pathological alterations in various target organs is an actual and perspective work.

Our aim was to study the changes in blood lipid spectrum, the state of MCB, the structural changes in the aorta and liver of guinea pigs at the early stages of atherogenesis.

MATERIALS AND METHODS

Experiments were carried out on random-bred male guinea pigs (n=60) weighing 300-400 g. The animals were kept in plastic-metal cages (2-5 per cage) with periodic illumination and food and water *ad libitum*. Experimental pigs (n=30) daily *per os* received CH suspension (ultra pure grade, Amresco) dispersed in milk (0.1 g/100 g body weight, fat content 3.2%). Controls received fresh milk (fat content 3.2%) in addition to the standard ration. Cardiac puncture was

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performed under ether anesthesia. Blood concentrations of total CH, HDL CH (LDL+VLDL)CH and triglycerides (TG) were determined. The content of total CH and TG in the serum was determined enzymatically [9,14] in an Airone 200 analyzer (Crony Instruments) using combined diagnostic kits (Biocon). The content of CH in HDL in the serum was measured in the same way as the level of total CH in the supernatant after sedimentation of LDL with sodium phosphotungstate (12 mM) and $MgCl_2$ (0.5 mM) [9]. The total CH fraction (LDL+VDLL)CH was calculated as the difference between total CH and HDL CH. Standardization and quality control of experimental research complied with requirements of Federal system of independent quality assessment of clinical studies. Atherogenic index (AI) was calculated as (VLDL+LDL)/HDL.

The morphological changes in the liver and aorta were examined on cryostat sections stained for lipids by the method of Goldman. Aortic macropreparations were stained with Sudan IV for lipids. The state of MCB was accessed in total film preparations of the small intestine mesentery impregnated with silver nitrate according to V. V. Kupriyanov [4]. Microvessels were morphometried using a Leitz-ASM semiautomatic image analyzer.

The data were processed statistically using two-sample Student's *t* test with Bonferroni correction for multiple comparisons. Analysis was performed using Exel software. The differences were significant at $p<0.05$.

RESULTS

ATD for 2 months increased the total CH in guinea pig almost 2-fold due to atherogenic fractions of lipoproteins (VLDL and LDL). After 4 months of ATD the concentration of total CH increased by 3 times in comparison with intact animals (Table 1). Similar changes occurred with atherogenic fractions of lipoproteins. AI increased with increasing the duration of ATD.

After 2 months of ATD the level of total CH in control animals was lower than that in intact animals, but after 4 months it slightly surpassed this level. It should be noted that milk markedly decreased AI in guinea pigs. At the same time, the level of TG in control pigs was higher than that in intact and experimental (ATD group) animals.

After 2 months of ATD, vascular, intravascular, and extravascular alterations in MCB were noted: enhanced tortuosity of microvessels, constriction of afferent vessels, local spasms of arterioles, dilation of venules, and microaneurysms. There was also a coincolumn erythrocyte aggregation or dense aggregation of erythrocytes characteristic of sludge syndrome. The extravascular alterations manifested by diapedesis of erythrocytes, cell infiltrations, and perivascular edema. The number of conduit postcapillaries and capillaries increased, which probably reflects adaptive reaction of the terminal vascular bed to the damaging factor. The morphometric indices of MCB also changed (Table 2).

TABLE 1. Effect of Atherogenic Diet on Lipid Metabolism in Guinea Pigs (*M*±*m*)

ATD duration and group		Biochemical indices				Atherogenic index
		total CH	HDL CH	(LDL+VLDL) CH	TG	
Intact animals (*n*=8)		0.91±0.19	0.37±0.23	0.55±0.24	0.82±0.30	2.40±2.05
After 2 months	control (*n*=8)	0.74±0.10	0.53±0.12	0.21±0.14	1.30±0.17	0.45±0.37
	ATD (*n*=10)	1.73±0.56*+	0.55±0.39	1.18±0.59*+	0.82±0.38	3.32±2.99*+
After 4 months	control (*n*=8)	1.07±0.13	0.47±0.12	0.59±0.05	1.56±0.45	1.31±0.36
	ATD (*n*=10)	2.95±0.88*+	0.33±0.13	2.67±0.90*+	0.71±0.34	9.85±6.93*+

Note. Here and in Tables 2: $p<0.05$ compared to *intact and +control animals.

TABLE 2. Effect of Atherogenic Diet on Microcirculatory Bed in Guinea Pigs (*M*±*m*)

ATD duration and group		Cross-section area of microvessels				
		arteriole	venule	precapillary	postcapillary	capillary
Intact animals (*n*=10)		186.9±9.7	238.5±11.2	36.7±2.5	69.9±5.8	30.5±2.9
After 2 months	control (*n*=8)	191.7±6.9	254.0±20.5	38.9±3.6	64.3±7.3	28.7±3.8
	ATD (*n*=10)	117.6±18.5*+	334.8±19.6*+	31.5±3.2+	72.8±4.1	21.8±2.1*+
After 4 months	control (*n*=8)	184.5±12.3	261.7±23.6	34.9±4.1	65.6±5.2	26.9±2.1
	ATD (*n*=10)	109.4±17.6*+	451.8±28.5*+	30.6±2.4*	78.9±3.8+	22.1±3.6*

The observed alterations were more pronounced after 4 months of ATD. At this term, the exchange elements of the vascular bed were damaged more severely: many capillaries were thrombotic and did not participate in circulation. In addition, the capillary network was rarefied. Probably, alterations in the terminal vascular bed aggravated the pathological process. At this stage, some animals demonstrated stratification and lipid infiltration of the aortic intima. The macroscopic signs of atherosclerosis in the aorta (sports and plagues) were observed in some animals after 6 months of ATD.

After 2 months, the circulatory abnormalities were observed together with signs of vacuolar, granular, and fatty degeneration accompanied by disintegration of liver trabecules. Hepatocytes with lipid inclusions occupied 8-10% section area and were located predominantly around the central venules. During the 4th month of ATD, hepatocytes with lipid degeneration occupied up to 50-70% section area with the same localization, while the abnormalities of trabecular structure increased. Starting from month 2, mononuclear infiltrations were observed together with perivascular and periductal edema.

Thus, at the early stages of atherogenesis, guinea pigs demonstrated microcirculatory disturbances characteristic of dyslipidogenic microangiopathy accompanied by degenerative changes in the liver and insignificant lesion of the aorta. These observations corroborate the leading role of microcirculatory disturbances in the development of regional pathology and atherosclerosis. Simulation of the early stages of atherogenesis in guinea pigs can be useful in the development of novel methods of prevention and therapy of atherosclerosis.

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