MORPHOLOGY AND PATHOMORPHOLOGY

Structural Changes in the Myocardium and Serum Lipid Spectrum in Experimental Hypercholesterolemia and Hypothyroidism


We studied the peculiarities of lipid spectrum of the blood and structural reorganization of the myocardium in experimental hypercholesterolemia with and without hypothyroidism. It was found that alimentary hypercholesterolemia accompanied by elevated total cholesterol, LDL, HDL, and triglyceride concentrations led to a decrease in body weight, heart weight, number of cardiomyocytes in the heart and induced pronounced lytic changes in cardiomyocytes, circulation disorders (sludge syndrome, echinocytosis of erythrocytes, lymphostasis), diffuse fibrosis of the stroma, and appearance of foam cells among diffuse mononuclear infiltrate cells. The combination of hypercholesterolemia with hypothyroid status caused more pronounced changes in the lipid spectrum and atherogenic index and more pronounced lytic and necrobiotic changes in cardiomyocytes. These findings suggest that elevated cholesterol concentrations in the blood, especially against the background of suppressed thyroid function, can directly induce considerable damage to cardiomyocytes, intramural vessels, and erythrocytes without the development of myocardial ischemia and in the absence of atherosclerotic plaques.

Key Words: hypercholesterolemia; hypothyroidism; myocardium; blood lipid spectrum; structural analysis

Lipid metabolism disorders (dyslipidemia) characterized by increased cholesterol and triglyceride content in the blood are the major risk factors of atherosclerosis development and related cardiovascular diseases [5,7]. In most studies of atherosclerosis, especially in recent works, the attention is primarily paid to evaluation of changes and elucidation of the molecular mechanisms of intima damage, formation of atherosclerotic plaques, their progress, possible stabilization or rupture. According to current views, atherosclerotic plaque formation depends on endogenous and exogenous atherogenic factors promoting accumulation and modification of apoB-lipoprotein in the vascular wall, activation of endothelial cells, migration and activation of inflammatory cells, especially macrophages, proliferation of smooth muscle cells, modulation of collagen biosynthesis and degradation, and activation of blood clotting factors and platelets [12,14].

The focus on structural and functional changes in the vascular wall in atherosclerosis and hypercholes-
terolemia led to predominance of the viewpoint that circulatory disturbances caused by coronary artery stenosis (i.e., changes in the myocardium caused by ischemia and probably reperfusion) are the leading event in the pathogenesis of cardiac failure in atherosclerosis. At the same time, structural and functional changes in the myocardium developing under conditions of hypercholesterolemia prior to atherogenic occlusion of coronary vessels and their correction received little attention, which contribute to low efficiency of postischemic therapy, development of severe cardiac failure, and frequent lethal outcomes.

High level of disability and mortality from CHD and other complications of atherosclerosis necessitates timely diagnostics of hypercholesterolemia-induced damage to the myocardium and, consequently, adequate modeling of this pathology. A promising approach for hypercholesterolemia modeling in small rodents promoting the development and intensifying of the pathological process is supplementation of atherogenic diet with antithyroid preparations, e.g., mercazolyl. Mercazolyl (thiamazole) reduces blood concentration of thyroxin, one of the main thyroid hormones reducing cholesterol content in the blood and excessive fat [10].

Here we studied structural reorganization of rat myocardium in experimental hypercholesterolemia induced by atherogenic diet and hypothyroid status.

**MATERIALS AND METHODS**

Experiments were carried out on male Wistar rats weighing 390-560 g. Group 1 rats (n=6) received atherogenic diet (alimentary hypercholesterolemia model): cholesterol in a dose of 25 mg/100 g body weight (Panreac Quimica SA) added to standard laboratory ration. Group 2 rats (n=6) received the same atherogenic diet and antithyroid preparation mercazolyl (Akrikhin) in a dose of 1 mg/100 g body weight added to the laboratory food. The animals were fed according to the following scheme: day 1 – food with cholesterol/cholesterol+mercazolyl; day 1 – food deprivation; water was given *ad libitum* every day. The control group (n=6) were maintained under standard conditions and received standard food every day. The animals were kept in individual cages. The experiments were performed with strict adherence to the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.

The rats were decapitated in 68 days after the start of the experiment (in the first half of the day). After decapitation, the heart was carefully separated from surrounding tissues, weighed, and examined visually. The myocardial specimens were fixed in 10% neutral formalin. Paraffin sections were stained with hematoxylin and eosin with Perls reaction and by the van Gieson method. The specimens were examined under a Leica DM 4000B microscope and photographed using a Leica DFC 320 camera and Leica QWin software.

In the blood serum collected from the cervical veins immediately after decapitation, parameters of lipid spectrum of blood plasma (LDL, HDL, and triglycerides) were measured by the enzymatic method on a Labsystem biochemical analyzer using Biocon kits. Total cholesterol (CH) was determined as the sum of LDL and HDL; atherogenic index was calculated as HDL/LDL ratio. For evaluation of the thyroid status, serum thyroxin (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>) concentrations were measured by the method of immunochromeluminescence on a LM-01A luminometer (Bekman Coulter Company) using Immunotech kits. Summary activity of MMP-2 and MMP-7 in blood serum was also measured using a fluorescent substrate MCA-Pro-Leu-Gly-Leu-DPA-Ala-Arg-NH<sub>2</sub> (ICN Biomedicals Inc., Calbiochem) on a Shimadzu RF-5301 PC spectrofluorometer at extinction and emission wavelengths of 325 and 393 nm. Activity was expressed in μmol substrate MCA/liter/min.

The data were processed statistically using Statistica 6.0 software; significance of differences in case of normal distribution was evaluated by Student *t* test.

**RESULTS**

The combination of atherogenic diet and starvation (every other day) was used, because starvation, being a potent stimulus creating nutrient deficiency, disordered metabolism. Mobilization of lipids for tissue stores and disorders of lipid and carbohydrate metabolism led to the development of endogenous hypercholesterolemia and aorta lipidosis, while additional administration of exogenous cholesterol aggravated plastic metabolism disturbances [4]. In both groups, the body weight and heart weight significantly decreased by the end of the experiment, this decrease was more pronounced in group 1 (by 27 and 25%, respectively) than in group 2 (by 16 and 12%, respectively, Table 1).

After 68-day atherogenic diet, changes in lipid spectrum of blood serum appeared typical of hypercholesterolemia [3]. In group 1 rats, serum concentration of total CH increased by 9%, LDL by 10%, HDL by 9% and triglycerides by 16%; index of atherogenic remained unchanged. The increase in HDL concentration indicates activation of liver enzymes, but the dose of exogenous cholesterol was high, which led to increased blood concentration of HDL and enhanced production of triglycerides.

Activity of MMP-2 (gelatinase A specific to type IV collagen, the major component of basement membranes) and MMP-7 (matrilysin, a protease cleav-