ACTION OF PROTAMINE ON BLOOD LIPOPROTEINS IN RABBITS IN THE EARLY PERIOD OF HYPERCHOLESTEROLEMIA

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The absolute cholesterol (ChS) concentration and its relative distribution in the composition of different classes of lipoproteins (LP) are not the only aspects to be considered in evaluation of the role of these blood particles in atherogenesis and in the development of related diseases. Determination of the ratio of the protein components of LP has been shown to be very important in the diagnosis of atherosclerosis and in the prognosis of the course of cardiovascular diseases [4]. Results obtained indicate that the degree of reduction of the fraction of alkaline blood serum proteins may characterize the course of atherosclerosis [1]. In the course of development of cholesterolosis in animals a period characterized by elevation of the level of these proteins has been found [3]. The aim of the present investigation was to study the action of one of the polypeptides of alkaline nature (protamine) on the composition of the blood serum LP in the early period of hypercholesterolemia (HchS).

EXPERIMENTAL METHOD

Noninbred male rabbits weighing 2-2.5 kg were used as experimental animals. The animals of one group received 0.5 g/kg body weight of twice recrystallized ChS, received a 1% solution of protamine, 10 mg/kg, intraperitoneally (Protamine sulfate for injections, USSR).

Another group of experiments was carried out on rats (males weighing 160-200 g). They were given ChS in a dose of 0.5 g/kg daily for 4 months, together with methimasole (12.5 mg/kg) for 30 days, starting with the 3rd month of the experiments.

Blood was taken from the marginal vein of the rabbits' ear under local anesthesia 1 day after stopping ChS and after starvation for 14 h, and centrifuged (2000 rpm, 10 min, 4°C) to obtain serum. LP were fractionated by preparative ultracentrifugation in a stepwise density gradient of salt solution [6]. Methods of determination of protein and ChS were described in detail by the writers previously [3]. Triglycerides (TG) were determined by recording optical density of the stained solution at 410 nm [5]. The results were subjected to statistical analysis by the usual methods.

EXPERIMENTAL METHOD

The data given in Table 1 are evidence that administration of protamine to the animals led to an increase in the total blood serum protein concentration (TSP). The TSP level also rose in the early period of isolated HchS. It is important to emphasize that this effect was observed in species of animals with a tendency to develop experimental atherosclerosis, but it is characteristic only of the initial stages of HchS, when the morphological features of atherosclerosis have not yet developed or are mild [2]. A considerable increase in the plasma ChS ratio was accompanied by formation of fibrous plaques on the inner surface of the large vessels. After 4-5 months of the experiments, nodular structures began to appear inside the aorta, formed by confluent plaques — the typical picture of experimental atherosclerosis was observed. This stage of atherogenesis differs from the initial stages in the sharp decrease in the TSP concentration. The changes were manifested more clearly in a study of protein fractions extracted by acid treatment of the animals' serum. Their level also was depressed in patients with atherosclerosis; posi-

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TABLE 1. Concentrations of Protein (in mg/ml), ChS, and Triglycerides (in Mm) and Blood Serum and LP of Rabbits under the Influence of Protamine in the Early Period of HchS ($\bar{x} \pm S_{\bar{x}}$)

Experimental conditions	LDL	HDL	Blood serum	VLDL	Chylomicron
			Protein		
Control	1,0±0,1	1,6±0,3	66,4±3,1	1	1
HChS + protamine	2,2±0,4*	6,0±0,4*	83,4±3,3*	7,7±0,6*	4,4±0,4*
HChS	1,0±0,2	5,1±0,8*	86,8±4,9*	2,8±0,2	5,8±0,5*
			Cholesterol		
Control	1,0±0,1	0,2±0,02	2,1±0,03	1	1
HChS + protamine	7,4±0,8*	0,3±0,03*	15,3±1,3*	15,3±0,3*	7,1±0,6*
HChS	3,0±0,4*	0,2±0,03	5,6±0,04*	2,9±0,2*	5,7±0,6*
			Triglycerides		
Control	0,4±0,05	0,1±0,02	1,1±0,1	1	1
HChS + protamine	1,0±0,07*	0,06±0,01	2,8±0,4*	1,1±0,1	0,6±0,1
HChS	0,6±0,06	0,06±0,01	1,4±0,1	0,8±0,1	0,7±0,1
			Protein/cholesterol		
Control	2,7	23,0	83,0	2,5	1,0
Experiment	0,8	45,7	14,0	1,2	0,6

Legend. Changes in VLDL and chylomicrons expressed as ratio to value in control; asterisk indicates statistically significant change compared with control; $\bar{x}$) arithmetic mean; $S_{\bar{x}}$) standard error of arithmetic mean.

TABLE 2. Changes in ChS Concentration (in mg/ml) in Liver and Blood Serum of Rats under the Influence of Protamine and against the Background of HchS ($\bar{x} \pm S_{\bar{x}}$)

Experimental conditions	Total ChS		ChS	LP
	blood serum	liver	LDL	HDL
Control	0,54±0,02	1,68±0,2	0,24±0,02	0,29±0,07
HChS	1,18±0,1*	2,49±0,2*	0,43±0,03*	0,39±0,06
HChS + protamine	1,40±0,04*	1,62±0,1**	0,63±0,06*	0,76±0,12*

Legend. Asterisk indicates statistically significant change compared with control, **) change significant compared with value observed during HchS.

tive correlation was found, moreover, between the intensity of the changes in the protein concentration and the depth of development of the atherosclerotic process [1].

Thus differences in the time course of HchS and atherogenesis are reflected in the pattern of changes in the total serum proteins, especially the fraction of alkaline nature. Marked atherosclerosis was accompanied by a decrease in the protein concentration, but in the early stages of HchS activation of biochemical mechanisms linked with accumulation of TSP is possible.

It follows from the data in Table 1 that the action of protamine against the background of HCS was accompanied by an increase in the protein content in the composition of chylomicrons and of all the classes of LP studied. This may indicate activation by protamine of the blood LP system. The intensity of the change in LP apoproteins was significantly greater than that of TSP, suggesting definite selectivity of the effect of protamine on accumulation of LP apoproteins.

The concentration of triglycerides (TG) in the particles studied was virtually unchanged, with the exception of LDL. A relative increase in the TG concentration (in % of the control) in the blood and LDL was identical (254 and 250%). It must accordingly be considered that after administration of protamine, TG accumulation in the blood serum depends on an increase in their content in the composition of LDL.

As a result of the action of protamine against a background of HchS, a varied increase in the ChS content was observed in the test fractions. As interesting aspect of the effect of protamine was that the protein/ChS ratio was unchanged compared

with the control in chylomicrons, reduced in VLDL and LDL, but increased in HDL. The sharp increase in protein in the composition of HDL, by contrast with the atherogenic fractions of LP was not accompanied by any proportional rise in ChS or TG. These data suggest that after administration of protamine elevation of the apoprotein A level was observed in the composition of HDL, but not an increase in the number of their particles. Since restoration of the normal structure of the plasma membranes of cells can take place under conditions of cholesterolosis as a result of removal of the excess of free ChS with the aid of apoproteins of HDL [4], this fact can be regarded as a manifestation of the antiatherogenic action of protamine.

Evidence in support of this view is given by data on recovery of the ChS concentration of the rat liver, when raised as a result of feeding the animals with stearin, in response to injection of protamine. Meanwhile, just as in rabbits, protamine did not prevent accumulation of ChS in the blood serum (Table 2).

Thus, in the early stages of HchS administration of protamine leads to changes in the composition of the blood serum LP. Compared with the control, a marked increase in the concentrations of protein components was observed in HDL, whereas in LDL the fractions of ChS and TG rose sharply.

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AFFINITY CHROMATOGRAPHY ON HEPARIN-SEPHAROSE UNDER REDUCING CONDITIONS AS A METHOD OF SELECTIVE ENRICHMENT WITH INDIVIDUAL ISOFORMS OF APOLIPOPROTEIN E

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One of the most important apoproteins of the plasma lipoproteins, namely apolipoprotein E (apo E), is involved in the maintenance of functional integrity of the plasma lipid transport system, by constant exchange between triglyceride-rich particles and high-density lipoproteins (HDL); this state of dynamic equilibrium, moreover, is shifted toward the latter. In the process of intravascular lipolysis of chylomicrons and very low-density lipoproteins (VLDL) apo E is transported to the heaviest HDL on the surface of which esterification of cholesterol takes place under the influence of lecithin-cholesterol acyltransferase. The reverse transport of apo E to VLDL is coupled with the transport of the cholesterol esters formed [1, 11]. The leading role of apo E in the final stages of lipoprotein metabolism consists of its ligand function during interaction of lipoproteins with specific apo E- or apo B,E-receptors of the liver and extrahepatic apo B,E-receptors [10]. The existence of multiple isoforms of human

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