THE RABBIT LIVER IN EXPERIMENTAL ATHEROSCLEROSIS SECRETES OXIDIZED LIPOPROTEINS

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One of the hypotheses explaining the development of atherosclerosis, namely the peroxide hypothesis, is based on the fact that processes of lipid peroxidation (LPO) are activated in the blood in cardiovascular diseases [10]. It has been suggested that the blood lipoproteins (LP) become involved in oxidative reactions. As we showed in experiments in vitro, this may lead to considerable disturbances of the structure of the surface proteolipid layer of LP [3, 4] and, as a result, to a disturbance of their lipid transport function [1, 5, 6, 8]. Oxidized low-density LP (LDL) transport cholesterol more intensively into cells [1, 5, 6, 8], whereas oxidized high-density LP (HDL) become virtually unable to accept an excess of cholesterol [6, 8]. The conditions are created for cholesterol to accumulate in the cells, and for their degeneration into foam cells. The presence of oxidized LP has now been recorded in human blood [7], but the ways of their appearance in the blood stream have not yet been explained. Does oxidative modification of LP take place in the blood or do the liver cells in atherosclerosis synthesize and secrete LP, containing lipoperoxides ab initio?

The aim of this investigation was to discover whether hepatocytes of animals affected with atherosclerosis secrete LP with an increased content of LPO products, using a model of the perfused liver.

EXPERIMENTAL METHOD

Experiments were carried out on male and female rabbits (weight 3-4 kg, age 9-10 months). The rabbits were divided into two groups: experimental (8) and control (6). Daily for 12 weeks the experimental animals were given cholesterol with their food in a dose of 0.25 g/kg. The atherogenic diet was then stopped and the animals put on an ordinary diet for 8 weeks (period of regression). At the end of the period on the atherogenic diet and also at the end of the period of regression, the cholesterol concentration in the rabbits' blood serum was determined on "Centrifichem-400" automatic analyzer (USA); the concentration of LPO products reacting with 2-thiobarbituric acid (TBA) also was determined. The fraction of apo-B-containing LP (very low density LDL + LDL) was isolated from the serum by the precipitation method [9] and the content of TBA-reactive products in it also was determined [15]. Next, each group of animals was divided into two equivalent subgroups (based on their serum cholesterol level). Animals of one group were used for determination of the concentrations of lipid oxidation products reacting with TBA in the liver [15], the other for perfusion of the liver by the method described in [2], without isolating the organ from the peritoneal cavity. Hanks' solution was used as the perfusion fluid, perfusion was carried out by recirculation, in a constant-temperature cupboard at 38°C for 30 min. The liver was first washed to remove blood. The total volume of perfusion fluid was 150 ml and its pH 7.4. To prevent any possible hypoxia and reoxygenation of the organ, the oxygen concentration in the perfusion fluid was kept constant by oxygenation. The rate of flow of the perfusion fluid was 25-30 ml/min.

After the end of perfusion EDTA and butylated hydroxytoluene (ionol) was added to the perfusion fluid up to a concentration of $5 \cdot 10^{-4}$ and 10^{-4} M respectively, in order to prevent possible oxidation in the future. Concentrations of

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TABLE 1. Concentrations of Cholesterol and LPO Products in Serum and in Apo-B-Containing LP (VLDL + LDL) Isolated from It in Rabbits of Control Group and with Alimentary Atherosclerosis (M ± m)

Group of animals	Serum cholesterol, mg %		MDA, μmoles/liter	
			Serum	VLDL + LDL
	A	B	A	A
Atherosclerosis (experiment)	920±50	491±117	0,57±0,05	0,72±0,28
Intact animals (control)	50±10	—	0,29±0,09	0,012±0,006

Legend. A) After stopping atherogenic diet, B) after period of regression. In all cases changes compared with control were significant ($p < 0.01$).

TABLE 2. Concentrations of LPO Products in Perfusion Fluid and in LP Isolated from It ($d < 1.065$ g/cm^3), and also in Liver Homogenate of Rabbits of Control Group and with Experimental Atherosclerosis (M ± m)

Group of animals	MDA				
	liver homogenate, μmoles/g wet wt. of tissue	perfusion fluid		LP	
		nmoles/liter	nmoles/g protein	nmoles/liter	nmoles/g protein
Atherosclerosis (experiment)	8,93±2,06	31,2±4,2	14,0±6,2	30,6±1,0	108,9±10,6
Intact animals (control)	5,80±0,45	12,2±3,2	4,8±3,4	15,3±1,2	53,5±24,2

Legend. In all cases differences compared with control were significant ($p < 0.05$).

protein [12] and of TBA-reactive lipid oxidation products [15] were determined in the perfusion fluid. Some of the perfusion fluid was used to isolate LP fractions from it. For this purpose, crystalline NaBr was dissolved in the perfusion fluid to increase its density up to 1.065 g/cm^3. This was followed by centrifugation at 6°C for 20 h at 120,000*g*. The LP fraction with a density of under 1.065 g/cm^3 thereupon floated [11]. Next, 1 ml of this fraction was withdrawn from the top of the centrifuge tube and concentrations of protein [12] and TBA-reactive products in it were determined [15]. Electrophoresis in 0.5% agarose gel showed that this fraction contained very low density lipoproteins (VLDL), secreted by the liver, but not HDL, which do not float under these conditions since their density exceeds 1.065 g/cm^3 [11]. No LDL could be found.

EXPERIMENTAL RESULTS

It is evident that keeping the experimental rabbits on an atherogenic diet led to marked hypercholesterolemia, compared with rabbits of the control group. Morphometric analysis showed that 52.9 ± 19.9% of the surface of the aorta of the experimental rabbits was affected by atherosclerotic lesions. Meanwhile the concentration of LPO products was increased in the serum (Table 1) and liver homogenate (Table 2). This is in good agreement with data in the literature [14] indicating intensification of LPO processes in the spleen and liver of animals in a state of hypercholesterolemia. It is important to note that under these conditions, as our experiments showed, the increase in the concentration of LPO products was increased by a considerable degree in apo-B-containing LP (by 60 times, whereas the increase in the serum was only twofold; Table 1).

An important function of the liver cells is to synthesize and secrete VLDL. It is perfectly logical to suggest that if during hypercholesterolemia LPO processes in the liver are intensified, hepatocytes can synthesize LP with an increased concentration of lipid peroxides. To study this problem we perfused the liver of experimental rabbits and determined the concentration of TBA-reactive LPO products in the perfusion fluid and also in the LP fraction isolated from it ($d < 1.065$ g/cm^3). It will be clear from Table 2 that the concentration of LPO products in the perfusion fluid of the experimental rabbits was 3 times higher than in the perfusion fluid of intact rabbits. Analysis of the concentration of LPO products in the LP fraction isolated from the perfusion fluid showed that rabbits with alimentary atherosclerosis contain twice as much of the LPO products secreted by the liver in their LP than rabbits of the control group.

We showed previously that oxidized VLDL, LDL, and HDL may be the cause of activation of the phagocytic cells of the blood monocytes and neutrophils [13]. Activation of phagocytes is accompanied by generation of active forms of oxygen (H_2O_2, $O_2^{\cdot-}$, OCl and $OH^{\cdot}$) by them, which promote intensification of LPO and stimulate oxidative destruction of LP. This may probably be responsible for the considerable increase in the content of LPO products which we found in the apo-B-containing LP of rabbits with experimental atherosclerosis (Table 1). These modified LP may be the cause of cholesterol accumulation in the cells, especially in monocytes [1], and their degeneration into foam cells, giving rise to atherosclerotic lesions in the vessels.

This investigation thus showed that the liver of animals with experimental atherosclerosis synthesizes and secretes LP with an increased content of LPO products, i.e., oxidized LP. This process may play an important role in the accumulation of modified LP in the blood and their involvement in the development of atherosclerotic degeneration of cells of the blood and vessel walls.

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