LOW-DENSITY LIPOPROTEINS ISOLATED FROM BLOOD OF CORONARY PATIENTS CAUSE LIPID ACCUMULATION IN HUMAN AORTIC INTIMAL CELLS

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Lipid accumulation in cells of blood vessel walls is a characteristic feature of spontaneous atherosclerosis in man and experimental atherosclerosis in animals. The mechanisms leading to elevation of the intracellular lipid level are not yet completely clear. Research on cell cultures has shown that sera, and also low-density (LDL) and very low-density lipoproteins (VLDL), obtained from animals with hypercholesterolemia, induce lipid accumulation in smooth-muscle cells (SMC) of the rabbit, pig, and monkey aorta [1, 2, 6, 12-14]. The writers showed recently that blood sera from patients with coronary heart disease (CHD), with either hyper or normocholesterolemia, and with angiographically documented coronary atherosclerosis, can cause an increase in the lipid concentration in SMC from unaffected human aortic intima [5].

The aim of this investigation was to study the effect of lipoproteins isolated from blood sera of CHD patients and normal individuals on lipid metabolism in human aortic intimal cells in culture.

EXPERIMENTAL METHOD

Blood was taken from three healthy individuals with no clinical manifestations of CHD and three CHD patients in classes II-IV according to the Canadian classification [4], with angiographically documented coronary atherosclerosis of one to three coronary arteries [7]. Blood was taken in the morning before breakfast from the cubital vein. LDL, VLDL, and high-density lipoproteins (HDL) were obtained by ultracentrifugation [8]. All classes of lipoproteins were recentrifuged, dialyzed for 48 h against 2000 volumes of phosphate buffer at 4°C, and sterilized by filtration (0.22 μ). Protein was determined by Lowry's method [9]. Intimal SMC were isolated from the aorta of men dying suddenly from myocardial infarction at the age of 40 to 51 years, 1-3 h after death. The cells were isolated by dispersion of the tissue with elastase and collagenase, and cultured as described in detail previously [11]. Lipids were extracted from the cells with a mixture of chloroform and methanol (1:2 *v/v*) [3]. Concentrations of triglycerides and of free and esterified cholesterol were determined by scanning densitometry [10]. Phospholipid concentrations were measured by Vaskovsky's method [15].

EXPERIMENTAL RESULTS

The results given in Fig. 1a show that LDL, isolated from the blood of patients with CHD, in a concentration of 100-1000 μg protein/ml led to a two-fivefold rise of the cholesterol ester level during incubation for 24 h with SMC from the intima of the unaffected human aorta. In a concentration of 500-1000 μg protein/ml, LDL increased the intracellular cholesterol concentration by 1.5-3 times and the triglyceride concentration by 1.5-2 times (Fig. 1b, c), but had no effect on the intracellular phospholipid concentrations (Fig. 1d). LDL taken from healthy individuals did not change the level of the main intracellular lipids.

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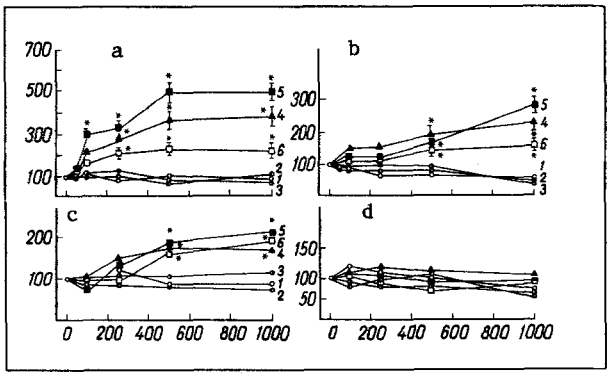

Fig. 1. Effect of LDL on concentrations of cholesterol esters (a), triglycerides (b), free cholesterol (c), and phospholipids (d) in culture of human aortic intimal cells. Abscissa, concentration of LDL (in μg/ml); ordinate, lipid concentration (in % of control). Here and in Fig. 2: sera 1, 2, and 3 were obtained from healthy individuals, 4, 5, and 6 from patients with CHD. The cells were cultured in the presence of 10% delipidized serum, acting as the control; $^*p < 0.05$ compared with control.

TABLE 1. Concentrations of Main Classes of Lipids in Cells Cultured in Presence of VLDL, Isolated from Blood of Healthy Individuals and Patients with CHD ($M \pm m$)

Serum	Lipid concentration, μg/10^5 cells			
	cholesterol	triglycerides	cholesterol	phospholipids
Control	7,8±0,5	3,9±0,2	5,7±0,6	52,4±6,1
Healthy subjects				
1	8,4±1,1	4,2±0,3	4,9±0,3	49,8±5,2
2	6,8±0,9	4,3±0,5	6,1±0,4	50,3±4,8
3	7,9±0,6	3,6±0,1	6,3±0,7	44,7±3,7
Patients with CHD				
4	7,7±0,5	4,5±0,3	5,5±0,6	51,1±4,8
5	17,4±1,5*	4,9±0,6	6,0±0,4	45,9±5,7
6	8,0±0,8	4,2±0,5	6,4±0,4	42,8±6,1

Legend. Cells cultured for 24 h in medium No. 199 containing 10% delipidized serum and VLDL in a concentration of 500 μg/ml; $^*p < 0.05$ compared with control.

VLDL taken from three healthy subjects and two patients, in a concentration of 25-250 μg protein/ml, did not change the lipid levels in the cells in culture. However, VLDL from one patient in a concentration of 500 μg protein/ml led to an increase of 2.5 times in the intracellular lipid concentration (Table 1).

HDL isolated from the blood of healthy subjects and patients with CHD, in a concentration of 25-250 μg protein/ml, had no effect on the lipid concentration in a culture of intimal cells from the unaffected human aorta (data not shown). HDL isolated from the blood of healthy donors and patients with CHD, in a concentration of 50-250 μg protein/ml, lowered the intracellular level of cholesterol esters by 33-50% in a culture of cells isolated from an atherosclerotic plaque (Fig. 2a, b).

The results showing the ability of HDL, both from patients and from healthy subjects, to cause a virtually equal fall of the intracellular level of cholesterol esters in a culture of cells taken from an atherosclerotic plaque, suggest that the atherogenic properties of the blood sera from patients with coronary atherosclerosis are not connected with changes in the lipid-acceptor system for HDL. The investigation also showed that LDL and, perhaps also, VLDL, isolated from the blood of patients with coronary atherosclerosis, but not from healthy individuals, possess atherogenic potential, i.e., are capable of causing intracellular accumulation of lipids in a culture of cells from the intact human aortic intima. We do not yet know what determines the atherogenic properties of lipoproteins, but it can be tentatively suggested that LDL circulating in the blood of patients with CHD, or some of them, are modified, although experimental verification of this hypothesis is required.

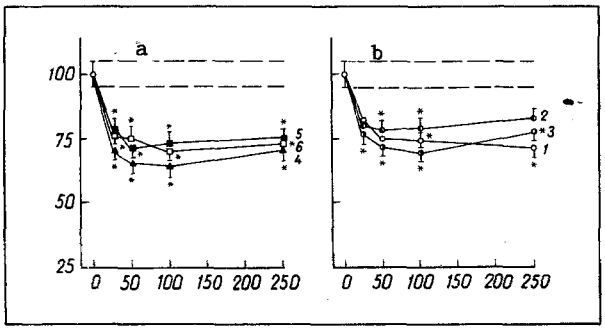

Fig. 2. Effect of HDL isolated from blood of patients with CHD (a) and healthy individuals (b), on concentration of cholesterol esters in cells from atherosclerotic plaque in culture. Abscissa, HDL concentration (in μg/ml): ordinate, concentration of cholesterol esters (in % compared with control).

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