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ATHEROGENIC LIPOPROTEINS FOUND IN THE BLOOD OF PATIENTS WITH CORONARY ATHEROSCLEROSIS ARE DESIALYLATED LOW-DENSITY LIPOPROTEINS

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The writers showed recently that low-density lipoproteins (LDL) isolated from the blood of most patients with coronary heart disease (CHD) and with angiographically verified coronary atherosclerosis verified differ from the LDL obtained from healthy human blood in their ability to induce intracellular cholesterol accumulation in a culture of smooth-muscle cells from the unaffected intima of the human aorta. It was suggested that LDL from CHD patients are modified lipoproteins, differing in their chemical composition from LDL isolated from normal individuals. The investigation described below established a significant decrease in the sialic acid content of LDL isolated from the blood of CHD patients with coronary atherosclerosis compared with LDL from normal individuals. The desialylation of native LDL obtained from normal subjects by neuraminidase makes these lipoproteins atherogenic, i.e., capable of causing an increase in the intracellular cholesterol concentration in cultures of smooth-muscled cells from the intima of the unaffected human aorta.

Sialic acid is known to be a component of native LDL [13] and to perform an important function in their metabolism [1, 4, 5].

EXPERIMENTAL METHOD

Experiments were carried out on blood from 22 healthy subjects with no clinical features of CHD of classes II-IV according to the Canadian classification [2] and with angiographically documented atherosclerosis of 1 to 3 coronary arteries [8]. Blood was taken from the cubital vein of the fasting subject before breakfast. LDL (d = 1.030-1.050 g/cm^3) were obtained by ultracentrifugation [3]. Protein B was determined as described previously [3]. Lipids were extracted from the cells with a mixture of chloroform and methanol (1:2, v/v [6]), and then fractionated by thin-layer chromatography on silica gel 60 plates (Merck, West Germany). The lipid content was determined by scanning densitometry [10]. The sialic acid level in LDL was measured by the method [14]. Total protein was determined by the method in [9]. LDL were treated with neuraminidase (Sigma, USA) as described in [12]. Intimal smooth-muscle cells (SMC) were isolated from the aorta of persons dying suddenly from myocardial infarction between the ages of 40 and 51 years, 1-3 h after death. The cells were isolated by dispersion of the tissue with elastase and collagenase ("Sigma") and were cultured as described previously [10]. The atherogenecity of the LDL was assessed by accumulation of cholesterol in cultured SMC compared with control cells, as described previously [11].

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TABLE 1. Characteristics of Atherogenic and Nonatherogenic LDL

Indicator	LDL	
	nonatherogenic	atherogenic
Atherogenicity (increase in intracellular cholesterol concn., % of control)	7±14	117±16*
Protein, %	21,8±1,4	22,3±1,1
Free cholesterol esters, %	13,4±1,1	14,3±0,9
Cholesterol esters, %	39,4±3,2	41,0±3,0
Triglycerides, %	7,0±0,8	6,6±0,5
Phospholipids, %	18,4±0,9	15,8±1,6
Sialic acid, μg/mg protein	30,4±1,6	9,9±0,9*

Legend. Asterisk denotes statistically significant difference from nonatherogenic LDL ($p < 0.05$).

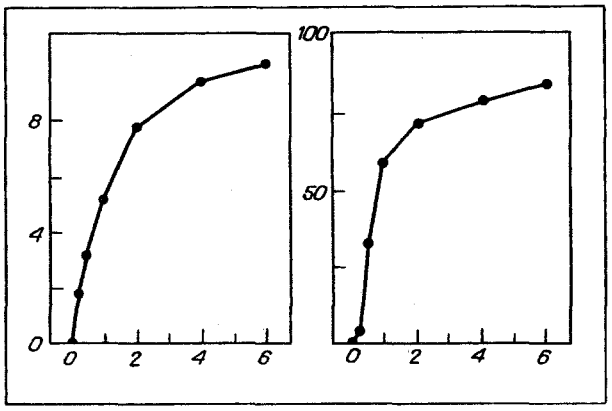

Fig. 1 Fig. 2

Fig. 1. Degree of desialylation of LDL from healthy individuals by sialidase depending on incubation time. Abscissa, incubation time (in h); ordinate, neuraminidase (in μg/mg protein).

Fig. 2. Increase in intracellular cholesterol concentration during incubation of SMC with desialylated LDL. Abscissa, incubation time (in h); ordinate, rise of intracellular cholesterol level (in μg/mg cell protein).

EXPERIMENTAL RESULTS

LDL isolated from blood plasma of patients with CHD and coronary atherosclerosis exhibited atherogenic properties in a culture of intimal cells, i.e., they caused a twofold increase in the total cholesterol concentration in the intimal cells. LDL obtained from healthy individuals possessed no atherogenic properties (Table 1). The sialic acid content in LDL obtained from patients with coronary atherosclerosis was only one-third of that in LDL from healthy individuals (Table 1). Moreover, a low level of sialylation was a characteristic feature of both the protein and lipid moities of LDL isolated from the blood of patients with CHD and coronary atherosclerosis. With respect to other parameters there was no difference between LDL from the patients and normal subjects (Table 1).

It can thus be tentatively suggested that the low degree of sialylation of LDL is connected with the atherogenic properties of the LDL, manifested as cholesterol accumulation in cultured human aortic intimal cells. To test this hypothesis, nonatherogenic LDL isolated from healthy human blood plasma were partially desialylated by means of neuraminidase. A significant degree of desialylation of LDL was achieved during neuraminidase treatment for 2 h (Fig. 1). Incubation of in vivo desialylated LDL with cultured cells isolated from the intima of the unaffected human aorta led to a twofold increase in the total intracellu-

lar cholesterol concentration (Fig. 2). The coefficient of correlation between the degree of desialylation of LDL and their atherogenicity was -0.96 ($p < 0.05$).

It can thus be concluded from these results that the low degree of sialylation of LDL isolated from blood plasma of patients with CHD and angiographically documented coronary atherosclerosis, compared with LDL from healthy individuals, is closely connected with the atherogenic properties of the LDL, i.e., their ability to induce accumulation of intracellular lipids in a culture of SMC. We know that LDL, modified chemically in vitro, can induce accumulation of intracellular cholesterol in cells in culture [7]. Modified LDL, present in the blood, are considered to facilitate the development of atherosclerotic damage to arteries [7]. In our view, desialylated LDL are one of the varieties of atherogenic modified LDL circulating in the bloodstream of patients with CHD and with angiographically documented coronary atherosclerosis.

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