ISOLATION OF DESIALYLATED LOW-DENSITY LIPOPROTEINS FROM BLOOD OF CORONARY HEART DISEASE PATIENTS BY AFFINITY CHROMATOGRAPHY

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Previously the writers reported that low-density lipoproteins (LDL) obtained from the blood of patients with coronary heart disease (CHD), but not from healthy subjects, can induce accumulation of lipids in cell cultures obtained from intact intima of the human aorta [9, 15]. It was later found that a low content of sialic acid is a distinguishing feature of LDL from CHD patients [10, 11]. The ability of LDL to increase the intracellular cholesterol concentration was found to correlate negatively with their sialic acid content. In addition, enzymic removal of sialic acid with the aid of neuraminidase leads to the appearance of ability to accumulate intracellular lipids in native LDL from healthy individuals [10, 11]. Desialylation is evidently one modification of LDL taking place in vivo.

Apoplipoprotein B (apo B) of LDL is known to have two types of polysaccharide chains, linked by an N-glycoside bond: oligomannoside and sialylated biantennal [14, 16]. Under these circumstances sialic acid is contained in chains of the second type, in which it is the terminal sugar. Glycosphingolipids, components of LDL, also contain terminal sialic acid [3]. Since the next sugar residue after the siatic acid in sialylated biantennal chains is galactose, desialylation of LDL ought to express galactose. On this basis we suggested that desialylated LDL can interact with galactose-specific lectins. One such lectin is the agglutinin ricin (R_{120}), which has high affinity for terminal β-galactose and low affinity for other sugar residues in the composition of the polysaccharide chains of LDL [2]. In the present investigation we used affinity chromatography on R_{120} immobilized on sepharose, in order to isolate desialylated lipoproteins and to study their effect on lipid accumulation in cells of the intact intima of the human aorta.

EXPERIMENTAL METHOD

To isolate LDL we used pooled blood from CHD patients (men aged 37-49 years) and clinically healthy persons (men aged 30-45 years). All the CHD patients had angiographically confirmed stenosis of 1-3 coronary arteries. The plasma cholesterol level was 5.2 ± 0.62 moles/liter for the group of healthy subjects and 5.04 ± 0.44 moles/liter for the CHD patients. None of the donors showed any signs of diabetes or of arterial hypertension.

LDL (1.019-1.063 mg/ml) were isolated by ultracentrifugation as in [5]. Desialylation of LDL was carried out with the aid of agarose-bound neuraminidase, as described previously [10]. The sialic acid content in LDL was determined as in [13]. R_{120} was isolated from the seeds of *Ricinus communis* as in [7].

Immobilization of R_{120} on BrCN-activated sepharose followed the standard method [1]. The concentration of the immobilized lectin was 2 mg/ml sepharose. To fractionate the LDL, columns containing 1-5 ml of R_{120}-sepharose were equilibrated with phosphate buffer (PBS), pH 7.0, containing 0.5 M NaCl.

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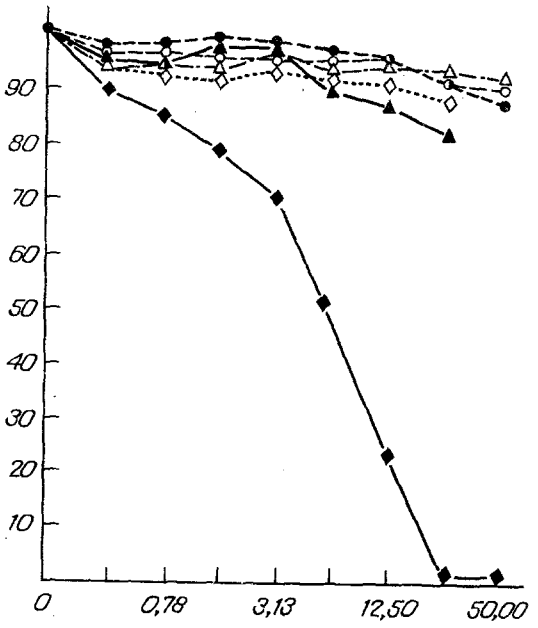

Fig. 1

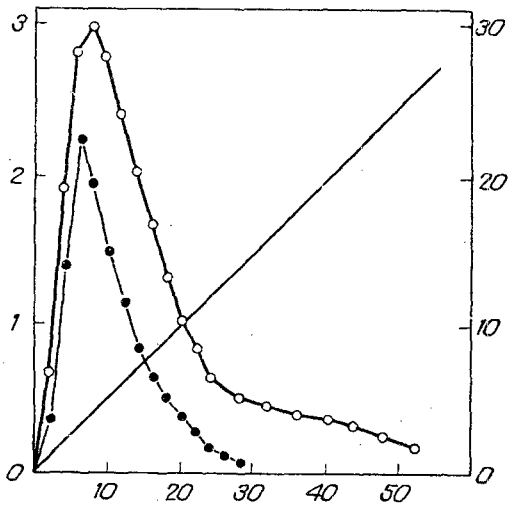

Fig. 2

Fig. 1. Displacement of LDL, bound with R_{120}-sepharose, by galactose (filled lozenges), mannose (empty lozenges), glucose (filled circles), N-actylglucosamine (empty circles), fucose (empty triangles), and sialic acid (filled triangles). Abscissa, sugar concentration (in mM); ordinate, quantity of LDL displaced, expressed as cholesterol (in % of quantity bound).

Fig. 2. Elution profile of LDL with linear galactose gradient. Abscissa, Nos. of fractions; ordinate, on right — galactose concentration (in mM), on left — LDL content. Filled circles denote healthy human LDL, empty circles — LDL from CDH patients.

After application of the specimen of lipoproteins the columns were rinsed with 5 volumes of PBS, containing 2% bovine serum albumin (BSA), and with 10 volumes of PBS, and the adsorbed LDL were elated with galactose solutions in the above concentrations. Bound and unbound fractions of LDL were concentrated by ultracentrifugation [5] and dialyzed against 1000 volumes of PBS.

Smooth-muscle cells from unaffected areas of the intima of the aorta from men dying from myocardial infarction were isolated by dispersion with cotlagenase and cultured in medium 199 containing 10% fetal calf serum and antibiotics, as described previously [8]. The medium was changed every 2nd day. On the 7th day of culture, medium 199 containing 10% of lipoprotein deficient serum, obtained by ultracentrifugation from a healthy donor (p > 1.250, g/ml) [5] and LDL preparations, freshly filtered through filters with pore diameter of 0.45 μ, were added to the cells. After incubation for 24 h the cells were washed 5 times with PBS. Lipids were extracted from the cells with a mixture of hexane—isopropanol (3:2, v/v) [4]. The total cholesterol concentration was determined by an enzymic method [12], using kits from "Boehringer Mannheim" (West Germany). The cell protein concentration was determined by the method in [6].

EXPERIMENTAL RESULTS

After application of LDL to the column with R_{120}-sepharose a certain part of the lipoproteins bound with the sorbent and was not elated by PBS/NaCl or PBS containing 2% BSA. Rinsing the column with solutions of galactose led to elution of the bound lipoproteins; with an increase in the galactose concentration, moreover, the quantity of LDL elated increased (Fig. 1). If 25 mM galactose was used, complete elution of the bound lipoproteins took place from the column. Other sugars in the composition of LDL, namely lysialic acid, D-mannose, D-glucose, L-fucose, N-acetylglucosamine, were unable to eluate the

TABLE 1. Effect of LDL Subtractions on Cholesterol Level in Cultured Cells of Intact Intima of Human Aorta

LDL fraction	Content, %	Sialic acid concentration, µg/mg protein	Cholesterol accumulation, % above control
Healthy individuals			
Total	100	24,5	12±7
Bound	8	12,8	74±7*
Unbound	92	26,9	8±8
LDL desialylated in vitro			
Total	100	9,6	254±18*
Bound	89	9,8	249±22*
CHD patients			
Total	100	14,7	154±17*
Bound	38	8,4	302±28*
Unbound	62	19,0	9±5

Legend. Data on cholesterol accumulation shown as mean of four determinations ($M \pm m$). Asterisk indicates significant difference from control, $p < 0.05$.

adsorbed LDL (Fig. 1). These results are evidence that the specific sites for LDL bound on R_{120}-sepharose are galactose residues.

Figure 2 shows the profile of elution of LDL obtained from healthy individuals and CDH patients by a linear galactose gradient. Clearly the quantity of LDL bound is higher in preparations obtained from the patients. The fraction of bound LDL isolated from the patients' blood was on average 28 ± 7% in the two cases studied (variations from 12 to 38%). Moreover, a subtraction of LDL elated from the column only by high (20-50 mM) concentrations of galactose, was present in these preparations (Fig. 2). The fraction of LDL obtained from healthy individuals, bound with R_{120}-sepharose, usually did not exceed 10%. Native healthy human lipoproteins, desialylated in vitro with neuraminidase, also were used in the experiments. About 90% of LDL desialylated in vitro was bound by the sorbent.

It will be clear from the data given in Table 1 that the sialic acid concentration in the total LDL preparation from healthy individuals was 24.5 µg/mg protein. Lipoproteins isolated from blood of healthy individuals, and LDL not bound on R_{120}-sepharose had the same sialic acid content as the original lipoproteins. Bound LDL from healthy human blood had a siatic acid level only half as high. Meanwhile the total LDL preparation obtained from patients with CHD contained just over half the amount of sialic acid compared with healthy human LDL. LDL obtained from patients, and bound with R_{120}-sepharose, had a content 1.8 times less, whereas the unbounded LDL had a content of sialic acid 1.3 times greater than the total fraction. The sialic acid content in LDL treated with neuraminidase was reduced by 2.5 times.

The results are evidence that with the aid of R_{120}-sepharose it is possible to isolate a subfraction of desialyated LDL from the total preparation of human lipoproteins, and that the appearance of lectin-accessible galactose evidently takes place as the result of loss of sialic acid.

Addition of the total LDL preparation obtained from healthy individuals and also of the LDL subfraction obtained from healthy individuals, and not bound on the sepharose sorbent, to the culture medium caused no change in the cholesterol content in cells of the intact intima of the human aorta (Table 1). Conversely, bound desialylated LDL from healthy human blood caused accumulation of intracellular cholesterol. LDL preparations from healthy individuals, treated with neuraminidase, caused a 3.5-fold increase in the cellular cholesterol concentration. A similar increase in cholesterol concentration was observed when cells were cultured with the subfraction of lipoproteins, desialylated in vitro, and bound on R_{120}-sepharose.

The total LDL preparation from CHD patients raised the cholesterol level in the cells by 2.5 times (Table 1). The subtraction of bound LDL had an even stronger (fourfold) effect. LDL from CHD patients, not bound with the sorbent, did not cause an increase in the intracellular cholesterol concentration.

Table 1 gives data obtained by the study of LDL preparations isolated from a single pool of blood from healthy individuals (10 persons) and a single pool of blood from CHD patients (10 persons). We obtained similar results in a study of

LDL preparations isolated from a further two pools of healthy human blood and three pools of patients' blood. In the LDL preparations tested the siatic acid content correlated negatively with their capacity ($r = -0.85$, $n = 21$, $p < 0.05$).

Thus the investigation described above showed that a subtraction of desialylated LDL isolated from blood of CDH patients can induce cholesterol accumulation in cultivated cells of the intima of the human aorta. Evidently desialylated LDL determined the atherogenic properties of the total LDL pool in CHD. Healthy human LDL likewise contain desialylated lipoproteins, but they induce accumulation of intracellular cholesterol by a lesser degree than desialylated LDL from the patients' blood. This may perhaps be connected with the lower degree of desialylation, which follows from the fact that bound LDL of healthy individuals are elated from the column by lower galactose concentrations. Total LDL preparations from healthy individuals are unable to increase the intracellular cholesterol concentration. It can be tentatively suggested that this is due to the weaker desialylation and the lower concentration of desialylated lipoproteins in the total LDL pool.

We hope that the method described above will enable the biochemical and physicochemical characteristics of desialylated lipoproteins to be studied in the future, and the pathways of their formation, mechanism of interaction with the cells, and the pathogenetic and diagnotic value of the presence of desialylated LDL in CDH patients to be investigated.

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