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Similarity Between Naturally Occurring Modified Desialylated, Electronegative and Aortic Low Density Lipoprotein

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The sialic acid content of electronegative low density lipoprotein (LDL) and LDL isolated from human aortic intima was measured. Sialic acid level in electronegative LDL of healthy subjects was 1.7-fold lower than in native LDL. Sialic acid content in electronegative LDL of coronary atherosclerosis patients was 3-fold lower than in native LDL. Lipoproteins isolated from grossly normal human aortic intima and from fatty streaks contained 20–56% less sialic acid as compared to blood plasma LDL. A negative correlation was established between the ability of electronegative and aortic LDL to stimulate lipid accumulation in cells cultured from uninvolved human aortic intima and lipoprotein sialic acid content. The results obtained indicate that electronegative and aortic LDLs have a low sialic acid content, i.e., are desialylated lipoproteins. Considered together with the fact that all known atherogenic LDLs have similar characteristics, our findings suggest that modified LDLs are the same lipoprotein particles subjected to multiple modification.

INTRODUCTION

We have recently found atherogenic multiple-modified low density lipoprotein (LDL) in the blood of patients with coronary atherosclerosis[1]. In contrast to native LDL, this LDL induces lipid accumulation in macrophages and human aortic intimal smooth muscle cells[2]. One of the main characteristics of multiple-modified LDL was a low sialic acid conent, therefore, this lipoprotein was called desialylated LDL[3].

In addition to multiple-modified desialylated LDL, other types of in vivo modified LDL were identified in human blood, namely, electronegative LDL[4] and small/dense LDL[5]. Besides, modified LDL was isolated from human aorta (aortic LDL)[6]. Similarly to desialylated LDL, electronegative, small/dense and aortic LDLs induce lipid accumulation in macrophages[7–9]. La Belle and Kraus reported that small/dense LDL has a low sialic acid content[10]. We have reported low sialic acid content of the most dense LDL subfractions[8]. Thus, small/dense LDL is desialylated lipoprotein. In this study we determined the sialic acid content of other naturally

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occurring modified lipoproteins: electronegative and aortic LDL. The ability of electronegative and aortic LDL to stimulate lipid accumulation in cells cultured from uninvolved human aortic intima was also studied.

MATERIALS AND METHODS

Donors

Plasma samples (1 mg/ml ethylenediamine tetraacetic acid (EDTA)) prepared from blood of 5 healthy male donors (35–46 yrs) and 5 patients (42–51 yrs) with angiographically documented coronary atherosclerosis were used. Thoracic aortas and blood plasma (EDTA, 1 mg/ml) were obtained by autopsy from 6 men (28–56 yrs) in one hour after sudden death from myocardial infarction.

Lipoprotein Isolation

Plasma low density lipoprotein (d = 1.019–1.063 g/cm^3) was isolated by ultracentrifugation according to Lindgren[11]. After ultracentrifugation, LDL preparation was dialyzed against phosphate buffered saline (PBS) and filtered through a polycarbonate filter (pore diameter, 0.45 μm). Electronegative LDL was isolated by ion exchange chromatography on DEAE Sepharose CL-4B column (1.5 × 10 cm) using a linear gradient of NaCl according to Avogaro *et al.*[4]. For isolation of aortic LDL, pieces of uninvolved intima and fatty streaks were separated into fibers with forceps. Samples were extracted overnight at 4°C with 0.15 M NaCl–0.01M phosphate buffer, pH 7.4, containing 0.1% EDTA, 0.5% ε-aminocaproic acid, 0.01mM pepstatin A and 0.01 mM leupeptin. LDL fraction (1.019–1.063 g/cm^3) was isolated from extract by gradient ultracentrifugation as descirbed earlier[12]. Aortic LDL preparations were purified by affinity chromatography on anti-apoB-agarose[3].

Cell Culture

Intimal smooth muscle cells were isolated from unaffected human aortic intima and cultured for 7 days as described elsewhere[13]. Cells were incubated for 24 h at 37°C in Medium 199 containing 10% lipoprotein-deficient serum from a healthy donor and 100 μg LDL protein/ml. Control cells were incubated in the absence of LDL. Cellular protein was measured according to Lowry *et al.*[14]. Lipids from cells were extracted according to Hara and Radin[15]. Total cholesterol content was measured colorimetrically using Boehringer Mannheim GmbH kit (Mannheim, Germany). Intracellular cholesterol content is expressed as percent of control.

Other Methods

The sialic acid content was determined according to Warren[16]. The level of thiobarbituric acid (TBA)-reactive substances in LDL preparations was determined according to Yagi[17]. Lipid peroxide level was measured by iodometric method according to Balla *et al.*[18].

Statistical Analysis

The significance of differences between group mean values was evaluated by multiple t-test of one-way analysis of variance using a BMDP statistical program package[19]. Significance of the correlation coefficient was evaluated using Fisher's Z-transformation[20].

RESULTS

Table I shows the sialic acid content of native and electronegative LDL. Electronegative LDL (LDL$^-$) of all studied healthy donors contained 18–60% less sialic acid than native LDL. The level of sialic acid in native LDL of patients with coronary atherosclerosis was the same as in native LDL of healthy subjects (Table I). In all studied

TABLE I Sialic Acid Level in electronegative LDL

Patient	Sialic Acid Level, nmol/mg Protein	
	Native LDL	Electronegative LDL
	Healthy subjects	
1.	42.5 ± 3.4	34.8 ± 2.3*
2.	42.0 ± 2.8	23.5 ± 1.4*
3.	41.6 ± 3.3	16.4 ± 1.I*
4.	37.2 ± 2.3	19.4 ± 1.2*
5.	35.5 ± 1.4	21.2 ± l.4*
	39.8 ± 1.4	23.1 ± 3.2*
	Coronary atherosclerosis patients	
1.	42.7 ± 2.3	11.5 ± 2.2*
2.	40.7 ± 1.7	18.7 ± 2.0*
3.	38.7 ± 1.5	19.4 ± 2.3*
4.	38.6 ± 1.1	10.5 ± 1.4*
5.	31.3 ± 1.0	8.6 ± 0.6*
	38.4 ± 1.9	13.7 ± 2.2*&

Data represent as the mean of three determinations ± standard error of mean.*, significant difference from native LDL, $p < 0.05$.&, significant difference from healthy subjects, $p < 0.05$.

cases, the sialic acid content in patients' LDL^- was 32–63% lower than that in native LDL. It should be noted that the mean sialic acid concentration in patients' LDL^- was significantly lower than that in LDL^- of healthy subjects (Table I).

The sialic acid content in LDL isolated from uninvolved aortic intima was 20–35% lower compared to that in LDL isolated from blood plasma of the same individual (Table II). The sialic acid content of LDL isolated from fatty streaks was 22–56% lower than that of plasma LDL. The level of sialic acid in LDL isolated from fatty streaks was lower than in LDL isolated from uninvolved aortic intima (Table II).

The ability of LDL to induce cholesterol accumulation was studied in a primary culture of smooth muscle cells isolated from uninvolved human aortic intima. Native LDL isolated from blood of healthy subjects and patients with coronary atherosclerosis did not stimulate total cholesterol accumulation in intimal smooth muscle cells (Table III). LDL^- of healthy donors caused

TABLE II Sialic Acid Level in Human Plasma and Aortic LDL

Case	Sialic Acid Level, nmol/mg Protein		
	Plasma LDL	Aortic LDL	
		Normal Intima	Fatty Streaks
1.	32.4 ± 2.6	20.9 ± 0.2	n.a.
2.	15.3 ± 0.5	n.a.	12.0 ± 0.2*
3.	29.2 ± 2.7	n.a.	15.1 ± 2.1*
4.	33.0 ± 1.0	21.3 ± 2.0*	14.5 ± 0.3*&
5.	43.6 ± 2.6	31.3 ± 1.5*	25.6 ± 1.3*&
6.	37.3 ± 2.3	27.5 ± 1.4*	21.4 ± 1.0*&

Data represent as the mean of three determinations ± standard error of mean. *, significant difference from plasma LDL, $p < 0.05$.&, significant difference from normal intima, $p < 0.05$. n.a., not analyzed.

TABLE III Effect of LDL Preparations on Cholesterol Content in Human Aortic Smoth Muscle Cells

LDL	Intracellular Cholesterol Content, % of Control
Control	100 ± 5
	Healthy subjects
Native LDL (5)	105 ± 4
Electronegative LDL (5)	164 ± 16*
	Coronary atherosclerosis patients
Native LDL (5)	111 ± 10
Electronegative LDL (5)	216 ± 26*
	Aortic LDL
Fatty streak (5)	186 ± 15*

Data are expressed as percent of control and represent the mean of three determinations ± standard error of mean. *, significant difference from control, $p < 0.05$.

1.6-fold increase of intracellular cholesterol level. LDL^- of patients with coronary atherosclerosis were more atherogenic causing 2.2-fold increase of cholesterol (Table III). Aortic LDL isolated from fatty streaks induced a 1.9-fold increase in the intracellular cholesterol content in smooth muscle cells (Table III).

Figure 1 shows correlation between the sialic acid content in LDL^- and aortic LDL and their ability to stimulate cholesterol accumulation in smooth muscle cells isolated from uninvolved human intima. There is a negative dependence between LDL atherogenicity and the sialic acid content ($r = -0.85$, $n = 15$, $p < 0.05$). We did not find any correlation between atherogenicity of LDL^- and aortic LDL and their contents of TBA-reactive substances ($r = 0.07$) and hydroperoxides ($r = 0.05$).

DISCUSSION

Data obtained in this study show that modified electronegative LDL has a lower sialic content

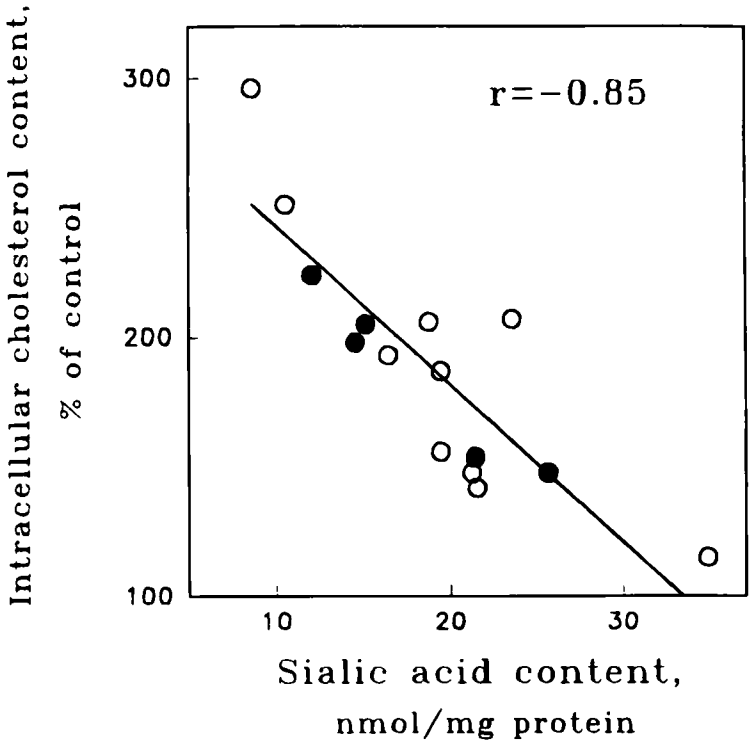

FIGURE 1 Correlation between sialic acid content of LDL and LDL ability to accumulate cholesterol in smooth muscle cells cultured from uninvolved intima of human aorta. (○), electronegative LDL; (●), LDL isolated from fatty streaks.

compared to native LDL. In LDL isolated from human aortic intima, the sialic acid content is lower than in plasma LDL of the same subjects and in native LDL. Thus, electronegative and aortic LDLs are desialylated lipoproteins like small/dense LDL[8,10] and desialylated LDL[1].

Previously we reported that desialylated LDL induce accumulation of neutral lipids, primarily cholesteryl esters, in smooth muscle cells cultured from uninvolved human aortic intima[2]. Then it was demonstrated that small/dense LDL also stimulates deposition of intracellular fat[8]. This study shows that two other naturally occurring modified LDLs (electronegative and aortic) stimulate cholesterol accumulation in aortic intimal smooth muscle cells. Thus, all known modified LDLs induce lipid accumulation in intimal smooth muscle cells and are desialylated.

The mechanisms responsible for intracellular lipid accumulation mediated by modified LDL so far remain obscure. The fact that different chemical and physico-chemical modifications of an LDL particle result in a unified response (intracellular lipid accumulation) suggests common step(s) in lipoprotein processing. Presumably, such a common feature is LDL aggregation with subsequent uptake of aggregates by phagocytosis. We have shown that desialylation, glycosylation and oxidation of LDL particles lead to their aggregation and accelerated uptake by intimal cells[23,24]. Apo B degradation, phospholipid modification and other processes also result in LDL aggregation[21,22]. Redistributions of the surface electric charge may be the main cause of LDL aggregation. Thus, any modification of an LDL particle involving its surface charge may render LDL atherogenic. In fact, there is a strong correlation between the sialic acid content and atherogenicity of LDL. On the other hand, we did not find any correlation between the ability of electronegative and aortic LDL to induce intracellular cholesterol accumulation and their content of lipid peroxidation products, such as TBA-reactive products and hydroperoxides. This can be explained at least by two facts that (1) LDL isolated from fatty streaks (but not from atherosclerotic plaques) and LDL- are not oxidized and (2) the lipid peroxidation products had been removed from LDL preparation during isolation. These suggestions require further investigation of stable oxidation products both in LDL- and in aortic LDL.

In addition to a low sialic acid content and the ability to cause accumulation intracellular lipids, naturally occuring modified LDLs have many similar and overlapping properties. All known forms of naturally occurring modified LDL may represent the same particle which had undergone a multiple modification. Multiple modification of an LDL particle is quite possible. It is exemplified by glycosylated LDL of diabetic patients, which is atherogenic modified lipoprotein. LDL of diabetic patients is not only glycosylated but also has a low sialic acid content[36]. The subfraction of desilaylated LDL was isolated from the blood of diabetics and proved to be glycosylated to a greater extent than normally sialylated LDL of the same patient[36]. In addition, the glycosylated LDL of diabetic patients has a higher susceptibility to oxidation as compared to that of native LDL[37]. Other specific features of glycosylated LDL similar to those of the known in vivo modified lipoproteins have been found[36]. These data indicate that multiple modification of a lipoprotein particle is principally possible.

It remains unclear whether multiple modification results from genetic alterations or LDL undergoes this modification in the blood or in peripheral organs. It is quite possible that multiple modification is a consequence of partial degradation of lipoprotein under the action of intra- and extra-lysosomal enzymes. Of importance is the question which alterations in an LDL particle emerge earlier and cause other modifications, including the changes of numerous physico-chemical properties of lipoproteins. In addition, it is obvious that not all changes in an LDL particle are atherogenic, i.e. induce atherosclerosis-related manifestations at the cellular level. These

and other questions determine further investigations of naturally occurring modified LDL.

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