Desialylation Decreases the Resistance of Apo B-Containing Lipoproteins to Aggregation and Increases Their Atherogenic Potential

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Subfractions of apo B-containing lipoproteins (VLDL and intermediate-density lipoproteins) with reduced content of sialic acid were found in human blood. These lipoproteins are characterized by high capacity to spontaneous association (aggregation) and stimulated accumulation of cholesterol in smooth muscle cells of human aortic intima. *In vitro* treatment of apo B-containing lipoproteins with α-2,6-sialidase and α-2,3-sialidase stimulated aggregation and increased the ability of these particles to potentiate cholesterol accumulation in smooth muscle cells of the intact human aortic intima. Probably, desialylation of various apo B-containing lipoproteins can occur in the blood; this process decreases their resistance to aggregation, and increases the ability of these particles to stimulate accumulation of cholesterol in human aortic intima cells, *i.e.* increases their atherogenic potential.

Key Words: *sialic acid; sialidase; desialylated lipoproteins; aggregation of lipoproteins; atherosclerosis*

Evaluation of the mechanisms of lipid deposition (mainly cholesterol and cholesterol esters) in vascular cells is an urgent problem in studying of the pathogenesis of atherosclerosis in humans. The subfraction of circulating strongly modified low-density lipoproteins (cmLDL) capable of stimulating cholesterol accumulation in cells of intact human aortic intima were identified and isolated from the blood of patients with coronary atherosclerosis [4,5,11,12]. As differentiated from native LDL (nLDL), cmLDL had a reduced level of sialic acid (desialylated LDL). A negative correlation was revealed between the ability of cmLDL to stimulate accumulation of intracellular cholesterol (atherogenicity of LDL) and concentration of sialic acid [6]. After *in vitro* desialylation with neuraminidase, nLDL gained the ability to accumulate intracellular cholesterol [4,5]. Strong accumulation of neuraminidase-desialylated LDL in cells was supported by other studies [1]. The loss of sialic acid (terminal sugar in N-chains of apo B and LDL glycosides) is an atherogenic modification. Our experiments showed that modified LDL gain atherogenicity after desialylation, which occurs in human blood [9]. Increasing the size of LDL particles (including desialylated LDL) is accompanied by an increase in their ability to induce accumulation of intracellular cholesterol [10]. Changes in physicochemical properties of LDL probably impair their resistance to aggregation. Aggregated LDL are strongly

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fixed in cells of the vascular intima and stimulate lipidosis.

Human blood also have other fractions of apo B-containing lipoproteins (apo B-LP), including very-low-density lipoproteins (VLDL) and intermediate-density lipoproteins (IDL).

This work was designed to detect desialylated VLDL and IDL in human blood. We evaluated whether desialylation of VLDL and IDL (as compared to LDL) affects their ability to stimulate cholesterol accumulation in cultured cells from the intact human aortic intima. It was interesting to determine whether atherogenicity of apo B-LP is associated with their resistance to aggregation and depends on the size of lipoprotein particles.

MATERIALS AND METHODS

Subfractions of apo B-LP were isolated by ultracentrifugation. The density of blood plasma was brought to 1.390 g/ml with NaBr (0.5 g/ml plasma). The plasma (4 ml) was put in a 16×76-mm centrifuge tube (Beckman Instruments, Inc.). NaBr (1.019 g/ml, 6 ml) was layered onto the plasma. The tube was centrifuged at 42,000 rpm for 2 h using a 50Ti rotor (Beckman Instruments, Inc.). The total fraction of VLDL, IDL, and LDL was isolated. NaBr in a concentration of 0.5 g'ml was added to the isolated fractions. The fraction of VLDL+IDL was separated into subfractions by repeated centrifugation at 42,000 rpm for 10 min. The subfraction of LDL was recentrifuged at 42,000 rpm and 4°C for 2 h. The preparations were dialyzed against a 4000-fold volume of phosphate buffer containing 10 mM ethylenediaminetetraacetic acid (pH 7.4) at 4°C for a night. Native and circulating strongly modified apo B-LP were separated by lecithin chromatography in a column packed with *Ricinus communis* agglutinin agarose (Boehringer Mannheim GmbH) [12].

Native lipoproteins were treated with sialidases from *Arthrobacter ureafaciens* (α-2,6-specific sialidase) and Newcastle Disease virus (α-2,3-specific sialidase, Oxford GlycocoScience). Lipoproteins were incubated in acetate buffer (pH 5.0) with 0.002 U/ml enzymes at 37°C for 2 h, isolated from the incubation medium by repeated centrifugation, and dialyzed against a 2000-fold volume of phosphate buffer (pH 7.4). The concentration of sialic acid was measured colorimetrically [8].

Smooth muscle cells from intact human aortic intima (7-day cultures) were incubated in a CO_2 incubator at 100% humidity and 37°C for 24 h. We used medium 199 (Gibco Europe) containing 10% lipoprotein-deficient serum from a healthy donor and essential amount of apo B-LP. Protein concentration in cells and lipoproteins was measured by the method of Lowry. The total concentration of intracellular cholesterol was estimated after lipid extraction [2].

The degree of lipoprotein aggregation was estimated by fluctuations in light transmission (laser beam, λ=860 nm) on a LA220 two-channel aggregometer (Biola). The relative dispersion of variations in optical density produced by random changes in the number of particles in the optical path of a laser beam reflects deviation from their mean size (*i.e.*, aggregation).

The results were processed by analysis of variance ($p<0.05$).

RESULTS

We studied *in vivo* and *in vitro* desialylated apo B-LP. *In vivo* desialylated apo B-LP were isolated from human blood. *In vitro* desialylated apo B-LP were obtained by treatment of apo B-LP characterized by normal level of sialic acid (sialylated lipoproteins) with sialidases specific for α-2,6- and α-2,3-bound sialic acids. Sialidases from *Arthrobacter ureafaciens* and Newcastle Disease virus desialylate carbohydrate chains in apo B and gangliosides, respectively.

Incubation of apo B-LP with sialidase from *Arthrobacter ureafaciens* at 37°C for 2 h decreased the concentration of protein-bound sialic acid in VLDL, IDL, and LDL by 1.8, 1.5, and 1.4 times, respectively (Table 1). It should be emphasized that the concentration of lipid-bound sialic acid remained unchanged under these conditions.

Sialylated VLDL and LDL in various concentrations had no effect on intracellular cholesterol content (Fig. 1). Sialylated IDL in a concentration of 40 μg/ml increased intracellular cholesterol content by 20%.

TABLE 1. Concentration of Protein- and Lipid-Bound Sialic Acid before and after Treatment of Apo B-LP with α-2,6-Sialidase from *Arthro-bacter ureafaciens*

Lipoproteins	Sialic acid	
	protein-bound, nmol/mg	lipid-bound, nmol/mg
VLDL sialylated		
before treatment	16±1	10±1
after treatment	9±1*	9±1
IDL sialylated		
before treatment	122±7	22±2
after treatment	79±5*	20±1
LDL sialylated		
before treatment	66±3	20±1
after treatment	46±2*	18±1

Note. Here and in Table 2: *$p<0.05$ compared to sialylated lipoproteins.

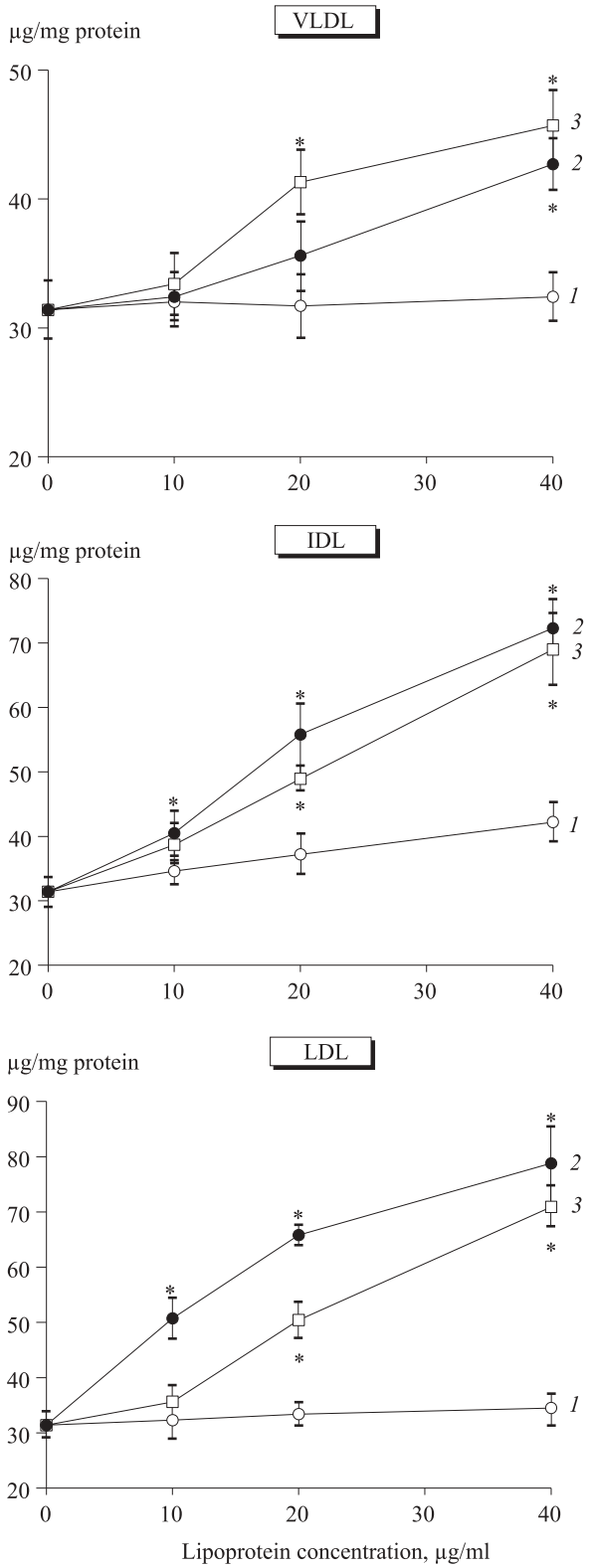

Fig. 1. Cholesterol content in smooth muscle cells of the intact human aortic intima depending on the concentrations of sialylated (*1*) and desialylated apo B-containing lipoproteins (apo B-LP) from human blood (*2*) and content of sialylated apo B-LP treated with α-2,3-sialidase (*3*). *p<0.05 compared to control sialylated lipoproteins.

Desialylated VLDL isolated from human blood plasma and applied in concentrations of 20 and 40 μg/ml stimulated accumulation of intracellular cholesterol. Desialylated IDL and LDL in various concentrations stimulated accumulation of cholesterol in aortic intima cells. VLDL treated with α-2,6-sialidase and used in a concentration of 40 μg/ml increased intracellular cholesterol content by 45%. IDL and LDL treated with α-2,6-sialidase and applied in concentrations of 20 and 40 μg/ml significantly increased cholesterol accumulation in aortic intima cells.

Sialidase from Newcastle Disease virus specific for 2,3-bound sialic acid significantly decreased sialic acid concentration in glycolipids of various glycoproteins (Table 2). Treatment with this sialidase did not significantly decreased the concentration of protein-bound sialic acid in apo B-LP (Table 2).

In various classes of lipoproteins the concentration of protein- and lipid-bound sialic acid was much lower compared to siallated apo B-LP (Table 2).

As differentiated from sialylated lipoproteins, VLDL and LDL (40 μg/ml) treated with α-2,3-sialidase increased the concentration of total cholesterol in smooth muscle cells from intact human aortic intima by 23 and 68%, respectively (Table 2). Sialylated IDL in a concentration of 40 μg/ml increased intracellular cholesterol content by 1.5 times (Table 2). IDL treated with α-2,3-sialidase increased intracellular cholesterol content by 2.1 times.

The mean size of VLDL treated with sialidases was greater compared to native lipoproteins (by 2.4 and 2.2 times, respectively). Treatment with glycolytic enzymes had a more pronounced effect on the mean size of IDL and LDL (3.1-3.9 and 6.0-6.2 times, respectively). The mean size of apo B-LP treated with sialidases did not differ from that of desialylated lipo-

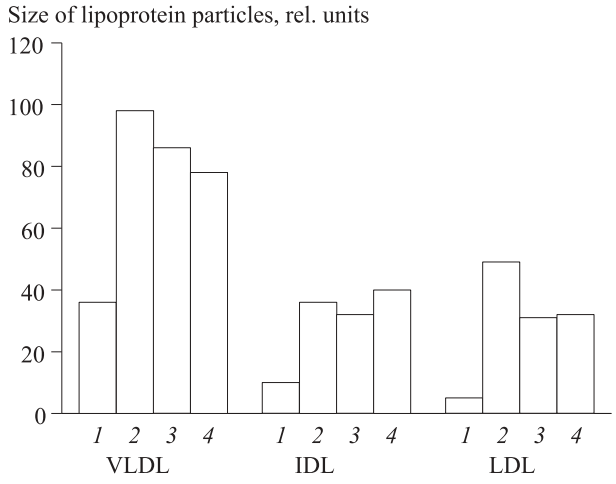

Fig. 2. Mean size of sialylated (*1*) and desialylated apo B-LP isolated from human blood (*2*) and sialylated apo B-LP treated with α-2,6-sialidase (*3*) and α-2,3-sialidase (*4*).

TABLE 2. Effect of α-2,3-Sialidase from Newcastle Disease Virus on the Concentration of Protein- and Lipid-Bound Sialic Acid in Apo B-LP and Ability of Apo B-LP to Stimulate Accumulation of Cholesterol in Smooth Muscle Cells of the Intact Human Aortic Intima

Lipoproteins		Sialic acid		
		protein-bound, nmol/mg protein	lipid-bound, nmol/mg phospholipid	Intracellular cholesterol, μg/mg protein
Control (without lipoproteins)				22.6±1.3
VLDL	sialylated	20±2	11±1	22.1±1.6
	after treatment	16±2	6±1*	27.8±0.9*
	desialylated	7±1*	6±1*	32.5±2.2*
IDL	sialylated	89±6	32±2	27.1±2.7
	after treatment	79±6	20±2*	48.6±4.0*
	desialylated	43±4*	24±2*	45.7±2.9*
LDL	sialylated	60±4	16±1	22.5±1.1
	after treatment	54±5	9±1*	37.9±3.0*
	desialylated	48±4*	8±1*	48.7±4.3*

proteins from human blood (Fig. 2). The coefficients of correlation between the mean size of sialidase-treated lipoproteins and accumulation of intracellular cholesterol induced by VLDL, IDL, and LDL were 0.55 (n=18), 0.68 (n=18), and 0.75 (n=36), respectively.

Thus, we showed for the first time that human blood contains subfractions of VLDL and IDL with reduced content of sialic acid (desialylated VLDL and IDL, respectively). Desialylation of carbohydrate chains of apolipoproteins and gangliosides in various classes of apo B-LP (VLDL, IDL, and LDL) induces aggregation of lipoprotein particles and stimulates cholesterol accumulation in smooth muscle cells of human aortic intima. Previous studied showed that human blood contains glycohydrolases (including those possessing sialidase activity) [7,9] and desialylated lipoproteins [3,4,11,12]. It can be hypothesized that desialylation of various classes of apo B-LP in human blood decreases the resistance of these particles to aggregation, which stimulates cholesterol accumulation in cells of human aortic intima (increase in atherogenicity of apo B-LP).

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