Multiply Modified Desialylated Low-Density Lipoproteins: Physicochemical Properties

V. V. Tertov, V. V. Kaplun, I. A. Sobenin, and A. N. Orekhov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 121, № 1, pp. 42-43, January, 1996
Original article submitted April 12, 1995

Sialylated and desialylated low-density lipoproteins from human blood are shown to differ markedly in physicochemical parameters. The latter lipoproteins have a smaller particle size, are denser and more electronegative, and tend to aggregate more readily than the former.

Key Words: *atherosclerosis; desialylated low-density lipoproteins; physicochemical properties*

We showed previously that human blood contains low-density lipoproteins (LDL) that are low in sialic acid (desialylated LDL) and cause lipid accumulation in cell culture [7]. The lipid and carbohydrate compositions of desialylated LDL were described in earlier reports. Here we examine the physicochemical characteristics of these lipoproteins.

MATERIALS AND METHODS

Pooled blood plasma from 24 men and 6 women (age range 28-48 years) suffering from ischemic heart disease with angiographically documented atherosclerosis was used. LDL (1.019-1.063 g/ml) were isolated by ultracentrifugation [4]. Sialylated and desialylated LDL were separated, as previously described [7], by lectin chromatography on ricin agglutinin (RCA_{120}) covalently bound to agarose.

The aggregation of LDL was estimated by measuring fluctuations in the optical density of a suspension of them [6]. The sizes of the aggregates, as well as of free LDL particles, were measured by the method of quasielastic laser scattering using an Autosizer 2 device (Malvern Instruments).

LDL were fractionated according to density by ultracentrifugation in a NaCl/KBr density gradient [5].

Cardiology Research Center, Russian Academy of Medical Sciences, Moscow (Presented by E. I. Chazov, Member of the Russian Academy of Sciences)

RESULTS

Measurements of particle size by quasielastic laser scattering yielded a mean value of 26.8 nm for sialylated LDL and 24.5 nm for desialylated LDL. Similar values were obtained using native electrophoresis in polyacrylamide gel: 26.2 nm and 22.9 nm for sialylated and desialylated LDL, respectively.

On agarose gel electrophoresis, desialylated LDL moved toward the anode 1.2 to 1.3 times as fast as

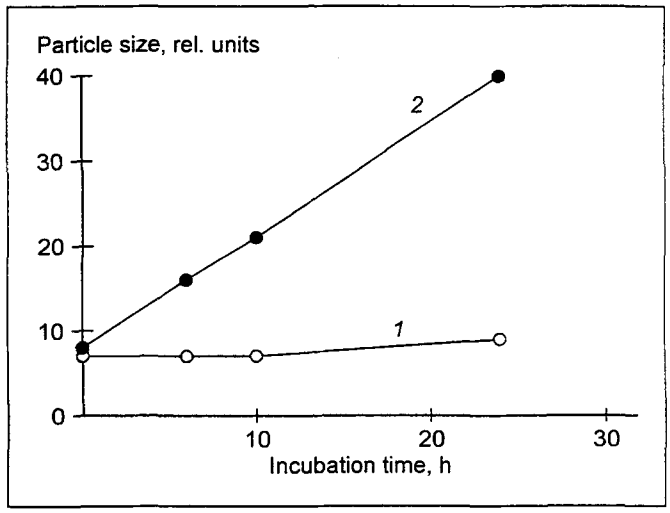

Fig. 1. Aggregation of sialylated (*1*) and desialylated (2) LDL from patients with ischemic heart disease. Lipoproteins were incubated in medium 199 containing 5% fetal calf serum, at 37°C. Mean particle size was determined by measuring fluctuations in the optical density of LDL suspensions.

0007-4888/96/0001-0038$15.00

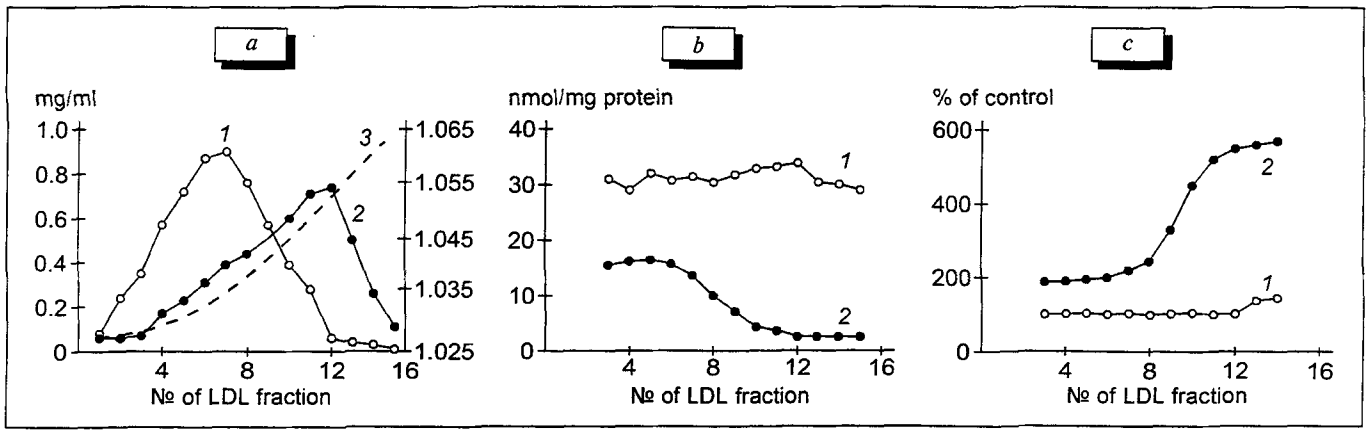

Fig. 2. Protein (*a*) and sialic acid (*b*) levels in sialylated (*1*) and desialylated (*2*) LDL fractions of different densities, and the effect of LDL density on intracellular cholesterol accumulation (*c*). LDL particle density values (g/ml) are shown on the right ordinate in *a*; LDL were fractionated according to density by gradient ultracentrifugation; *3*: density gradient.

did sialylated LDL, which indicates that their electronegativity is greater.

The rates at which LDL aggregate were estimated during their incubation at 37°C in medium 199 supplemented with 5% fetal calf serum. The mean particle size of sialylated LDL remained virtually unchanged throughout the 24-h incubation period, whereas that of desialylated LDL had increased approximately fivefold by the end of this period (Fig. 1), indicating that the latter LDL are prone to aggregate.

The distributions of LDL by density are shown in Fig. 2. In the case of desialylated LDL there is a clear shift toward higher densities (Fig. 2, *a*). Particle density has little effect on the sialic acid content of sialylated LDL (Fig. 2, *b*). In the low density region (1.019-1.035 g/ml), the sialic acid content of desialylated LDL is approximately half that of sialylated LDL; it decreases markedly with increasing particle density (Fig. 2, *b*).

Figure 2, *c* shows how LDL of different densities affected cholesterol accumulation in cultured smooth muscle cells from human aorta. It can be seen that the "dense" desialylated LDL (1.035-1.063 g/ml) increased cholesterol accumulation by the cells 2-6 times, whereas sialylated LDL had little or no effect on cholesterol accumulation.

Thus, sialylated and desialylated LDL show marked differences in their physicochemical properties. Desialylated LDL have smaller particle sizes than sialylated LDL and are denser. Their high electrophoretic mobility points to their greater electronegativity.

Austin *et al.* [1] and our studies [3] have demonstrated the existence of small/dense LDL low in sialic acid. As found in our laboratory, the "electronegative" LDL, first described by Avogaro *et al.* [2], contain lower sialic acid concentrations [3]. These findings, together with the results of the present study, suggest that the lipoprotein particles of various modified LDL have similar physicochemical parameters and possibly represent the same multiply modified low-density lipoproteins.

REFERENCES

1. M. A. Austin, J. L. Breslow, C. H. Hennekens, *et al.*, *J. Am. Med. Assoc.*, **260**, 1917-1921 (1988).
2. P. Avogaro, B. G. Bittolo, and G. Cazzolato, *Arteriosclerosis*, **8**, 79-87 (1988).
3. O. Jaakkola, T. Solakivi, V. V. Tertov, *et al.*, *Arteriosclerosis*, **10**, 858 (1990).
4. F. T. Lindgren, in: *Analysis of Lipids and Lipoproteins*, New York (1975), pp. 205-224.
5. B. Teng, G. R. Thompson, A. D. Sniderman, *et al.*, *Proc. Nat. Acad. Sci. USA*, **80**, 6662-6666 (1983).
6. V. V. Tertov, I. A. Sobenin, Z. A. Gabbasov, *et al.*, *Biochem. Biophys. Res. Commun.*, **163**, 489-494 (1989).
7. V. V. Tertov, I. A. Sobenin, A. G. Tonevitsky, *et al.*, *Biochem. Biophys. Res. Commun.*, **167**, 1122-1127 (1990).