REVIEWS

Multiple Modifications of Low-Density Lipoproteins in the Blood of Patients with Atherosclerosis

A. N. Orekhov, V. V. Tertov, and V. L. Nazarova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 120, № 8, pp. 118-121, August, 1995
Original article submitted August 17, 1994

Atherogenic low-density proteins (LDL) found in human blood - desialylated, electronegative, and small dense LDL - share many chemical and physical characteristics and appear to represent the same subfraction of multiply modified lipoproteins.

Key Words: *atherosclerosis; desialylated, electronegative, and small dense low-density lipoproteins*

Epidemiological studies have revealed a close association between elevated concentrations of low-density lipoproteins (LDL) in human blood plasma and early development of atherosclerosis [11], suggesting that LDL are blood components responsible for cholesterol accumulation in the vessel wall and for certain other manifestations of atherosclerosis. More recently, modified rather than native LDL were found to be responsible for cholesterol buildup in the arterial wall. Studies were therefore undertaken to detect modified LDL in human blood, and several types of such LDL have now been identified. However, by no means all modifications of LDL particles are atherogenic, i.e., capable of imparting to the lipoproteins a capacity to induce atherosclerotic manifestations. Glycosylated LDL found in the blood of patients with diabetes mellitus have an atherogenic potential, for they stimulate cholesterol accumulation in cultured macrophages and smooth muscle cells [19,29]. Atherogenic modified LDL have also been detected in the blood of patients with diseases other than diabetes mellitus. While it is generally believed that oxidized LDL represent the main types of modified LDL occurring *in vivo*, so far such LDL have not been detected in human blood, aside from the LDL recovered from atherosclerotic lesions in vessels (so-called "tissue" LDL with properties very similar to those of oxidized LDL) [14,38]. On the other hand, several types of naturally occurring modifications of LDL circulating in the blood have been identified, namely, small dense LDL, electronegative LDL, desialylated LDL, and LDL with heightened susceptibility to oxidation which possibly accumulate in areas of atherosclerotic vascular lesions as "tissue" LDL. The types of modified LDL with atherogenic properties have many similar and overlapping properties which are listed in Table 1 and discussed below.

1. Atherogenic properties of circulating LDL are determined by their ability to potentiate cholesterol accumulation in cells, as was demonstrated for cultured smooth muscle cells and macrophages. Thus, cholesterol accumulation in cultured smooth muscle cells [15,34] is caused by small dense LDL, and these LDL have a direct relation to atherosclerosis [1,17]. A subfraction of electronegative LDL isolated from the blood of healthy indi-

Institute of Experimental Cardiology, Cardiology Research Center, Russian Academy of Medical Sciences, Moscow (Presented by D. S. Sarkisov, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0008-0759$12.50

viduals induced cholesterol accumulation within macrophages [2]. Desialylated LDL (those with low levels of sialic acid) were isolated directly from the blood of patients with angiographically documented atherosclerosis [23] and subsequently were also found in the blood of healthy people, though in much lower concentrations [37]. Desialylated LDL stimulate lipid accumulation in cultured cells and induce cell proliferation and production of extracellular matrix, i.e., they elicit atherosclerosis-associated phenomena at the cellular level [22]. Lipoproteins oxidized *in vitro* and "tissue" LDL stimulate cholesterol accumulation in macrophages [6,26]. Modified LDL of all types isolated directly from the blood of patients with coronary atherosclerosis have proved to be more susceptible to oxidation than are LDL from healthy individuals [5]. This indicates that LDL are potentially capable of undergoing oxidation *in vivo*. Evidence of a correlation between the degree of LDL susceptibility to oxidation and the severity of coronary atherosclerosis has been obtained [27].

2. Low sialic acid levels is a characteristic feature not only of desialylated LDL but also of the small dense LDL [18]. Moreover, as we have found, sialic acid is also present at a reduced level in circulating electronegative LDL: 7.4±0.5 *vs.* 47.5±5.2 nmol/mg apolipoprotein B (apoB) in native LDL ($p<0.001$). Since oxidized LDL as such have not been isolated so far, their sialic acid content remains unknown. Studies have shown that sialic acid levels in "tissue" LDL are lower than in native LDL (39.1±5.8 *vs.* 57.8±4.2 nmol/mg apoB; $p<0.025$).

3. *In vivo* isolated modified LDL have a higher negative surface charge than native LDL. A subfraction of electronegative LDL was isolated from human blood directly by ion-exchange chromatography [2]. Examination of desialylated and small dense LDL showed these to have a higher electrophoretic mobility, i.e., a higher negative charge, than native LDL [15,34]. The "tissue" LDL recovered from lesioned vessel walls exhibited increased electrophoretic mobility [13].

4. Desialylated LDL particles are much smaller and much denser than native LDL particles [34]. There is thus a similarity between desialylated LDL and small dense LDL. No data on the sizes of electronegative LDL and "tissue" LDL particles have been reported, due to the difficulty of measuring the diameters of these particles because of their aggregation [2,14]. It has been established, however, that the density of lipoproteins increases upon their oxidation *in vitro* [31], and that "tissue" LDL isolated from the vessel wall are denser than native LDL [20].

5. It is widely known that modified LDL circulating in the blood are highly susceptible to oxidation. This is equally true of desialylated [34], small dense [8,22], and electronegative [3] LDL. The high susceptibility of LDL to oxidation may be either a cause or a consequence of lowered levels of endogenous antioxidants, including vitamin E, in modified lipoprotein particles. Lowered vitamin E levels have been detected in desialylated [34], small dense [9], and electronegative [3] LDL. The content of this vitamin also decreases as a result of LDL oxidation *in vitro* [10].

TABLE 1. Characteristics of *in Vivo*–Isolated Modified LDL

	Desialylated LDL	Small dense LDL	Electronegative LDL	"Tissue" LDL
Intracellular lipid accumulation	+	+	+	+
Sialic acid level	low	low	low	low
Electronegative charge	high	high	high	high
Size reduced	reduced	?	?	
Density high	high	?	high	
Susceptibility to oxidation	high	high	high	high
Content of:				
vitamin E	low	low	low	low
lysophospholipids	high	?	high	high
hydroxysterols	high	?	high	high
cholesterol esters	low	low	low	low
phospholipids	low	low	low	low
Aggregation	high	high	high	high
Autoantibodies to modified LDL	+	?	?	+
Binding to scavenger receptor	+	?	?	+
Binding to B,E–receptor	lowered	lowered	lowered	lowered

6. Desialylated and electronegative LDL display some of the properties of oxidized LDL. Desialylated LDL [34], electronegative LDL [3], "tissue" LDL [7,30], and *in vitro*-oxidized LDL [30,31] are high in lysophospholipids and hydroxysterols, which are probably oxidation products. No data have been reported on levels of lysophospholipids or hydroxysterols in small dense LDL.

7. All types of modified LDL isolated *in vivo*, as well as *in vitro*-oxidized LDL, have lowered levels of phospholipids and cholesterol esters [2,3, 15,28,31,34].

8. Desialylated LDL [31,35], small dense LDL [15,34], electronegative LDL [2], and "tissue" and *in vitro*-oxidized LDL [14] all tend to aggregate.

9. Autoantibodies to desialylated LDL have been detected in human blood [21]. In addition, autoantibodies interacting with "tissue" LDL [38] and with LDL modified by malonic dialdehyde [25] have been identified, which indicates that the antigens for these autoantibodies are oxidized LDL. To date, no autoantibodies to small dense or electronegative LDL have been identified.

10. Desialylated LDL [24], "tissue" LDL [28], and *in vitro*-oxidized LDL [12] are taken up by the scavenger receptor of macrophages, which results in decreased binding of the modified LDL to the B,E-receptor [3,16,24,28,32,33].

The overview presented above leads to the conclusion that modified LDL of all known types isolated from human blood directly have similar properties and may be interpreted as the same particles which have undergone multiple modifications. That multiple modifications are a real possibility cannot be denied. Two pertinent examples are the subfractions of glycosylated LDL isolated from the blood of patients with diabetes mellitus and the electronegative LDL isolated from the blood of healthy subjects; both types of LDL are modified atherogenic lipoproteins. The lipoproteins from diabetic patients not only are glycosylated but also contain a lowered level of sialic acid [29]. On the other hand, the desialylated LDL from these patients are more glycosylated than the normal sialylated LDL from the same patients [29]. Furthermore, the glycosylated LDL from diabetic patients resemble all modified LDL isolated *in vivo* so far [29]; in particular, they show heightened susceptibility to oxidation [4]. Recently, we compared sialic acid levels in the fractions of native and electronegative LDL (isolated by Dr. Bittolo Bon with ion-exchange chromatography) and found the electronegative LDL to contain 6 times less sialic acid than native LDL (see item 2). This finding, together with the data on glycosylated desialylated LDL from patients with diabetes mellitus, indicates that multiple modifications of lipoprotein particles are possible.

It remains to be established whether multiple modifications result from defects at the gene level or whether LDL undergo such changes in the blood and peripheral organs. It may well be that multiple modifications are a consequence of partial LDL degradation under the action of intra- and extralysosomal enzymes. One of the most important issues is which change in the LDL particle occurs first and triggers subsequent modifications, including alterations in several physicochemical properties of the lipoproteins. It should also be recognized that not all modifications in LDL particles are atherogenic, i.e., capable of including atherosclerosis-related manifestations. This and other aspects require further study of *in vivo*-circulating modified LDL.

Since we have been focusing on the similarities between modified LDL of different types, we have not discussed the existing differences between them because these do not contradict the concept of multiple modifications. To date, a satisfactory homogeneous fraction of such modified LDL has not been isolated. The observed lack of concordance in characteristics among different types of modified LDL may be explained by the presence of unmodified LDL in the preparations.

REFERENCES

1. M. A. Austin, J. L. Breslov, C. H. Hennekens, *et al.*, *JAMA*, **260**, 1917 (1988).
2. P. Avogaro, G. Bittolo Bon, and G. Cazzolato, *Arteriosclerosis*, **8**, 79 (1988).
3. P. Avogaro, G. Cazzolato, and G. Bittolo Bon, *Atherosclerosis*, **91**, 163 (1991).
4. A. Bowie, D. Owens, P. Collins, *et al.*, *Atherosclerosis*, **102**, 63 (1993).
5. H. C. Chiu, J. R. Jeng, and S. M. Sheih, *Biochim. Biophys. Acta*, **1225**, 200 (1994).
6. B. A. Clevidence, R. E. Morton, G. West, *et al.*, *Arteriosclerosis*, **4**, 196 (1983).
7. A. Daugherty, B. S. Zweifel, B. E. Sobel, and G. Schonfeld, *Arteriosclerosis*, **8**, 768 (1988).
8. J. DeGraaf, H. L. M. Hak-Lemmers, M. P. C. Hectors, *et al.*, *Arterioscler. Thromb.*, **11**, 298 (1991).
9. S. Deiager, E. Bruckert, and M. J. Chapman, *J. Lipid Res.*, **34**, 295 (1993).
10. H. Esterbauer, G. Striegl, H. Puhl, and M. Rothender, *Free Radic. Res. Commun.*, **6**, 67 (1989).
11. J. W. Gofman, W. Young, and R. Tandy, *Circulation*, **34**, 679 (1966).
12. M. E. Haberland, A. M. Fogelman, and P. A. Edvards, *Proc. Nat. Acad. Sci. USA*, **79**, 1712 (1982).
13. H. F. Hoff, W. A. Bradley, C. L. Heideman, *et al.*, *Biochim. Biophys. Acta*, **573**, 361 (1979).
14. H. F. Hoff and J. O'Neil, *Arterioscler. Thromb.*, **11**, 1209 (1991).

15. O. Jaakkola, T. Solakivi, V. V. Tertov, *et al.*, *Artery Dis.*, **4**, 379 (1993).
16. Y. Kleinman, S. Eisenberg, Y. Oschry, *et al.*, *J. Clin. Invest.*, **75**, 1796 (1985).
17. P. O. Kwiterovich Jr., *Clin. Chem.*, **34**, 71 (1988).
18. M. La Belle and R. M. Krauss, *J. Lipid Res.*, **31**, 1577 (1990).
19. T. J. Lyons, R. L. Klein, J. W. Baynes, *et al.*, *Diabetologia*, **30**, 916 (1987).
20. R. E. Morton, G. A. West, and H. F. Hoff, *J. Lipid Res.*, **27**, 1124 (1986).
21. A. N. Orekhov, V. V. Tertov, A. E. Kabakov, *et al.*, *Arterioscler. Thromb.*, **11**, 316 (1991).
22. A. N. Orekhov, V. V. Tertov, S. A. Kudryashov, and V. N. Smirnov, *Circ. Res.*, **66**, 311 (1990).
23. A. N. Orekhov, V. V. Tertov, D. N. Mikhin, and I. A. Mikhailenko, *Biochem. Biophys. Res. Commun.*, **162**, 206 (1989).
24. A. N. Orekhov, V. V. Tertov, I. N. Sobenin, *et al.*, *J. Lipid Res.*, **33**, 805 (1992).
25. W. Palinski, M. E. Rosenfeld, S. Yla-Herttuala, *et al.*, *Proc. Nat. Acad. Sci. USA*, **86**, 1372 (1989).
26. S. Parthasarathy, U. P. Steinbrecher, J. Barnet, *et al.*, *Proc. Nat. Acad. Sci. USA*, **82**, 3000 (1985).
27. J. Regnstorm, J. Nilsson, P. Tornvall, *et al.*, *Lancet*, **339**, 1183 (1992).
28. M. Shaikh, S. Martini, J. R. Quiney, *et al.*, *Atherosclerosis*, **69**, 165 (1988).
29. I. A. Sobenin, V. V. Tertov, T. Koschinsky, *et al.*, *Atherosclerosis*, **100**, 41 (1993).
30. U. P. Steinbrecher and M. Lougheed, *Arterioscler. Thromb.*, **12**, 608 (1992).
31. U. P. Steinbrecher, S. Parthasarathy, D. S. Leake, *et al.*, *Proc. Nat. Acad. Sci. USA*, **81**, 3883 (1984).
32. U. P. Steinbrecher, J. L. Witztum, S. Parthasarathy, and D. Steinberg, *Arteriosclerosis*, **1**, 135 (1987).
33. B. Teng, A. Sniderman, R. M. Krauss, *et al.*, *J. Biol. Chem.*, **260**, 5067 (1985).
34. V. V. Tertov, I. A. Sobenin, Z. A. Gabbasov, *et al.*, *Lab. Invest.*, **67**, 665 (1992).
35. V. V. Tertov, I. A. Sobenin, Z. A. Gabbasov, *et al.*, *Biochem. Biophys. Res. Commun.*, **163**, 489 (1989).
36. V. V. Tertov, I. A. Sobenin, Z. A. Gabbasov, *et al.*, *Circ. Res.*, **71**, 218 (1992).
37. V. V. Tertov, I. A. Sobenin, A. G. Tonevitsky, *et al.*, *Biochem. Biophys. Res. Commun.*, **167**, 1122 (1990).
38. S. Yla-Herttuala, W. Palinski, M. E. Rosenfeld, *et al.*, *J. Clin. Invest.*, **84**, 1086 (1989).