EFFECT OF COMPONENTS OF THE EXTRACELLULAR MATRIX ON LIPID ACCUMULATION IN HUMAN CELLS

V. É. Kotelyanskii, A. N. Orekhov, V. V. Tertov, Kh. A. Khashimov, M. A. Glukhova, M. G. Frid, O. I. Ornatskaya, T. D. Vasilevskaya, and V. N. Smirnov

UDC 616.13-004.6-092:616.13-008.939.15

KEY WORDS: low-density lipoproteins; cholesterol accumulation; extracellular matrix

Lipid accumulation in cells of the blood vessel wall is one of the most important early manifestations of atherosclerosis. It is generally considered that the control of lipoprotein hemostasis is effected by lipoprotein receptors, exposed on the cell surface. Lipoprotein receptors play a key role in the regulation of cholesterol and triglyceride catabolism; they have been found on the surface of fibroblasts, smooth-muscle cells, macrophages, leukocytes, liver cells, etc. [7, 10]. However, during lipid accumulation by cells of the vascular wall, accompanying atherogenesis, the most important role is played by processes in which lipoprotein receptors are not directly involved. Lipid-loaded (foam) cells of the vascular wall affected by atherosclerosis are macrophages of the monocytic series and smooth-muscle cells. The source of the lipids is evidently plasma low-density lipoproteins (LDL), which can pass through the monolayer of endothelial cells into the subendothelial intima. The mechanism of LDL-mediated lipid accumulation in cells of the subendothelial intima is not clear. During incubation of macrophages or smooth-muscle cells in medium with high concentrations of native LDL lipids do not accumulate in the cells. A significant quantity of LDL is taken up by the cells only if chemically modified LDL are used. Since most of the LDL found in the vessel wall lie outside the cells, it has been suggested that LDL uptake and catabolism by cells of the vascular wall may be influenced by components of the extracellular matrix. It has recently been reported that LDL, incorporated into insoluble complexes with components of the extracellular matrix — fibronectin (FN), heparin (H), and gelatin (G) — and denatured collagen are actively phagocytosed by mouse peritoneal macrophages, as a result of which cholesterol esters accumulate in the cells [5].

The aim of this investigation was to study the effect of components of the extracellular matrix of lipid accumulation by various human cells.

EXPERIMENTAL METHOD

FN was isolated from human blood plasma [4]. Human plasma LDL (density 1.019-1.063 g/ml)

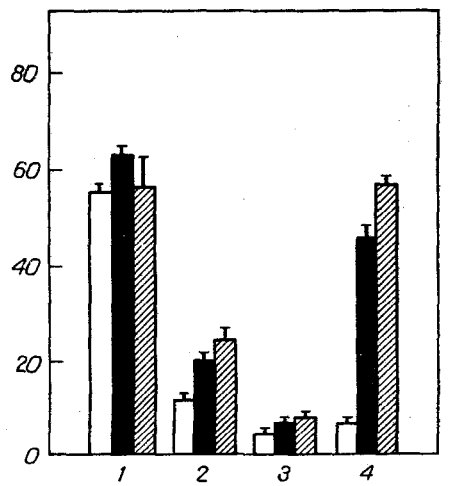

Fig. 1. Lipid content in cells of subendothelial intima of human aorta after incubation with LDL or with LDL—H—FN—G for 24 h. Ordinate, lipid concentration (in $\mu g/10^5$ cells). Unshaded columns — native LDL added to the cells, black columns — LDL—H—FN complexes added, obliquely shaded columns — LDL—H—FN—G added. 1) Phospholipids; 2) free cholesterol; 3) triacylglycerides; 4) cholesterol esters.

All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 11, pp. 562-564, November, 1987. Original article submitted December 25, 1986.

 0007-4888/87/0011-1544$12.50

TABLE 1. Effect of Components of Extracellular Matrix on Cholesterol Accumulation by Cells Isolated from Human Aorta

Material	Total cholesterol concentration, μg/10⁵ cells	
	cells from intima (primary culture)	cells from media, subcultured
Nothing added	20,3±1,8	5,2±0,4
LDL	20,5±1,6	5,5±0,3
LDL + H	18,1±0,7	4,3±0,4
LDL + FN	19,6±2,6	4,3±0,4
LDL + G	31,5±2,6	5,5±0,9
LDL + H + FN	32,0±2,4	7,4±1,7
LDL—H—FN (insoluble complex)	153,7±8,3	13,8±1,3
LDL—H—FN—G (insoluble complex)	195,7±4,3	17,3±1,3

TABLE 2. Cholesterol Accumulation by Various Human Cells after Incubation for 24 h with LDL or with Insoluble LDL—H—FN—G Complexes

Type of cells	Total cholesterol concentration, μg/10⁵ cells		
	intact LDL	LDL—H—FN—G	LDL/LDL—H—FN—G
Monocytes	1,7±0,2	7,6±1,1	4,4
Peritoneal macrophages	7,5±0,7	46,6±3,9	6,2
Subendothelial aortic intima (primary culture)	20,3±1,8	195,7±4,3	9,7
Medial of aorta (primary culture)	5,2±0,4	17,3±1,3	3,3
Medial of aorta (subculture, 7th passage)	4,6±0,6	14,5±2,4	3,2
Endothelium of aorta	8,2±0,7	25,3±3,0	3,1
Skin fibroblasts	5,1±0,2	7,7±0,3	1,5
Hepatocytes	4,6±0,6	13,0±0,5	2,8

were obtained by the flotation method [8]. To prepare insoluble complexes the LDL (0.6 ml protein) were diluted with a 5% solution of G to 6 ml, after which 0.2 mg of H (1 mg/ml), 1 ml of 0.5 M $CaCl_2$ solution, and 1.4 mg FN were added. The mixture was allowed to stand overnight at 4°C, the complexes were sedimented by centrifugation for 15 min at 1500g, and the residue was suspended in medium 199 [5]. Cells of the intima of the human aorta were obtained by treatment of the tissue with collagenase, and cells of the media were obtained by treatment with collagenase and elastase [11]. Cultures of endothelial cells from the human aorta and of cells from the media of the human aorta were obtained at the 5th-7th passage by the methods in [6, 9]. Human skin fibroblasts were isolated by the method in [3]. Hepatocytes were obtained by perfusion of the liver with collagenase [1]. Peritoneal macrophages were isolated from the ascites fluid of a patient with cardiovascular failure. Monocytes were obtained from human blood in a Percoll gradient [13]. To study interaction between LDL-containing insoluble complexes and cells the latter were transplanted into 24-well culture panels, and 3 days later (20,000-40,000 cells) insoluble complexes (100 μg LDL protein/ml) were added to them in medium 199 with 10% calf serum, previously delipidized (1 ml suspension per well). After incubation for 24-48 h the cells were washed with warm (37°C) medium and 0.02% trypsin, removed from the substrate with 0.5% gypsin, washed again with medium, and assayed for their lipid content [2, 12].

EXPERIMENTAL RESULTS

In the experiments of series I cells of the subendothelial intima and media of the human aorta were incubated with LDL in the presence of FN, G, or H for 24 h, after which their total cholesterol content was determined. It will be clear from Table 1 that the addition of FN, G, or H together with LDL to the cell culture had no significant effect on the cholesterol concentration in the cells. By contrast with this, after incubation of the cell culture with insoluble LDL—H—FN—G or LDL—H—FN complexes the cholesterol concentration in the cells was considerably increased (eight-tenfold in the case of cells from the intima). It will be clear from Fig. 1 that mainly cholesterol esters accumulated in cells of the subendothelial intima, and their concentration was increased by 7-9 times. The concentration of free cholesterol almost doubled, that of triacylglycerides increased by 1.5-1.8 times, whereas the phospholipid concentration in the cells remained virtually unchanged.

In the experiments of series II the comparative ability of the different human cells to accumulate lipids during incubation with insoluble LDL—H—FN—G complexes was studied. The results of these experiments are summarized in Table 2. The greatest degree of cholesterol accumulation was observed in cells of the intima and in peritoneal macrophages. Significant quantities of cholesterol also were accumulated by monocytes, endothelial cells, and also by smooth-muscle cells from the media of the human aorta. The least cholesterol was accumulated by

human skin fibroblasts. Thus all types of cells studied were able to accumulate lipids when incubated with complexes containing LDL, FN, H, and G, although the ability of the cells to accumulate lipids depended on the type of cells. It must be emphasized that the greatest quantity of lipids was accumulated by cells of the subendothelial intima, i.e., by the layer of the vessel wall which undergoes pathological changes during atherogenesis. It can thus be tentatively suggested that components of the extracellular matrix play an important role in the formation of lipid-loaded foam cells, which accumulate in regions of the arterial wall affected by atherosclerosis.

Consequently components of the extracellular matrix can disturb LDL catabolism in human cells. During classical receptor binding LDL are quickly taken up by lysosomes and are hydrolyzed, and during endocytosis of insoluble LDL—H—FN—B complexes processes of LDL degradation are disturbed, probably due to ineffective uptake of LDL by lysosomes.

LITERATURE CITED

1. E. Jaffe (ed.), Biology of Endothelial Cells, Boston (1984), p. 1.
2. D. M. Bissell and P. S. Guzelian, Ann. New York Acad. Sci., 349, 85 (1980).
3. E. G. Blight and W. J. Dyer, Can. J. Biochem., 37, 911 (1959).
4. M. S. Brown, S. E. Dana, and J. L. Goldstein, J. Biol. Chem., 249, 789 (1979).
5. E. Engvall and E. Ruoslahti, Int. J. Cancer, 20, 1 (1977).
6. D. J. Falcone, N. Mated, H. Shio, et al., J. Cell Biol., 99, 1266 (1984).
7. M. A. Glukhova, A. E. Kabakov, D. I. Ornatsky, et al., FEBS Lett., 189, 291 (1985).
8. J. L. Goldstein and M. S. Brown, Annu. Rev. Biochem., 46, 897 (1977).
9. R. J. Havel, H. A. Eder, and J. H. Blagdon, J. Clin. Invest., 34, 1345 (1955).
10. R. W. Mahley and T. L. Innerarity, Biochim. Biophys. Acta, 737, 197 (1983).
11. A. N. Orekhov, V. A. Kosykh, V. S. Repin, and V. N. Smirnov, Lab. Invest., 48, 749 (1983).
12. V. V. Tertov, A. N. Orekhov, V. S. Repin, and V. N. Smirnov, Biochem. Biophys. Res. Commun., 109, 1228 (1982).
13. S. D. Wright and S. C. Silverstein, J. Exp. Med., 156, 1149 (1982).

SPECTROPHOTOMETRIC DETERMINATION OF OSMOTIC RESISTANCE OF LYSOSOMES ISOLATED FROM ANIMAL TISSUES

S. P. Syatkin and V. A. Frolov

UDC 612.014.462.1.014.2:576.314

KEY WORDS: lysosomes; osmotic resistance

The fact that lysosomes have a single semipermeable and closed membrane endows them with properties of an osmotic system [5]. Low-molecular-weight substances damage them by a greater degree than compounds with high molecular weight [3]. Realization of the osmotic properties of lysosomes is observed in various pathophysiological states and exposure to physicochemical factors. Reoxygenation of the anoxic heart [4], ischemia of the liver [6], and myocardium [9], and also overloading of lysosomes with lysosomotropic preparations [1] lead to vacuolar degeneration of these particles. This is the reason why determination of the osmotic resistance of lysosomes is important as a convenient and informative test when studying the dynamics of the state of the lysosomal apparatus of the cell in experiments conducted in the field of molecular biology, cellular biochemistry, biochemical pharmacology, and clinical medicine. However, quantitative estimation of the osmotic resistance of lysosomes is difficult because of the lack of a rapid and direct method of determining the structural integrity of these particles. This property is evaluated indirectly by determining the balance between different forms of activity of acid hydrolases, marker enzymes for these organelles [2, 7]. Nonsedimented activity of the enzyme as a fraction (in per cent) of total activity is regarded as a measure of integrity of the lysosomes. The disadvantages

Department of Pathological Physiology, Medical Faculty, Patrice Lumumba People's Friendship University, Moscow.

Translated from Éksperimental'noi Biologii Meditsiny, Vol. 104, No. 11, pp. 564-567, November, 1987. Original article submitted February 20, 1987.

0007-4888/87/0011-1546$12.50