The findings suggest that glucocorticoids have an important regulatory role directed toward the maintenance of the optimal level of binding and internalization of HDL_3 by hepatocytes. Their stimulatory effect on the expression of HDL "receptors" is independent of the CH content in parenchymal liver cells.

REFERENCES

1. A. V. Bocharov, I. B. Frdina, V. G. Spirov, *et al., Byull. Eksp. Biol. Med.*, **109**, No. 6, 590-592 (1990).
2. V. S. Repin and V. N. Smirnov, *Basic Research in Atherosclerosis. Review* [in Russian], Moscow (1989).
3. V. B. Rosen, *Fundamentals of Endocrinology* [in Russian], Moscow (1984).
4. M. Bradford, *J. Biol. Chem.*, **72**, 248-256 (1976).
5. M. L. Fernandez and D. J. McNamara, *Metabolism*, **40**, 127-134 (1991).
6. N. Fidge, A. Kagami, and M. O'Connor, *Biochem. Biophys. Res. Commun.*, **129**, 759-765 (1985).
7. B. A. Gottlieb and J. B. March, *Atherosclerosis*, **67**, 251-259 (1987).
8. D. L. Graham and J. F. Oram, *J. Biol. Chem.*, **262**, 7439-7442 (1987).
9. K. Henze, B. J. Kudchodkar, A. Chait, *et al., Biochim. Biophys. Acta*, **666**, 199-204 (1981).
10. P. B. Hylemon, E. C. Gurley, R. T. Stravitz, *et al., J. Biol. Chem.*, **267**, 16866-16871 (1992).
11. L. Keso, M. Lukka, *et al., FEBS Lett.*, **215**, 108 (1987).
12. A. S. McFarlane, *Nature*, **182**, 53 (1958).
13. T. G. Redgrave, D. C. K. Roberts, and E. West, *Ann. Biochem.*, **65**, 42-49 (1975).
14. B. M. Richard and R. C. Pittman, *J. Lipid Res.*, **34**, 571-579 (1993).
15. G. Scatchard, *Ann. New York Acad. Sci.*, **51**, 660-672 (1949).
16. W. H. Sperry and M. Webb, *J. Biol. Chem.*, **187**, 497-509 (1950).
17. D. D. Sviridov, M. Y. Pavlov, I. G. Safonova, *et al., Atherosclerosis*, **31**, 1821-1830 (1990).

Interaction between Multiply Modified (Desialylated) Low-Density Lipoproteins Isolated from Blood of Atherosclerotic Patients and Cell Receptors

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It is shown that binding of native LDL to fibroblasts expressing the B,E-receptors is twice as high as that of desialylated LDL. An excess of acetylated LDL inhibits binding, uptake, and degradation of ^{125}I-desialylated LDL by macrophages, while an excess of desialylated LDL inhibits binding, uptake, and degradation of acetylated LDL. Desialylated LDL may interact with both B,E and scavenger receptors.

Key Words: *low-density lipoproteins; sialic acid; cell culture; metabolism of low-density lipoproteins*

Previously we found that blood serum of patients with coronary atherosclerosis possesses an atherogenic potential, i.e., it is capable of stimulating lipid accumulation by cultured cells from intact aorta intima [3,6]. The atherogenicity of the serum was shown to be due to the presence of a subfraction of low-density lipoproteins (LDL) with a reduced content of sialic acid - desialylated LDL, which, unlike native (sialylated) LDL, induce lipid

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TABLE 1. Binding (ng/mg Protein per 2 h) of Native and Modified ^{125}I-LDL by Human Skin Fibroblasts and P388D1 Mouse Macrophages ($M \pm m$)

Competitor	^{125}I-LDL	^{125}I-dsLDL	^{125}I-acLDL
	Fibroblasts		
Control	7.8±0.2*	3.5±0.2	–
nLDL	0.6±0.1*	0.3±0.1*	–
dsLDL	2.0±0.1*	0.3±0.1*	–
	Macrophages		
Control	12.4±1.8	73.9±2.9	300.8±6.9
nLDL	2.3±0.2*	58.4±1.3	286.7±14.9*
dsLDL	2.5±0.2*	43.4±4.2	200.7±16.5*
acLDL	11.7±0.5*	35.4±1.8	42.4±4.3*

Note. Here and in Table 2: nLDL – native LDL, dsLDL – desialylated LDL, acLDL – acetylated LDL. *: $p<0.05$ in comparison with cells incubated without unlabeled LDL.

accumulation in cultured cells [7,8]. Further investigation showed that desialylated lipoproteins differ from native LP in a number of chemical and physicochemical characteristics, i.e., they are multiply modified [10].

In the present study we investigated the interaction of native and desialylated lipoproteins with both LDL-specific and scavenger receptors. These experiments were carried out using human skin fibroblasts possessing LDL receptors, and P388D1 mouse macrophages carrying both LDL and scavenger receptors.

MATERIALS AND METHODS

Blood plasma was pooled from 10 patients aged 35-50 with coronary heart disease (CHD) and effort angina pectoris of functional classes II-IV and from 10 healthy subjects with no history of CHD and without signs of the disease upon medical examination. LDL (d=1.019-1.063 g/cm^3) were isolated by preparative ultracentrifugation in a NaBr density gradient [5]. The subfraction of desialylated LDL was isolated from pooled plasma of CHD patients by column chromatography on CL-4B-Sepharose with immobilized *Ricinus communis* agglutinin (RCA120) [1,11]. Acetylated LDL were prepared as previously described [2]. LDL were labeled with ^{125}I after Shepherd *et al.* [9]. P388D1 macrophages and human skin fibroblasts were isolated and grown as described elsewhere [3,12]. For determination of the uptake and degradation of LDL the cells were incubated in the presence of 10 μg/ml ^{125}I-LDL at 37°C over 5

TABLE 2. Uptake and Degradation (ng/mg Protein per 2 h) of Native and Modified ^{125}I-LDL by Human Skin Fibroblasts and P388D1 Mouse Macrophages ($M \pm m$)

Competitor	^{125}I-LDL	^{125}I-dsLDL	^{125}I-acLDL
	Uptake		
Fibroblasts			
Control	66.1±7.6	61.5±3.3	–
nLDL	4.6±0.5*	14.7±2.8*	–
dsLDL	45.8±0.7*	30.9±3.3*	–
P388D1			
Macrophages			
Control	41±1*	623±39	1107±50
nLDL	7±2*	485±13*	1080±60
dsLDL	30±2*	346±10*	516±28*
acLDL7	49±5	260±25*	295±12*
	Degradation		
Fibroblasts			
Control	136.4±9.8	121.4±8.0	–
nLDL	20.0±2.0*	47.6±12.4*	–
dsLDL	86.5±6.7*	60.0±14.1*	–
P388D1			
Macrophages			
Control	189±15	707±83	4477±350
nLDL	64±2*	468±17*	3829±155
dsLDL	138±5*	293±17*	2198±115*
acLDL	158±11	113±27*	1940±194*

hours [2,4], after which the cells were washed with isotonic phosphate buffer saline and dissolved in 0.1 M NaOH, and the uptake (binding and internalization) was determined by measuring the radioactivity of the extract [2,4]. The level of degradation was judged from ^{125}I-tyrosine radioactivity in the medium [2,4]. In the binding experiments the lipoproteins were incubated with the cells at 4°C for 2 hours [2,4]. Radioactivity was measured after the cells were dissolved in 0.1 M NaOH.

RESULTS

When the labeled native and desialylated LDL were incubated with fibroblasts possessing the B,E receptors, the binding of native LDL was twice as high as that of desialylated LDL (Table 1). A 20-fold excess of unlabeled native LDL reduced the binding of desialylated LDL by 91%, while the binding of native LDL was 74% suppressed in the presence of an excess of unlabeled desialylated LDL.

In a study of the uptake and degradation of the native and desialylated LDL in fibroblasts, these parameters were found to be similar for both LDL types (Table 2). An excess of unlabeled native LDL inhibited the uptake and degradation of desialylated by 4.4 and 2.7 times, respectively, whereas an excess of unlabeled desialylated LDL suppressed the binding and degradation of native LDL by 1.5 and 1.6 times, respectively.

The binding of desialylated LDL by P388D1 macrophages was 6 times more effective than that of native LDL (Table 1). At the same time, the uptake and degradation of desialylated LDL was elevated 15- and 3.7-fold in comparison to those of native LDL (Table 2). Binding of ^{125}I-desialylated LDL was 50% suppressed by a 20-fold excess of acetylated LDL (Table 1), and the uptake and degradation were suppressed in this case by 58% and 84%, respectively (Table 2). A less pronounced inhibition (by 22-34%) of the binding, uptake, and degradation of desialylated LDL was observed in the presence of a 20-fold excess of native LDL. The excess of desialylated LDL reduced the binding 1.5-fold and inhibited the uptake and degradation of ^{125}I acetylated LDL 2-fold. The addition of a 20-fold excess of desialylated LDL inhibited the binding 5-fold and reduced the uptake and degradation of native LDL by P388D1 macrophages 1.4-fold.

The obtained results suggest that desialylated LDL, like native LDL, are able to bind the B,E receptors. However, the efficacy of such binding is considerably lower than that of native LDL. In light of this it may be surmised that the modification affects the sites of apolipoprotein B responsible for interaction with the B,E receptors on the cell surface. Macrophages bind, take up, and degrade the desialylated LDL much more intensively then native LDL. This indicates that desialylated LDL are metabolized not only through the LDL-specific receptors, but also through scavenger receptors. This assumption is confirmed by the fact that desialylated LDL compete with acetylated LDL for binding with scavenger receptors in macrophages. Thus, the desialylated LDL which occur in the blood of patients with coronary atherosclerosis represent a natural ligand for scavenger receptors.

REFERENCES

1. J. V. Baenzinger and D. Fiete, *J. Biol. Chem.*, **19**, 9795 (1979).
2. S. K. Basu, J. L. Goldstein, R. G. V. Anderson, and M. S. Brown, *Proc. Nat. Acad. Sci. USA*, **73**, 3178 (1976).
3. E. I. Chazov, V. V. Tertov, A. N. Orekhov, *et al.*, *Lancet*, **2**, 595 (1986).
4. J. K. Goldstein, Y. K. Ho, and S. K. Basu, *Proc. Nat. Acad. Sci. USA*, **76**, 333 (1979).
5. F. T. Lindgren, *American Oil Chemical Society*, New York (1975), p. 205.
6. A. N. Orekhov, V. V. Tertov, S. N. Pokrovsky, *et al.*, *Circulat. Res.*, **62**, 421 (1988).
7. A. N. Orekhov, V. V. Tertov, D. N. Mukhin, and I. A. Mikhailenko, *Biochem. Biophys. Res. Commun.*, **162**, 206 (1989).
8. A. N. Orekhov, V. V. Tertov, and D. N. Mukhin, *Atherosclerosis*, **86**, 153 (1991).
9. S. H. Shepherd, O. K. Bedford, and N. K. Morgan, *Clin. Chim. Acta*, **66**, 97 (1976).
10. V. V. Tertov, I. A. Sobenin, A. G. Tonevisky, *et al.*, *Biochem. Biophys. Res. Commun.*, **167**, 1122 (1990).
11. V. V. Tertov, I. A. Sobenin, Z. A. Gabbasov, *et al.*, *Lab. Invest.*, **67**, 665 (1992).
12. D. P. Via, A. L. Plant, I. F. Craig, *et al.*, *Biochim. Biophys. Acta*, **833**, 417 (1985).