Vol. 102, No. 2, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 30, 1981 Pages 636-639

UPTAKE OF Lp(a) LIPOPROTEIN BY CULTURED FIBROBLASTS

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Received August 11,1981

Summary

Lp(a) lipoprotein is bound and taken up by cultured human fibroblasts and increases cell cholesterol content and cholesterol esterification. The increase in cell cholesterol content and cholesterol esterification in low density lipoprotein receptor negative cells was minimal, which suggests that Lp(a) lipoprotein was taken up by the low density lipoprotein receptor pathway.

Introduction

The Lp(a) lipoprotein [Lp(a)] closely resembles low density lipoprotein (LDL) of d 1.019-1.063 in lipid composition (1,2). Furthermore, the major structural apoprotein of Lp(a) is apolipoprotein B, the principal protein of LDL (3,4). However, Lp(a) is immunochemically and chemically distinct from LDL, since it contains a specific Lp(a) antigen and its content of hexose, hexosamine and sialic acid are significantly higher than that of LDL (2,3). Lp(a) is present in nearly all individuals and its levels are largely genetically determined (5,6). Lp(a) and LDL appear to be under separate metabolic and genetic control (7). Increased levels of Lp(a) lipoprotein are associated with premature coronary heart disease (5,8). In the present study, we report that Lp(a), like LDL, are taken up and degraded by cultured human skin fibroblasts.

Methods

Human skin fibroblasts were grown in Dulbecco-Vogt medium containing 10% pooled human serum as described (9). Fibroblasts were seeded in 35 mm dishes (5×10^4 cells) and the cells were used for experiments when grown to subconfluency. Cells were then preincubated 24 or 48 h in lipoprotein-deficient serum from pooled human serum (d 1.25 g/ml) to increase LDL-receptor activity. Two strains of normal fibroblasts and one strain of LDL-receptor negative cells were used (GM 488 from the Genetic Mutant Cell Repository, Camden, NJ).

Abbreviations: Lp(a), lipoprotein (a); LDL, low density lipoprotein.

0006-291X/81/180636-04$01.00/0

Table 1. Increase in Cell Cholesterol Content and Cholesterol Esterification with Lp(a) in LDL Receptor-Positive Cells

	LDL-receptor-positive cells		LDL-receptor-negative cells	
	Cell cholesterol ug TC/mg protein	Cholesterol-Esterification[a]	Cell Cholesterol ug TC/mg protein	Cholesterol-Esterification[a]
0 h control	32.0±2.4 (4)[b]		30.1±0.7 (5)	-
24 h control	37.8±0.9 (4)	1.43±0.01 (4)	32.9±0.8 (6)	0.32±0.02 (6)
LDL 25 ug TC/ml	54.0±1.2 (3)	6.21±0.12 (3)	36.5±0.7 (5)	0.38±0.01 (5)
Lp(a) 25 ug TC/ml	43.3±1.0 (3)	3.01±0.26 (3)	36.8 (2) (35.0, 38.6)	0.41 (2) (0.40, 0.42)

TC: total cholesterol

[a] nmol [^{14}C]oleic acid incorporated into cholesteryl ester/mg cell protein

[b] mean±S.E.M., number of dishes within parentheses

Fibroblasts were preincubated in 5% lipoprotein-deficient human serum for 48 h with one change after 24 h. Lipoproteins were then added to growth medium containing 10% lipoprotein-deficient fetal calf serum and incubated for 24 h.

LDL (d 1.019 to 1.063 g/ml) and Lp(a) (d1.063 to d1.090 g/ml) were isolated by sequential ultracentrifugation as described (7,9). LDL and Lp(a) were iodinated with ^{125}I by the McFarlane monochloride method as modified for lipoproteins (10). Analysis of lipoprotein binding, internalization, and degradation was performed as previously described (11). Lipoprotein cholesterol was determined (12) and cell cholesterol was measured in chloroform extracts of cells scraped with methanol:water, after washing the cells 5 times with phosphate-buffered saline (2 ml), using an enzyme method (13). Esterification of cholesterol was measured by addition of [1-^{14}C]oleic acid (Amersham/Searle) complexed to fatty acid-free albumin (Sigma Chemical Co.) in Krebs-Ringer phosphate buffer (pH 7.4) for 2 h at 37°C as described earlier (7). Two uM compactin was added during the 2 h pulse with the [^{14}C]oleic acid-albumin complex to suppress incorporation of labeled acetate from [^{14}C]oleic acid into the sterol ring. Protein was measured by the Lowry method (14).

Results and Discussion

Lp(a) stimulated esterification approximately half as much as did LDL and Lp(a) increased cholesterol content less than did LDL in LDL receptor-positive cells (Table 1). These data are consistent with findings with iodinated lipoproteins which showed that, compared with ^{125}I-labeled LDL, 1/3 as much Lp(a) lipoprotein was taken up and degraded by LDL receptor-positive skin fibroblasts when the two lipoproteins were added at equal protein concentration (7.5 ug/ml) and incubated with cells for 24 h (Table 2). Lp(a) cholesterol appeared to be

Table 2

Binding, Internalization, and Degradation of Lp(a) in LDL Receptor-positive Cells[a]

	% Medium Lipoprotein Protein/10^6 Cells	
	^{125}I-LDL	^{125}I-Lp(a)
4 h binding	0.061	0.241
24 h net uptake	0.190	0.067
24 h degradation	1.325	0.672

[a]Fibroblasts were preincubated in 10% lipoprotein-deficient human serum for 24 h. Labeled lipoproteins were then added to the growth medium containing 10% lipoprotein-deficient fetal calf serum and incubated for 4 or 24 h as indicated. Results expressed as mean of duplicate dishes.

delivered to cells via the LDL-receptor pathway, since Lp(a), like LDL, had little effect on cholesterol esterification or cholesterol content in LDL-receptor-negative cells (Table 1).

This study thus suggests that Lp(a) mainly enters fibroblasts via the LDL-receptor pathway, but is taken up to a lesser extent than LDL. The decreased uptake of Lp(a) may depend on the high content of sialic acid in Lp(a) since it has been suggested that sialic acid inhibits uptake of LDL by fibroblasts (15). However, another study failed to support a role for sialic acid in LDL uptake (16). Alternatively, the larger size and altered surface distribution of the apolipoprotein B determinants of the Lp(a) lipoprotein may play a role in its decreased uptake.

Acknowledgments

We thank Dr. Akira Endo for the gift of compactin. Vilma Fernandez, Carole Brewer, Weiling King and Maria Culala provided excellent technical assistance. We thank Sharon Kemp for preparing the manuscript for publication.

These studies were supported by NIH grants HL 18645, AM 02456, and contract HV 12157A, Lipid Metabolism Branch, NHLBI, and a grant from Reynolds

Industries, and performed during the tenure of an Established Investigatorship (JJA) from the American Heart Association with funds contributed by the Washington State Affiliate.

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