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In vivo studies on the binding sites for lipoprotein (a) on parenchymal and non-parenchymal rat liver cells

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The direct correlation between lipoprotein (a) (Lp(a)) concentrations and atherosclerosis stimulated us to investigate the in vivo interaction of Lp(a) with the liver and the various liver cell types. In untreated rats the serum decay of Lp(a) is comparable to that of LDL. By estrogen treatment the interaction of LDL with parenchymal liver cells is increased 17-fold whereas only a 2-fold effect on Lp(a) is found. The decay of Lp(a) in estrogen-treated rats is slower than for LDL. The data indicate that Lp(a) in vivo shows a less efficient interaction than LDL with the estrogen-induced apo-B,E receptor on parenchymal liver cells. It is suggested that the inability of Lp(a) to interact efficiently with the LDL removal system of the liver might be related to its atherogenic action.

17α-Ethinylestradiol; Lipoprotein (a); (Parenchymal liver cell, Non-parenchymal liver cell)

1. INTRODUCTION

Lipoprotein (a) can be detected in the blood of most humans. It has gained renewed interest because a number of studies have indicated a positive correlation between the serum level of Lp(a) and coronary vascular diseases [1–4]. Recently an induction of Lp(a) by fat feeding was reported [5].

After density gradient ultracentrifugation of human sera, Lp(a) is found in the density range of 1.055–1.110 g/ml which borders low density lipoprotein (LDL) and coincides partly with that of high density lipoprotein (HDL). Lp(a) resembles LDL in lipid composition [6] and also contains apo-B as the major apoprotein [7]. However, Lp(a) can be distinguished from LDL by the presence of a unique Lp(a) apoprotein and by its high content of hexose, hexosamine and sialic acid [6,7].

A number of in vitro studies have shown that Lp(a) can bind to the apo-B,E receptor on fibroblasts [8–10], although it was also reported that Lp(a) does not interact with the apo-B,E receptor [11]. Recently we described the intrahepatic cellular localization of lipoprotein receptors in rats. The presence of an apo-B,E receptor on parenchymal and Kupffer cells [12,13] was demonstrated and the acetyl-LDL (scavenger) receptor appears to be very active on liver endothelial cells [14]. Our cell isolation method, which yields pure fractions of parenchymal, Kupffer or endothelial cells, now enables us to test which receptors are involved in the catabolism of Lp(a) in vivo. In order to determine the interaction of Lp(a) with the apo-B,E receptor, one can make use of the fact that the number of receptors can be selectively increased on parenchymal liver cells [12] by estrogen treatment.

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

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2. MATERIALS AND METHODS

Human LDL ($1.024 < d < 1.055$ g/ml) was isolated by 2 repetitive centrifugations according to Redgrave et al. [15] as described [12]. The human LDL preparation used in this study contains almost exclusively apolipoprotein-B (99.97%); no degradation products were detectable as checked by polyacrylamide gel electrophoresis using 2.5–25% slab gels with 0.1% SDS according to [16]. With a high LDL concentration (5 mg apolipoprotein/ml) in a radial immunodiffusion system according to [17], apolipoprotein-E was noticeable at the detection limit and contributed maximally 0.02–0.03% of the total apolipoprotein.

Radioiodination of LDL was done according to the ICl method described in [18], using carrier-free ^{125}I or ^{131}I.

Lp(a) ($1.050 < d < 1.110$ g/ml) was isolated by ultracentrifugation from the pooled plasma of 5–7 highly positive donors. Purification of Lp(a) was done according to Gaubatz et al. [19] with the following preservatives present during all steps of purification: 1 mg EDTA/ml; 0.1 mg chloramphenicol/ml; 100 KIE Trasylol/ml; 10 μM phenylmethanesulfonyl fluoride and 5 mM iodoacetate. Due to these preservatives, the tendency of isolated Lp(a) to precipitate upon cooling was kept to a minimum [20]. A typical gel pattern of apo-Lp(a) is shown in fig.1 and is in agreement with data published by Gaubatz et al. [19].

Radioiodination of the Lp(a) preparations was done according to the ICl method [18]. After iodination the Lp(a) preparation was dialyzed 3 times against 0.024 M NaBr, 0.01 M Tris-HCl, pH 8.0, and 2 times against 0.15 M NaCl, 0.3 mM EDTA, pH 7.0.

Reductive methylation of the lipoproteins was done according to [21]. 0.5 ml lipoprotein (approx. 2.5 mg apolipoprotein/ml) was mixed with 0.38 ml of 0.3 M borate buffer, pH 9.0. At $t = 0$, 0.5 mg $NaBH_4$ and 0.5 μl formaldehyde were added, thereafter 0.5 μl formaldehyde was added every 6 min (5 times). The extent of methylation of lysine residues was >80% as determined by the trinitrobenzenesulfonic acid method [27].

2.1. *Animals*

12-week-old male Wistar rats were used throughout the study. 17α-Ethinylestradiol in propyleneglycol at a dose of 5 mg/kg body wt [13] was injected subcutaneously every 24 h for 3 days. Control rats received equal volumes of the solvent.

2.2. *Materials*

17α-Ethinylestradiol was obtained from Brocacef (Maarssen, The Netherlands); collagenase (type I) from Sigma (St Louis, USA); pronase B-grade from CalBiochem-Behring (La Jolla, USA); metrizamide was purchased from Nyegaard (Oslo, Norway); trasylol from Bayer (Leverkusen, FRG); Ham F-10 medium from Gibco (Paisley, Scotland) and ^{125}I and ^{131}I (carrier-free) from Amersham (Amersham, England).

2.3. *In vivo uptake studies*

Rats were anesthetized by intraperitoneal injection of 2 mg nembutal. The abdomen was opened and the radiolabeled lipoproteins were injected in the inferior vena cava at the level of the renal veins. After the indicated circulation time the liver was perfused with an oxygenated Hanks buffer at 8°C. After 8 min perfusion, a lobule was tied off for determination of the total liver uptake. To determine the uptake by the various cell types, the cell separation was carried out by low temperature procedures [14,23].

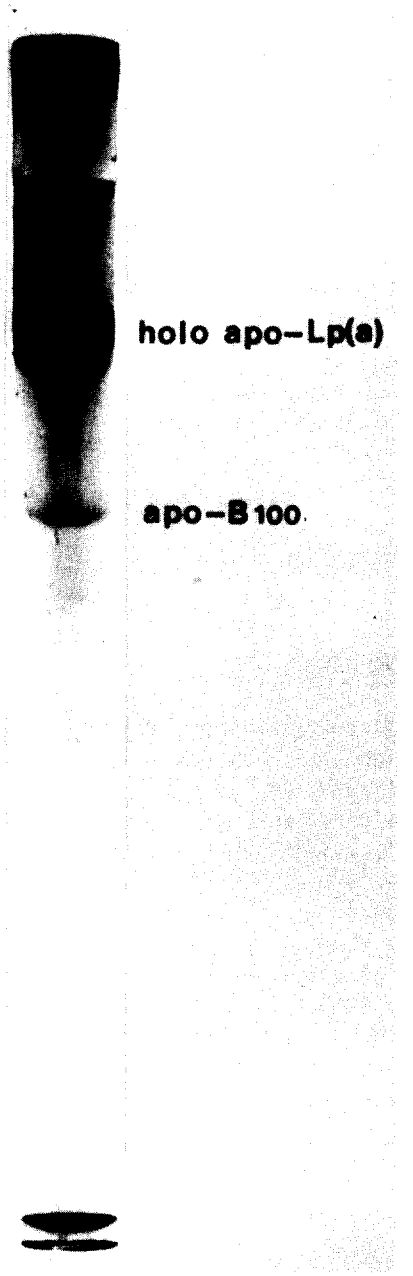

Fig.1. Delipidated Lp(a) (200 μg/gel) run on a non-reducing 3.75% polyacrylamide gel containing 0.1% SDS (according to [19]).

3. RESULTS

The radiolabeled lipoproteins were injected into rats and after 30 min parenchymal and non-parenchymal cells were isolated and purified. During the 30 min circulation the serum decay of the lipoproteins was determined (fig.2). The serum decay of Lp(a) is comparable to that of LDL. In order to determine the involvement of the parenchymal apo-B,E receptor in the clearance of Lp(a), rats were treated with 17α-ethinylestradiol. This treatment accelerated the clearance rate for native LDL (review [12,13]), but influenced the decay of Lp(a) to a much lower extent.

Fig.3A shows the in vivo uptake of Lp(a) and of LDL by the liver in control and estrogen-treated rats. It is found that the liver uptake of Lp(a) is enhanced by the estrogen treatment to a lesser extent than that of LDL. In control rats the association of Lp(a) with the liver is higher than for LDL. However, the higher liver-association of Lp(a) is probably not LDL-receptor-mediated, since methylation of the lipoproteins inhibits the liver association of Lp(a) to a lower extent than that of LDL (hereby the interaction with LDL-receptors can be blocked [12,13]). Fig.3B,C displays the amount of lipoprotein associated in vivo with the parenchymal and non-parenchymal cells. It is clear that the increased liver association of Lp(a) as compared to LDL is caused by an increased association with parenchymal cells. Estradiol treatment increases specifically LDL-receptors in parenchymal cells and therefore a 17-fold increase in the amount of LDL associated with parenchymal cells, that can be blocked by methylation of the particle, is noticed. On the other hand, the stimulation of the Lp(a) uptake by estrogen treatment was only 2-fold while methylation of Lp(a) also inhibited the increased uptake in the estradiol-treated rats. In non-parenchymal cells the cell association of Lp(a) and LDL is comparable both in control and in estrogen-treated rats. From these data it can be calculated (table 1) that in control rats, parenchymal cells play a quantitatively more important role in the uptake of Lp(a) than in the uptake of LDL (51 versus 32%, respectively). After estrogen treatment the relative contribution of parenchymal cells to the uptake of LDL exceeds that of Lp(a) (90 versus 67%, respectively).

A further subdivision of non-parenchymal cells

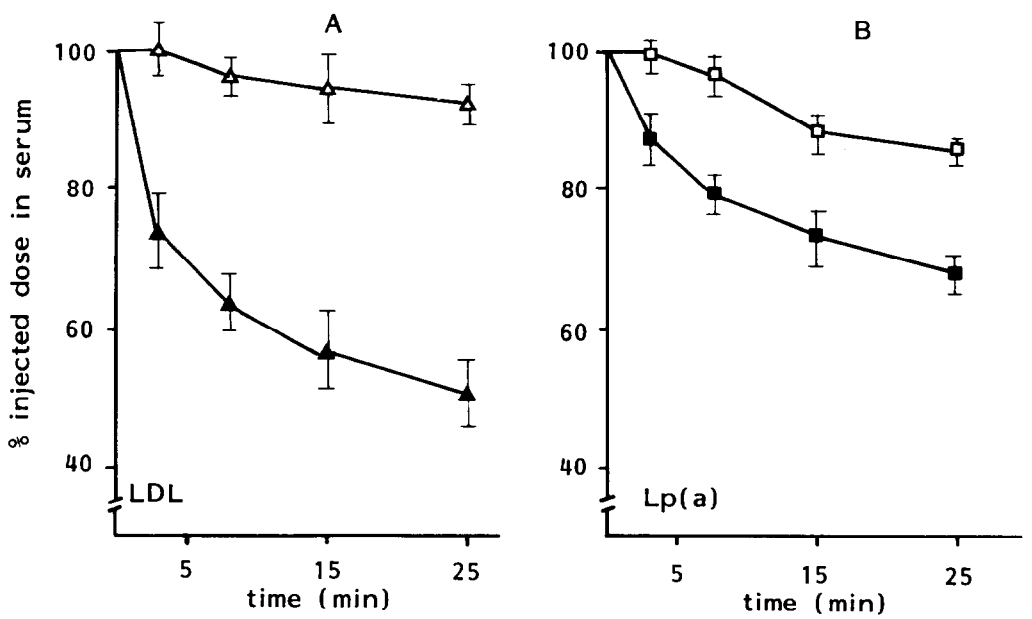

Fig.2. Serum decay of LDL (A) or Lp(a) (B) in estrogen-treated (closed symbols) and control (open symbols) rats. 3, 8, 15 and 25 min after injection of the ^{125}I-labeled lipoproteins, blood samples were drawn and the radioactivity in the serum determined. Each symbol represents the mean of 3–4 experiments (± SE).

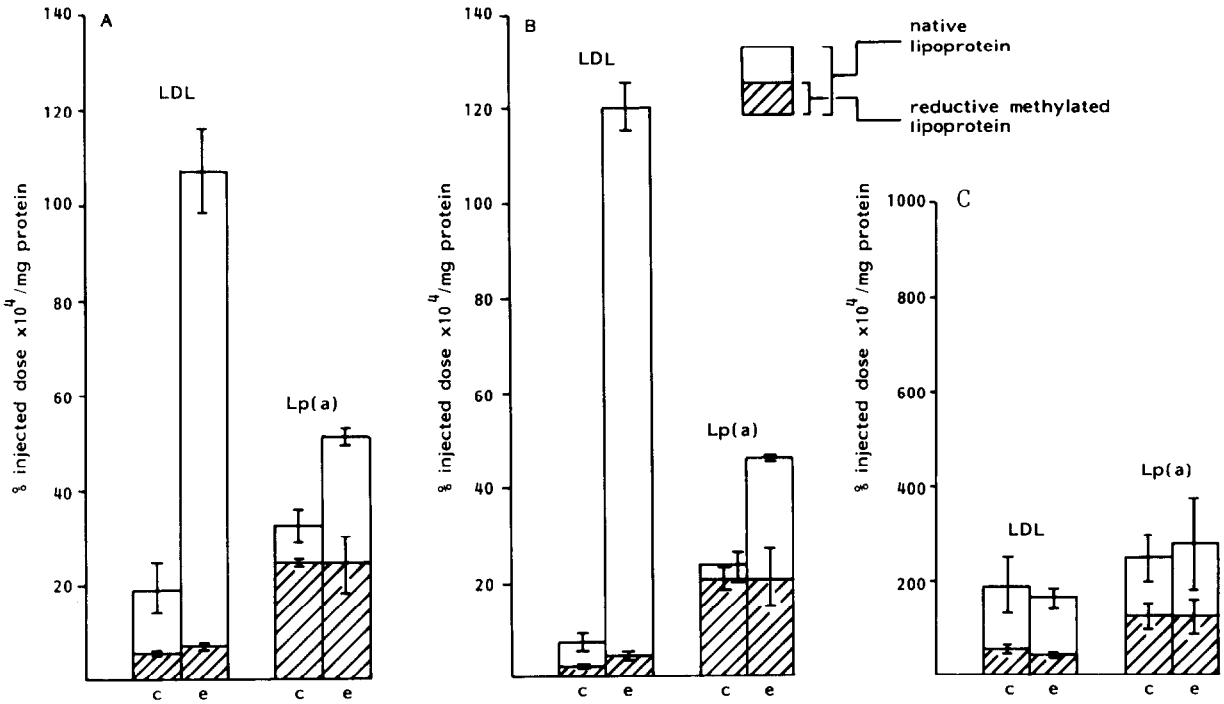

Fig.3. In vivo lipoprotein association with liver (A), parenchymal (B) and non-parenchymal cells (C) in control (c) and ethinylestradiol-treated rats (e). The radioiodinated lipoproteins were injected intravenously into rats and after 30 min of circulation, the liver cells were isolated. The open plus hatched bars represent association of the native lipoproteins; the hatched bars, association of reductively methylated lipoproteins. The values are calculated as the mean of 3–6 experiments (± SE).

in endothelial and Kupffer cell fractions shows that Lp(a) has a slightly increased in vivo cell association with the endothelial and Kupffer cells as compared to LDL (table 2). However, when compared to the association of acetylated LDL (a specific substrate for the scavenger acetyl-LDL receptor), there is no specific increase in the uptake by endothelial cells so that probably the acetyl-LDL receptor does not actively interact with Lp(a).

Table 1

Percentual contribution of parenchymal and non-parenchymal cells to the total liver uptake of Lp(a) and LDL, 30 min after injection in estrogen-treated and control rats

	Parenchymal cells		Non-parenchymal cells	
	Control	Estrogen	Control	Estrogen
Lp(a)	51 ± 4	67 ± 2	49 ± 4	33 ± 4
LDL	32 ± 3	90 ± 2	68 ± 6	10 ± 1

The amount of radioactivity/mg cell protein in the isolated cell fractions was multiplied with the amount of protein that each cell type contributes to total liver protein. The values are calculated as the mean of 4–6 experiments (± SE)

Table 2

In vivo uptake of LDL, Lp(a) and acetyl-LDL in liver endothelial and Kupffer cells at 30 min after injection

	% injected dose × 10^4/mg cell protein	
	Endothelial cells	Kupffer cells
LDL	32 ± 2 (3)[a]	232 ± 15 (3)
Lp(a)	82 ± 18 (5)	571 ± 163 (5)
Acetyl-LDL[b]	4700 ± 500 (3)	630 ± 100 (3)

[a] Mean ± SE; *n* in parentheses
[b] As shown earlier in our laboratory [14]

4. DISCUSSION

The present study provides data on the in vivo interaction of Lp(a) with the liver and the various liver cell types. The results allow some conclusions

which may be relevant to an understanding of the atherogenic nature of this lipoprotein. (i) Lp(a) can interact with the apo-B,E receptor in vivo, although apparently less efficiently than LDL. This conclusion is mainly based on the modest increase in cell association to parenchymal cells upon estrogen treatment. These data are in accord with recent studies on the interaction of Lp(a) with the fibroblast apo-B,E receptor, which indicates that apo(a) can suppress the LDL character of Lp(a) [24]. (ii) Besides the inefficient interaction with the apo-B,E receptor on parenchymal cells, Lp(a) shows a high (unspecific) interaction with parenchymal cells. This conclusion is based on the high association of Lp(a), as compared to LDL, with parenchymal cells in control rats, which is not affected by methylation. (iii) Lp(a) is not actively taken up by the acetyl-LDL receptor on liver endothelial cells. This indicates that in vivo Lp(a) does not interact with the scavenger receptor. This conclusion is similar as drawn earlier from studies with macrophages in vitro [25].

The diminished interaction of Lp(a) as compared to LDL with the apo-B,E receptor of the liver, as noticed in estrogen-treated rats, may lead to a less efficient removal of Lp(a) from the circulation in animals including humans with high receptor activity on hepatocytes. Furthermore the high unspecific interaction of Lp(a) with cells might lead to an extracellular disposition which could explain the atherogenic character of this lipoprotein.

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REFERENCES

[1] Albers, J.J., Adolphson, J.L. and Hazzard, W.R. (1977) J. Clin. Invest. 18, 331–338.
[2] Berg, K. (1963) Acta Pathol. Microbiol. Scand. 59, 369–382.
[3] Berg, K., Dahlen, G. and Frick, M.H. (1974) Clin. Genet. 6, 230–235.
[4] Dahlen, G., Berg, K., Ramberg, U. and Tamm, A. (1974) Acta Med. Scand. 196, 327–331.
[5] Bersot, T.P., Innerarity, T., Pitas, R.E., Rall, S.C., Weisgraber, K.H. and Mahley, R.W. (1986) J. Clin. Invest. 77, 622–630.
[6] Jürgens, G. and Kostner, G.M. (1975) Immunogenetics 1, 560–569.
[7] Ehnholm, C., Garoff, H., Renkonen, O. and Simons, K. (1972) Biochemistry 11, 3229–3232.
[8] Havekes, L., Vermeer, B.J., Brugman, T. and Emeis, J. (1981) FEBS Lett. 132, 169–173.
[9] Floren, G.H., Albers, J.J. and Bierman, E.L. (1981) Biochem. Biophys. Res. Commun. 102, 636–639.
[10] Krempler, F., Kostner, G.M., Roschner, A., Haslauer, F., Bolzano, K. and Sandhofer, F. (1983) J. Clin. Invest. 71, 1431–1441.
[11] Maartman-Moe, K. and Berg, K. (1981) Clin. Genet. 20, 352–362.
[12] Harkes, L. and Van Berkel, T.J.C. (1983) FEBS Lett. 154, 75–80.
[13] Harkes, L. and Van Berkel, T.J.C. (1984) Biochim. Biophys. Acta 794, 340–347.
[14] Nagelkerke, J.F., Barto, K.P. and Van Berkel, T.J.C. (1983) J. Biol. Chem. 258, 12221–12227.
[15] Redgrave, T.G., Roberts, D.C.K. and West, C.E. (1975) Anal. Biochem. 65, 42–49.
[16] Laemmli, U.K. (1970) Nature 227, 680–686.
[17] Mancini, G., Carbonara, A.P. and Heremans, J.F. (1965) Immunochemistry 2, 235–254.
[18] McFarlane, A.S. (1958) Nature 182, 53.
[19] Gaubatz, J.W., Heideman, C., Gotto, A.M. jr, Morriseet, J.D. and Dahlen, G.H. (1983) J. Biol. Chem. 285, 4582–4589.
[20] Jürgens, G. (1983) Clin. Chem. 29, 1856.
[21] Weisgraber, K.H., Innerarity, T.L. and Mahley, R.W. (1978) J. Biol. Chem. 253, 9053–9062.
[22] Habeeb, A.F.S.A. (1966) Anal. Biochem. 14, 328–336.
[23] Van Berkel, T.J.C., Dekker, C.J., Kruijt, J.K. and Van Eijk, H.G. (1987) Biochem. J. 243, 715–722.
[24] Armstrong, V.W., Walli, A.K. and Seidel, D. (1985) J. Lipid Res. 26, 1314–1323.
[25] Krempler, F., Kostner, G.M., Roscher, A., Bolzano, K. and Sandhofer, E. (1984) J. Lipid Res. 25, 283–287.