Cellular localization of the receptor-dependent and receptor-independent uptake of human LDL in the liver of normal and 17α-ethinyl estradiol-treated rats

Leen Harkes and Theo J.C. van Berkel

Department of Biochemistry I, Medical Faculty, Erasmus University, PO Box 1738, 3000 DR Rotterdam, The Netherlands

Received 28 December 1982

The cellular localization in the liver of the receptor-dependent and -independent uptake of human low density lipoprotein (LDL) in normal and 17α-ethinyl estradiol-treated rats was investigated by the simultaneous in vivo injection of human ^{131}I-LDL and human reductive methylated ^{125}I-LDL. The cells were subsequently isolated by a low temperature method. In untreated rats, after 30 min of in vivo circulation of human LDL, 57% of the receptor-dependent liver-association of human LDL occurs in non-parenchymal cells and 43% in parenchymal cells. Estradiol treatment of rats for 3 days selectively increases the receptor-dependent cell-association of human LDL with hepatocytes (17-fold), while the receptor-dependent cell-association with non-parenchymal cells is not affected.

Ethinyl estradiol *Low density lipoprotein* *Non-parenchymal liver cell* *Parenchymal liver cell* *Reductive methylated LDL*

1. INTRODUCTION

Administration of 17α-ethinyl estradiol in pharmacological amounts to rats produces a profound hypolipidemia [1]. The hepatic uptake and catabolism of both rat and human low density lipoprotein is stimulated many-fold in these rats [2]. This enhanced uptake is associated with an increased number of high affinity binding sites on membranes derived from the whole liver [3]. The binding site involved is described to be a functional lipoprotein receptor that recognizes lipoproteins, containing apoprotein B or E [4]. From autoradiographic studies it was concluded that estrogen-treatment enhances the normal mechanism by which LDL is taken up by the liver and that both in control and estrogen-treated rats the parenchymal cell is the predominant cell type for LDL interaction [5].

Abbreviations: LDL, low density lipoprotein; Me-LDL, reductive methylated LDL

Here, we have applied a low-temperature cell isolation method to determine the tissue site of the estradiol-stimulated lipoprotein receptor in the rat liver. A discrimination between receptor-dependent and -independent association was made by the simultaneous injection of native human ^{131}I-LDL and human reductive methylated ^{125}I-LDL (Me-^{125}I-LDL). As reductive methylation of at least 30% of the lysyl residues blocks receptor–lipoprotein interaction [6], the difference between the amount of cell-association of native and Me-LDL can be defined as receptor-mediated cell-association or uptake.

2. MATERIALS AND METHODS

17α-Ethinyl estradiol was obtained from Brocacef BV (Maarssen); collagenase (type I) from Sigma (St Louis MO); pronase B-grade from CalBiochem Behring Corp. (La Jolla CA); sodium [^{125}I]- and [^{131}I]iodide (carrier-free) was purchased from the Radiochemical Centre (Amersham).

Published by Elsevier Science Publishers
00145793/83/0000–0000/$3.00

12-Week-old male Wistar rats (250 g av. body wt) which had free access to water and food (standard laboratory chow), were used. Rats were injected subcutaneously with 17α-ethinyl estradiol dissolved in propylene glycol at 5 mg/kg body wt [2] every 24 h for 3 days. At this time the maximal decrease in plasma cholesterol concentration [3,7] and increase in LDL receptor-activity [3] is attained. Control rats received equal volumes of the solvent.

2.1. *Preparation of lipoproteins*

Human LDL ($1.024 < d < 1.055$ g/ml) was isolated as in [8], the isolated LDL was subjected to a second identical centrifugation to avoid any contamination with other lipoproteins. Apo E content of this LDL fraction was < 0.03% of the total apoprotein [9]. Radioiodination of LDL was done by a modification [10] of the ICl method in [11]. Reductive methylation of LDL was done as in [6]. About 80% of the lysyl residues from human LDL were methylated as determined by using the 2,4,6-trinitrobenzene sulfonic acid colorimetric assay [12]. Human ^{131}I-LDL and human Me-^{125}I-LDL were always prepared from the same LDL-preparation, specific radioactivity of both preparations varied from 100–500 cpm/ng apoprotein.

2.2. *Fate of LDL in rat serum and liver*

Rats were anesthesized by intraperitoneal injection of 20 mg nembutal. The abdomen was opened and about 40 μg human ^{131}I-LDL and 40 μg human Me-^{125}I-LDL in a measured volume (usually 500 μl) of 0.15 M NaCl, 0.3 mM EDTA (pH 7.0) was injected in the inferior vena cava at the level of the renal veins. After 3, 8, 15 and 25 min 0.2 ml blood was taken from the inferior vena cava at least 2 cm distal of the injection point. ^{125}I and ^{131}I were determined in the serum and the results expressed as percentage of the ^{125}I or ^{131}I in the sample taken 3 min after the injection. In some experiments 500 μl 12% trichloroacetic acid was added to 100 μl serum samples to determine the serum acid-soluble and acid-precipitable radioactivity.

After 30 min circulation of the radiolabeled lipoproteins the vena porta was cannulated and the liver was preperfused with an oxygenated Hanks buffer at 8°C. After 8 min perfusion a lobule was tied off for determination of the total liver uptake. Subsequently, the liver was subjected to a low temperature (8°C) perfusion with 0.25% pronase for the isolation of nonparenchymal cells or a low temperature (8°C) collagenase (0.05%) perfusion for the isolation of parenchymal cells based upon [13] and extensively described in [28]. The parenchymal cells were completely free from non-parenchymal cells as checked microscopically. The non-parenchymal cell preparation was completely free from parenchymal cells as checked microscopically and biochemically by the pyruvate kinase assay [14]. The purity of the cell preparation is furthermore illustrated by the described selective increase of radioactivity in parenchymal cell preparations after estrogen treatment.

2.3. *Other determinations*

Protein determination was done as in [15]. Liver wet weight for the studied rats is 3.75% of the body weight [16] and as the protein concentration in both parenchymal and non-parenchymal cells is identical (in mg/ml cell volume) [17], the relative protein contribution of parenchymal and non-parenchymal cells to total liver will be 92.5% and 7.5%, respectively [16,18]. These calculations based upon morphometric data are further sustained by our earlier studies on enzyme distribution between the different cell types [19].

3. RESULTS

The isolation of parenchymal and non-parenchymal cells was performed at a low temperature to prevent degradation of lipoproteins during the isolation procedure.

A comparison of the low temperature procedure with the method exerted at 37°C [20] indicates that the recovery of radioactivity in the isolated cells, as compared to total liver, is quantitative for the cells isolated at low temperature (table 1). The increase in recovery from 39 to 105% is caused by a 2-fold higher amount of radioactivity recovered in parenchymal cells and a 4-fold higher value in non-parenchymal cells leading to a doubling of the ratio of specific radioactivity of non-parenchymal over parenchymal cells.

In ethinyl estradiol-treated rats the disappearance rate of human LDL from the plasma is markedly increased as compared to untreated rats (fig.1) while the uptake of human LDL in the liver is 5-fold higher (table 2). The removal from serum

Table 1

Distribution of human ^{131}I-LDL between parenchymal and non-parenchymal liver cells, isolated at low (8°C) and high (37°C)[a] temperature 30 min after intravenous injection

	% × 10^4 of the injected dose/ mg cell protein[b]	
	8°C method	37°C method[a]
Whole rat liver	15.0 ± 1.2 (6)	14 ± 1
Parenchymal cells (PC)	5.8 ± 1.3 (3)	3 ± 0
Non-parenchymal cells (NPC)	138.3 ± 6.8 (3)	33 ± 6
Ratio NPC/PC	23.8	11
Recovery (%)[c]	105.3 ± 3.3 (3)	39 ± 4

[a] Values obtained from [20]
[b] Mean ± SE, *n* in parentheses
[c] The mean recovery of the radioactivity in the cells isolated by the 8°C method as compared to whole liver with inclusion of estradiol-treated rats and Me-LDL is 104.5 ± 3.6% (n = 10)

and uptake in liver of human Me-LDL is not influenced by estradiol treatment. For human LDL and human Me-LDL the radioactivity in plasma is for 98.3% and 99.4% trichloroacetic acid-precipitable, respectively. This percentage remains constant during the 30 min of circulation both for control and estradiol-treated rats.

Estradiol-treatment of rats leads to a 13-fold higher amount of human LDL associated with parenchymal cells, while no effect is seen on the association with non-parenchymal cells (table 3). For the human Me-LDL there is only a slight increment in the parenchymal cell-associated radioactivity. The difference in the amount of cell-association of native LDL and reductive methylated LDL can be considered to represent the receptor-mediated uptake [6]. This receptor-dependent uptake is clearly present in both parenchymal and non-parenchymal cells from untreated rats and increased 17-fold in parenchymal cells from estradiol-treated rats, while there is no significant effect of estradiol-treatment on the non-parenchymal cell uptake. The increased uptake of human LDL by parenchymal cells is not simply caused by the reduced mass of native rat LDL in ethinyl estradiol-treated rats, because the

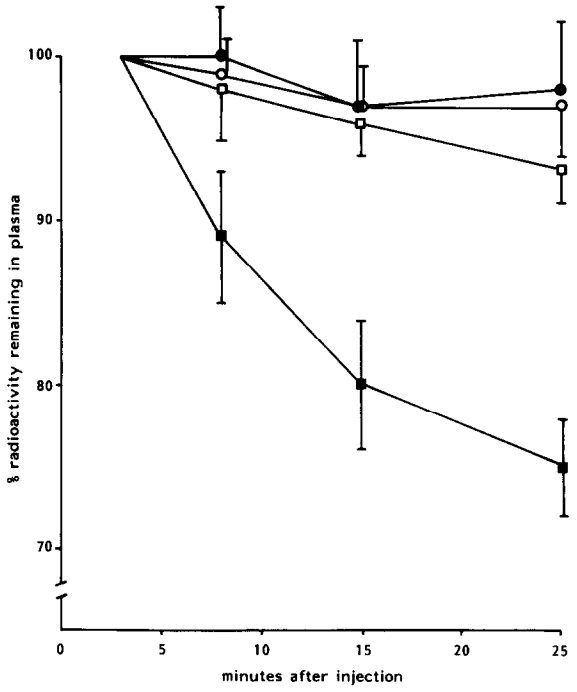

Fig.1. Removal from blood plasma of human ^{131}I-LDL and reductive methylated human ^{125}I-LDL in control and 17α-ethinyl estradiol-treated rats. Human ^{131}I-LDL (□,■) and reductive methylated human ^{125}I-LDL (○,●) were injected intravenously in control (open symbols) and estradiol-treated rats (closed symbols). Radioactivity was determined in 50 μl samples of serum. Each point ± SE represents the mean value from 6 rats.

simultaneous injection of a 10-fold excess of human LDL did not change the relative uptake in control and estradiol-treated rats (table 4).

Taking into account the relative protein contribution of parenchymal and non-parenchymal

Table 2

Effect of estradiol treatment on the liver uptake of human ^{131}I-LDL and human Me-^{125}I-LDL 30 min after intravenous injection

	% of the injected dose[a]	
	Human LDL	Human Me-LDL
Untreated	2.8 ± 0.2 (6)	1.0 ± 0.1 (7)
Estradiol-treated	13.5 ± 1.2 (7)	1.0 ± 0.0 (8)

[a] Mean ± SE, *n* in parentheses

Table 3

Distribution of human ^{131}I-LDL and human Me-^{125}I-LDL between parenchymal and non-parenchymal cells in control and 17α-ethinyl estradiol-treated rats 30 min after intravenous injection

	% × 10^4 of the injected dose/mg cell protein[a]		
	Human LDL	Human Me-LDL	Receptor-dependent[b] cell association
Whole liver			
untreated	15.0 ± 1.2 (6)	5.8 ± 0.5 (6)	9.3 ± 1.0 (6)
estradiol-treated	82.2 ± 5.4 (6)	5.9 ± 0.2 (6)	75.4 ± 5.4 (6)
Parenchymal cells			
untreated	5.8 ± 1.3 (4)	1.5 ± 0.1 (3)	4.3 ± 1.2 (3)
estradiol-treated	78.0 ± 3.7 (4)	2.7 ± 0.1 (4)	75.0 ± 3.7 (4)
Non-parenchymal cells			
untreated	138.3 ± 6.8 (3)	67.4 ± 8.0 (3)	70.9 ± 8.7 (3)
estradiol-treated	126.7 ± 13.3 (3)	40.9 ± 6.8 (3)	85.9 ± 6.8 (3)

[a] Mean ± SE, n in parentheses
[b] Receptor-dependent cell association is the difference between human ^{131}I-LDL and human Me-^{125}I-LDL

cells to total liver it can be calculated that in the estrogen-treated rats, the parenchymal cells form the major tissue site for receptor-dependent liver uptake of human LDL (92%). In contrast, in untreated rats the non-parenchymal cells are quantitatively more important with 57% of the total receptor-dependent cell-association for human LDL.

Table 4

Effect of a 10-fold excess of unlabeled human-LDL on the uptake of human ^{131}I-LDL and human Me-^{125}I-LDL in parenchymal cells of untreated and 17α-ethinyl estradiol-treated rats, 30 min after intravenous injection[a]

	% × 10^4 of the injected dose /mg cell protein[b]	
	Human LDL	Human Me-LDL
Whole liver		
untreated	16.5 ± 0.8	8.9 ± 1.6
estradiol-treated	86.1 ± 15.2	13.5 ± 3.9
Parenchymal cells		
untreated	8.1 ± 0.2	3.9 ± 0.2
estradiol-treated	102.9 ± 1.4	8.0 ± 4.4

[a] Containing: (1st expt) 34 μg human ^{131}I-LDL, 19 μg human Me-^{125}I-LDL and 335 μg unlabeled human LDL; (2nd expt) 51 μg human ^{131}I-LDL, 19 μg human Me-^{125}I-LDL and 670 μg unlabeled human LDL
[b] Mean ± SE, n = 2

4. DISCUSSION

The cellular localization of the liver uptake of human LDL in vivo could be determined quantitatively by taking 2 precautions to prevent loss of lipoprotein degradation products from the cells.

(1) A circulation time of 30 min was chosen. In this time interval no increase of trichloroacetic acid-soluble products in serum occurs, nor is there any difference in the amount of the acid-soluble products between treated and control rats. A similar lag phase for human LDL degradation in rats is found in [2].

(2) A cell isolation procedure for parenchymal and non-parenchymal cells was performed in which no loss of degradation products occurs. This was achieved by maintaining a low temperature during the initial liver perfusion and subsequent isolation of the liver cells. The data indicate that this procedure leads to a quantitative recovery of the total liver-associated radioactivity in the subsequently isolated cells.

The simultaneous circulation of native and Me-LDL enables us to discriminate between receptor-dependent and receptor-independent uptake [6]. By applying a sucrose-label [27] in total liver 67.4% of the total LDL uptake was shown to be receptor-mediated. For whole rat liver we obtain a value of 61.3%. The present results indicate that in normal rats both in parenchymal and non-parenchymal cells a receptor-mediated uptake mechanism for human LDL is present. These data obtained in vivo, confirm data obtained in vitro, which showed the presence of a human LDL receptor in freshly isolated parenchymal cells [9] and non-parenchymal cells [21] from untreated rats. Estradiol treatment of the rats selectively increases the receptor-mediated uptake of human LDL in parenchymal cells, while the uptake in non-parenchymal cells is not affected. The parenchymal liver cells are therefore solely responsible for the increased liver-association of human LDL in estradiol-treated rats and form then the major liver site for receptor-dependent cell-association of human LDL with 92% of the total liver amount. This value agrees with autoradiographic data [5], which indicated that 5–15% of the grains of radiolabeled human LDL were seen over non-parenchymal cells after estrogen-treatment. In untreated rats, however, the non-parenchymal cells are quantitatively an important liver site for receptor-dependent cell-association of human LDL with 57% of the total liver uptake. The receptor-independent uptake of human LDL in rat liver is not influenced by estrogen-treatment and is mainly exerted by the non-parenchymal cells.

The properties of the estradiol-stimulated lipoprotein receptor of rat liver are extensively described in [2–4] and it appears that it reflects the LDL receptor characterized on extrahepatic cells (review [22]). However, in these studies membrane preparations from total liver or liver perfusions are used and it was not possible to decide if the estradiol-induced LDL binding sites and the enhanced LDL uptake occurs in parenchymal or non-parenchymal cells [3]. Our data indicate that selectively parenchymal cells show an increased uptake of human LDL as a result of estrogen-treatment and consequently the metabolism of LDL inside the liver is not only quantitatively but also in relation to cellular sites greatly changed.

With parenchymal liver cells isolated from untreated rats, we investigated the properties of a binding site for human LDL [9]. It was found that human LDL is bound with high affinity and the binding site recognizes both apo B as well as apo E containing lipoproteins and resembles the inducible apo B,E receptor [23]. These properties differ from the receptor which mediates the uptake of rat very low density lipoprotein (VLDL)-remnants [24] or rat chylomicron-remnants [25,26]. This remnant receptor, recognizing apo E, does not interact with human LDL. The ability to induce selectively the apo B,E receptor in parenchymal liver cells and not in non-parenchymal cells may form an important tool to determine the relative importance of the different cell types and receptors for liver lipoprotein metabolism.

ACKNOWLEDGEMENTS

We thank Kar Kruijt and Koen Barto for their excellent technical assistance and Cecile Hanson for typing the manuscript. This research was supported by a grant from the Netherlands Heart Foundation, grant No. 79.001/HA.

REFERENCES

[1] Davis, R.A. and Roheim, P.S. (1978) Atherosclerosis 30, 293–299.
[2] Chao, Y.-S., Windler, E.E.T., Chen, G.C. and Havel, R.J. (1979) J. Biol. Chem. 254, 11360–11366.
[3] Kovanen, P.T., Brown, M.S. and Goldstein, J.L. (1979) J. Biol. Chem. 254, 11367–11373.
[4] Windler, E.E.T., Kovanen, P.T., Chao, Y.-S., Brown, M.S., Havel, R.J. and Goldstein, J.L. (1980) J. Biol. Chem. 255, 10464–10471.
[5] Chao, Y.-S., Jones, A.L., Hradek, G.T., Windler, E.E.T. and Havel, R.J. (1981) Proc. Natl. Acad. Sci. USA 78, 597–601.
[6] Weisgraber, K.H., Innerarity, T.L. and Mahley, R.W. (1978) J. Biol. Chem. 253, 9053–9062.
[7] Arbeeny, C.M. and Eder, H.A. (1980) J. Biol. Chem. 255, 10547–10550.
[8] Redgrave, T.G., Roberts, D.C.K. and West, C.E. (1975) Anal. Biochem. 65, 42–49.
[9] Harkes, L. and Van Berkel, Th.J.C. (1982) Biochim. Biophys. Acta 712, 677–683.
[10] Van Tol, A., Van 't Hooft, F.M. and Van Gent, T. (1978) Atherosclerosis 29, 449–457.
[11] McFarlane, A.S. (1958) Nature 182, 53.

[12] Habeeb, A.F.S.A. (1966) Anal. Biochem. 14, 328–336.
[13] Praaning-Van Dalen, D.P. and Knook, D.L. (1982) FEBS Lett. 141, 229–232.
[14] Van Berkel, Th.J.C. and Kruijt, J.K. (1977) Eur. J. Biochem. 73, 223–229.
[15] Lowry, O.H., Rosebrough, R.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[16] Blouin, A., Bolender, R.P. and Weibel, E.R. (1977) J. Cell Biol. 72, 441–455.
[17] Munthe-Kaas, A.C., Berg, T. and Sjeljelid, R. (1976) Exp. Cell Res. 99, 146–154.
[18] Groot, P.H.E., Van Berkel, Th.J.C. and Van Tol, A. (1981) Metabolism 30, 792–797.
[19] Van Berkel, Th.J.C., Kruijt, J.K. and Koster, J.F. (1975) Eur. J. Biochem. 58, 145–152.
[20] Van Tol, A. and Van Berkel, Th.J.C. (1980) Biochim. Biophys. Acta 619, 156–166.
[21] Van Berkel, Th.J.C., Nagelkerke, J.F., Harkes, L. and Kruijt, J.K. (1982) Biochem. J. 208, 493–503.
[22] Brown, M.S., Kovanen, P.T. and Goldstein, J.L. (1981) Science 12, 628–635.
[23] Hui, D.Y., Innerarity, T.L. and Mahley, R.W. (1981) J. Biol. Chem. 256, 5646–5655.
[24] Van Berkel, Th.J.C., Kruijt, J.K., Van Gent, T. and Van Tol, A. (1981) Biochim. Biophys. Acta 665, 22–33.
[25] Cooper, A.D., Erickson, S.K., Nutik, R. and Shrewsbury, M.A. (1982) J. Lipid Res. 23, 42–52.
[26] Kita, T., Goldstein, J.L., Brown, M.S., Watanabe, Y., Hornich, C.A. and Havel, R.J. (1982) Proc. Natl. Acad. Sci. USA 79, 3623–3627.
[27] Carew, T.E., Pittman, R.C. and Steinberg, D. (1982) J. Biol. Chem. 257, 8001–8008.
[28] Nagelkerke, J.F., Barto, K.P. and Van Berkel, Th.J.C. (1982) in: Sinusoidal Liver Cells (Knook, D.L. and Wisse, E. eds) pp.319–326, Elsevier Biomedical, Amsterdam, New York.