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SATURABLE HIGH AFFINITY BINDING OF LOW DENSITY AND HIGH DENSITY LIPOPROTEIN BY PARENCHYMAL AND NON-PARENCHYMAL CELLS FROM RAT LIVER

Theo J.C. Van Berkel, Johan K. Kruijt, Teus Van Gent and Arie Van Tol

Department of Biochemistry I, Faculty of Medicine, Erasmus University, P.O.Box 1738, 3000 DR Rotterdam, The Netherlands

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SUMMARY

Freshly isolated parenchymal liver cells bind both low density lipoprotein (LDL) and high density lipoprotein (HDL). With increasing concentrations of LDL and HDL the amount of cell-associated radioactivity approaches saturation and a linear double-reciprocal plot for the binding is obtained. The binding of LDL and HDL to isolated non-parenchymal liver cells is also saturable and the maximal binding of LDL and HDL per mg cell protein is 4-5 times higher than with parenchymal cells. It is suggested that the presence of a 4-5 fold higher concentration of lipoprotein receptor (for LDL and HDL) on non-parenchymal cells as compared to parenchymal cells explains the 4-5 times higher uptake of lipoproteins by the non-parenchymal liver cells, observed in vivo.

The liver plays an important role in serum lipoprotein catabolism[1] and is the only organ where cholesterol and/or cholesterolesters can be removed from the circulation and degraded to bile acids[2]. Studies with cultured human fibroblasts[3], aortic smooth muscle cells[4,5] and isolated rat liver parenchymal cells[6] resulted in a model for the catabolism of lipoproteins by the individual cell types[7]. This model includes binding of lipoprotein to membrane receptors, active endocytosis and lysosomal degradation of the protein and cholesterolesters by the action of cathepsins and acid cholesterolesterase, respectively. The liver is suggested to be a site for the catabolism of LDL[8-10] and HDL[11,12]. Recent studies from our laboratory indicate that in addition to parenchymal cells, non-parenchymal cells are also important for the hepatic uptake of LDL- and HDL-apoprotein[13] and cholesterolesters[14] in vivo. After intravenous injection of both cholesterolester-labeled or apoprotein-labeled LDL or HDL, the subsequently isolated non-parenchymal liver cells contain about 4 times more radioactivity per mg cell protein than the parenchymal cells[13,14]. In the light of these data and the aforementioned model of lipoprotein uptake, it is important to compare the binding of serum lipoproteins to parenchymal and non-parenchymal liver cells in order to determine if the presence of different

Abbreviations: VLDL, very low density lipoprotein (density <1.006 g/ml); LDL, low density lipoprotein (density range 1.019-1.050 g/ml); HDL, high density lipoprotein (density range 1.050-1.13 g/ml).

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levels of lipoprotein receptors in the different liver cell types explains the higher concentration of labeled lipoproteins in non-parenchymal cells as compared to parenchymal cells.

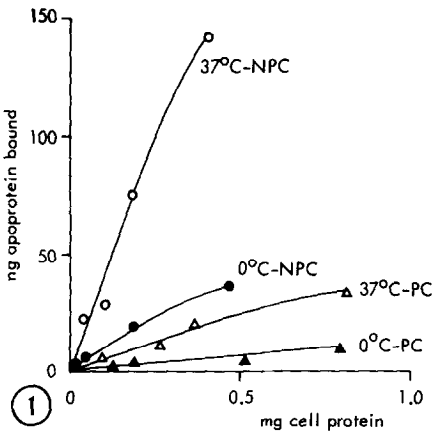
MATERIALS AND METHODS

Preparation of iodine-labeled lipoproteins. Rat LDL and HDL were isolated from serum of male Wistar rats fed a semi-synthetic carbohydrate-rich diet for two weeks[15]. The use of this diet restricted the labeling of lipoprotein phospholipids to less than 4%. The rats were bled under ether anesthesia after an overnight fast and LDL and HDL were isolated by the method of Redgrave et al.[16]. LDL and HDL were isolated from the density ranges 1.019-1.050 and 1.050-1.13 g/ml, respectively. It is known that rat LDL has a lower density than human LDL[17]. Rat HDL isolated between densities 1.050 and 1.13 g/ml does not contain apoprotein B, as checked by polyacrylamide gel electrophoresis of the delipidated apolipoproteins. The isolated lipoproteins were iodinated at pH 10 by the ICl method[18], as described earlier[15,19]. 3-4% of the radioactivity in the preparation was free, 3-5% was present in phospholipids and 92-94% was protein-bound. The characterization of iodinated LDL and HDL is described in detail elsewhere[15,19].

Preparation of liver cells and assay of lipoprotein binding. Isolation of pure and intact rat parenchymal and non-parenchymal cells was performed by perfusion of the liver with collagenase (0.05%) followed by isolation of the liver cells by differential centrifugation, exactly as described elsewhere[13]. The non-parenchymal cell preparation was isolated without the use of pronase (the NPC_2 fraction as described in ref. 13). Incubations of freshly isolated liver cells with the indicated amounts of lipoproteins and cells were performed in Ham's F-10 medium (modified), containing 5% (v/v) human lipoprotein-deficient serum (LPDS; final protein concentration 2.5 mg/ml). The incubations were carried out in plastic Eppendorf tubes in a total volume of 1 ml. After 10 min of incubation, at the indicated temperatures, the cells were centrifuged in an adapted Eppendorf centrifuge for 2 min at 3 000 rpm. The pellets were suspended in 1 ml of medium containing 50 mM Tris-HCl pH 7.4, 0.15 M NaCl and 2 mg bovine serum albumin, incubated for 5 min at 4°C and centrifuged again. This washing procedure was repeated twice. The last washing was performed with 0.15 M NaCl only, in order to enable a reliable protein determination. The cell-associated radioactivity and 0.5 ml of the different supernatants were counted in a LKB-Wallace Ultrogamma counter. The radioactivity in the last supernatant was less than 10% of the cell-associated radioactivity. To 0.5 ml of the first supernatant 0.2 ml 35% trichloroacetic acid was added followed by incubation for 15 min at 37°C; subsequently the mixture was centrifuged for 2 min at 15 000 rpm. To 0.5 ml of the supernatants 5 µl 40% KI and 25 µl 30% H_2O_2 were added. After 5 min at room temperature 0.8 ml $CHCl_3$ was added and the mixture was shaken for another 5 min. After centrifugation for 2 min at 15 000 rpm, 0.4 ml of the aqueous phase (containing iodinated amino acids and small peptides) and 0.5 ml of the $CHCl_3$ phase (containing I_2 formed from I^- by oxidation with H_2O_2) were counted [19,20]. In the corresponding blanks the lipoproteins were incubated in the absence of cells.

RESULTS

Fig. 1 shows the amount of cell-associated radioactivity after incubation of different amounts of parenchymal and non-parenchymal cells with 10 µg/ml iodine-labeled rat LDL at 37°C or 0°C. It is evident that the extent of binding is linear with the amount of cell protein up to 0.4 mg protein/ml



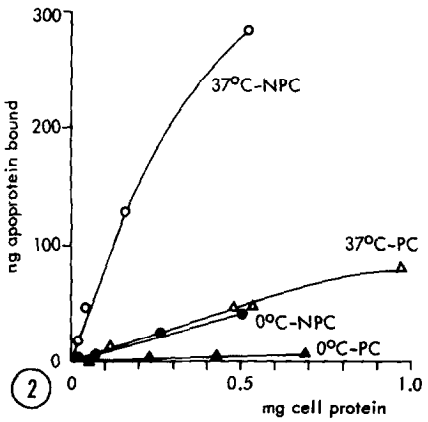


Fig. 1. Binding of ^{125}I-LDL to increasing amounts of parenchymal and non-parenchymal rat liver cells at 37°C and 0°C. Different amounts of cells were incubated in a final volume of 1 ml containing 10.0 µg/ml ^{125}I-LDL. The cell-associated radioactivity is given as ng apoprotein bound. NPC are non-parenchymal cells, PC are parenchymal cells.

Fig. 2. Binding of ^{125}I-HDL to increasing amounts of parenchymal and non-parenchymal rat liver cells at 37°C and 0°C. Different amounts of cells were incubated in a final volume of 1 ml containing 10.1 µg/ml ^{125}I-HDL. The cell-associated radioactivity is given as ng apoprotein bound. NPC are non-parenchymal cells, PC are parenchymal cells.

for parenchymal cells and 0.2 mg/ml for non-parenchymal cells. Protein dependency of lipoprotein binding has rarely been reported because most workers use cultured cells in which the whole content of the culture dish is incubated with lipoproteins. The two reports[6,21], using parenchymal cells for studying HDL binding measured binding with 2-5 mg cell protein/ml. When, however, the capacities of two different cell populations for lipoprotein binding are compared, it is essential that the binding is linear with respect to cell protein for both cell types. Comparison of the binding clearly indicates that the capacity of non-parenchymal cells to bind LDL per mg of cell protein is much higher than for parenchymal cells. Fig. 1 also shows that the binding for LDL is temperature-dependent. Decreasing the temperature from 37°C to 0°C results in a 70-75% drop of cellular radioactivity in both cell types. Less than 0.5% of the added lipoprotein was recovered in the aqueous phase as TCA-soluble non-iodide radioactivity during the 10 min incubation. This low degradation was independent of the amount of cell-associated lipoprotein. No evidence for cell-dependent deiodination was obtained because the amount of free I^- did not increase during incubation, as concluded from the radioactivity measured in the chloroform phase. 95-100% of the cell-associated radioactivity is precipitated by trichloroacetic acid and is mainly protein-bound (less than 10% was extractable by chloroform[22]).

Fig. 2 shows that similar data are obtained for the binding of iodine-labeled HDL to parenchymal and non-parenchymal rat liver cells. Also for HDL

the binding is clearly in favour of the non-parenchymal cells. At 0°C binding of HDL to both parenchymal and non-parenchymal cells is only 8-10% of the value at 37°C. Evidence that binding is measured, rather than binding plus uptake, is obtained from experiments in which, after initial binding, the cells were subsequently incubated in a lipoprotein-free medium. After 1 h of incubation 70-80% of the cell-associated radioactivity is released from the cells and recovered in the medium in a TCA-precipitable form (less than 2% is TCA-soluble), indicating that degradation of HDL-apoprotein is minimal under these conditions. The release of cell-associated radioactivity can be increased to 90-95% by including 0.05% collagenase in the lipoprotein-deficient medium. This binding appears to be specific, according to the definition of Ho et al.[23] because inclusion of an excess of unlabeled LDL (95 µg/ml) or HDL (65 µg/ml) to the corresponding labeled lipoprotein leads to a decrease in cell-bound radioactivity by more than 70%.

The effect of increasing LDL and HDL to the extent of binding to parenchymal cells is indicated in Fig. 3A and 3B. With increasing concentrations of LDL and HDL the amount of cell-associated radioactivity approaches saturation and a linear double-reciprocal plot is obtained (Fig. 3B). These data indicate saturable high affinity binding of both LDL and HDL to parenchymal liver cells.

Fig. 4 shows the effect of increasing amounts of LDL and HDL on the extent of binding to non-parenchymal liver cells. The high affinity binding sites for LDL and HDL present on non-parenchymal cells are also clearly saturable. The

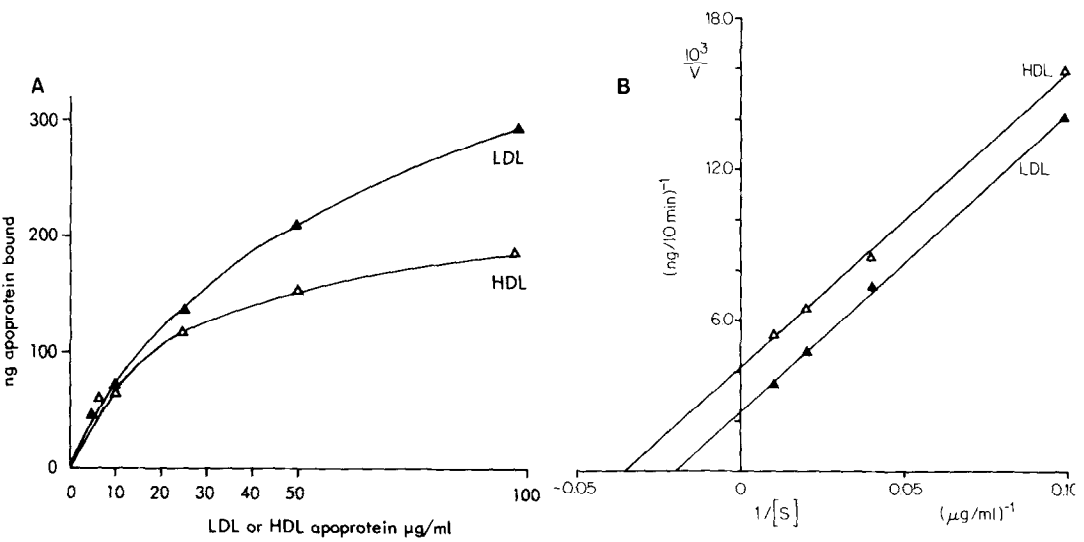


Fig. 3 A, B. Relation of the concentration of ^{125}I-LDL or ^{125}I-HDL to the extent of binding to parenchymal liver cells (A) and the double-reciprocal plot of these data (B). The amount of LDL or HDL apoprotein in the assay was varied as indicated and plotted against the cell-associated radioactivity (ng apoprotein bound/mg cell protein). The cell protein concentration was approximately 0.4 mg/ml.

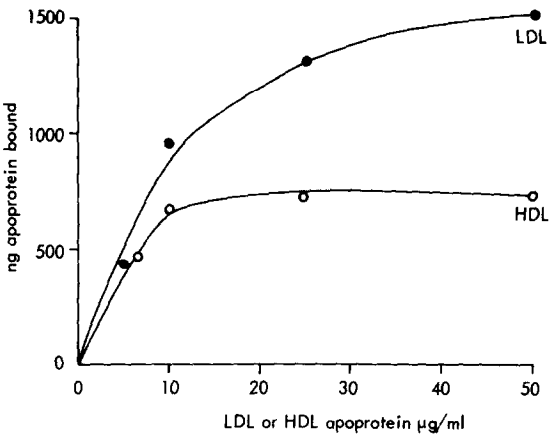


Fig. 4. Relation of the concentration of ^{125}I-LDL or ^{125}I-HDL to the extent of binding to non-parenchymal liver cells. The amount of LDL or HDL apoprotein in the assay was varied as indicated and plotted against the cell-associated radioactivity (ng apoprotein bound/mg cell protein). The cell protein concentration was approximately 0.15 mg/ml.

TABLE I

BINDING OF LOW DENSITY AND HIGH DENSITY LIPOPROTEIN TO PARENCHYMAL AND NON-PARENCHYMAL RAT LIVER CELLS

Apoprotein concentration	LDL binding				HDL binding			
	10 µg/ml		50 µg/ml		10 µg/ml		50 µg/ml	
		(n)		(n)		(n)		(n)
Parenchymal cells	73±15	(7)	270±56	(3)	96±16	(7)	254±52	(3)
Non-parenchymal cells	528±103	(7)	1404±190	(3)	781±181	(7)	1089±153	(3)
Ratio $\frac{\text{Non-parenchymal cells}}{\text{Parenchymal cells}}$	7.7±1.1		5.4±0.5		8.6±1.4		4.5±0.4	

The amount of bound LDL and HDL is given as ng apoprotein per mg cell protein (± S.E.M.). The amount of different cell preparations used (n) is indicated in parenthesis while 3 different lipoprotein preparations were applied.

maximal amount of LDL and HDL bound per mg cell protein is 4-5 times higher than with parenchymal cells. The binding to parenchymal and non-parenchymal cells is further quantified in Table I. It can be seen that the ratio of binding to non-parenchymal cells over parenchymal cells is about five at a relatively high lipoprotein concentration (50 µg/ml) while at a lower lipoprotein concentration (10 µg/ml) this ratio is even higher.

DISCUSSION

Saturable binding of cholesterolester-labeled chylomicron-remnants has been shown to occur in the perfused liver[24,25], on isolated hepatocytes[26] and on isolated liver plasma membranes[27]. Saturable binding sites for HDL are also

present on isolated hepatocytes[6,21]. Specific binding sites for LDL are suggested to be present on hepatocytes[28], but Soltys and Portman[29] were unable to show such sites on freshly isolated cells. The present experiments indicate that in addition to high affinity sites for HDL, freshly isolated hepatocytes also contain saturable high affinity binding sites for LDL. In this respect it is of interest that hepatocytes isolated by the method used in this study, are also still sensitive for low concentrations of glucagon indicating that also the glucagon receptor is not influenced by the isolation procedure used[30].

Earlier we showed that when apoprotein- and cholesterolester-labeled LDL and HDL are injected intravenously, the subsequently isolated non-parenchymal cells contain about 4 times more radioactivity per mg cell protein than parenchymal cells[13,14]. Goldstein and Brown[7] concluded from studies with cultured fibroblasts that the number of receptor sites on the cell membrane determines the uptake of LDL. The present experiments suggest that the high uptake of lipoproteins in non-parenchymal liver cells can be explained by the presence of a high concentration of saturable high affinity sites on the membrane of non-parenchymal liver cells. The ratio of *in vivo* uptake by non-parenchymal cells over that by parenchymal cells for the different lipoproteins tested sofar (VLDL-remnants, LDL and HDL) is similar and varies between 4-5[13,14]. It is remarkable that the *in vitro* binding ratio, shown here for LDL and HDL, of non-parenchymal cells over parenchymal cells, is also 4-5 at saturating levels of both lipoproteins. This suggests that also in liver cells the *in vivo* uptake is determined by the concentration of receptor sites. This raises the question in how far different receptors are involved in the uptake of the different lipoproteins, or that one lipoprotein receptor mediates the uptake of the different classes of lipoproteins. Studies are in progress to clarify this point.

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