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Uptake of lactosylated low-density lipoprotein by galactose-specific receptors in rat liver

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The liver contains two types of galactose receptors, specific for Kupffer and parenchymal cells respectively. These receptors are only expressed in the liver, and therefore are attractive targets for the specific delivery of drugs. We provided low-density lipoprotein (LDL), a particle with a diameter of 23 nm in which a variety of drugs can be incorporated, with terminal galactose residues by lactosylation. Radioiodinated LDL, lactosylated to various extents (60–400 mol of lactose/mol of LDL), was injected into rats. The plasma clearance and hepatic uptake of radioactivity were correlated with the extent of lactosylation. Highly lactosylated LDL (> 300 lactose/LDL) is completely cleared from the blood by liver within 10 min. Pre-injection with *N*-acetylgalactosamine blocks liver uptake, which indicates that the hepatic recognition sites are galactose-specific. The hepatic uptake occurs mainly by parenchymal and Kupffer cells. At a low degree of lactosylation, approx. 60 lactose/LDL, the specific uptake (ng/mg of cell protein) is 28 times higher in Kupffer cells than in parenchymal cells. However, because of their much larger mass, parenchymal cells are the main site of uptake. At high degrees of lactosylation (> 300 lactose/LDL), the specific uptake in Kupffer cells is 70–95 times that in parenchymal cells. Under these conditions, Kupffer cells are, despite their much smaller mass, the main site of uptake. Thus not only the size but also the surface density of galactose on lactosylated LDL is important for the balance of uptake between Kupffer and parenchymal cells. This knowledge should allow us to design particulate galactose-bearing carriers for the rapid transport of various drugs to either parenchymal cells or Kupffer cells.

INTRODUCTION

In the liver, both Kupffer and parenchymal cells have receptors on their plasma membranes that specifically bind and internalize ligands with terminal D-galactose residues. The receptor on parenchymal cells is the classical asialoglycoprotein receptor originally described by Ashwell & Morell [1]. The receptor on Kupffer cells, which was described more recently [2], is different from the receptor on parenchymal cells [3]. Recent evidence suggests that the receptor on Kupffer cells might be identical with serum amyloid P, a circulating lectin [4].

The two receptor systems have similar carbohydrate-binding specificities, but it has been suggested that there is a difference in their capacity to internalize ligands of various sizes [5]. Unlike parenchymal cells, Kupffer cells are unable to internalize small galactose-terminated molecules such as asialofetuin (diameter 4.5 nm) via their galactose-specific receptors [6,7]. Gold particles coated with asialofetuin are, however, taken up readily by Kupffer cells [2,7].

Since the galactose-specific receptors are only expressed on Kupffer and liver parenchymal cells and not at other sites in the body, they are attractive targets for the specific delivery of drugs and other material to these liver cells. To be able to direct drugs specifically with an adequate carrier to either receptor, it is essential to have more detailed information about the specificities of the two receptor systems for potential carriers. In the present study, we have investigated the role of the density of galactose residues on a particle in uptake via the two receptor systems. We have used for our studies low-density lipoprotein (LDL) because it forms a homogeneous population of particles with a diameter of 23 nm, to which various amounts of D-galactose residues can be coupled by reductive lactosamination. LDL is an attractive transport vehicle, as it is an endogenous particulate carrier that is not rapidly removed from the circulation by the reticuloendothelial system. Furthermore, it has been demonstrated that it can accommodate up to 100 drug molecules in its oily core [8].

EXPERIMENTAL

Reagents

Collagenase type I, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, BSA (fraction V), agarose-bound neuraminidase (from *Clostridium perfringens*; type VI-A) and fetuin (type IV) were obtained from Sigma, St. Louis, MO, U.S.A. ^{125}I (carrier free) and [D-*glucose*-1-^{14}C]lactose were supplied by Amersham International, Amersham, Bucks., U.K. Lactose was supplied by Merck, Darmstadt, Germany. Sodium cyanoborohydride was from Aldrich, Brussels, Belgium. Gadolinium chloride ($GdCl_3$) was obtained from Janssen, Beerse, Belgium. All other chemicals were analytical grade.

Fetuin was desialylated enzymically by incubating the protein, dissolved in 0.1 M-sodium acetate buffer, pH 5.5, with agarose-bound neuraminidase (20 m-units/ml) for 4 h at 37 °C. A minimum of 80% of the sialic acid residues, assayed as described previously [9], was removed by this procedure.

Isolation, radiolabelling and lactosylation of LDL

Human LDL ($1.019 < d < 1.063$) was isolated by two repetitive centrifugations as described previously [10]. The lipoprotein was labelled with ^{125}I as described previously [11]. Less than 1% of the labelled material was trichloroacetic acid-soluble. LDL was lactosylated by incubating the lipoprotein (at 2 mg/ml in 20 mM-sodium phosphate buffer, pH 7.0, containing 1 mM-EDTA) at room temperature with lactose and sodium cyanoborohydride to final concentrations of 100 mg/ml and 50 mg/ml respectively. After 8–360 h, the reaction mixture was diluted 10-fold with cold phosphate-buffered saline [10 mM-

Abbreviation used: LDL, low-density lipoprotein.

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sodium phosphate (pH 7.4)/150 mM-NaCl], containing 1 mM-EDTA, and dialysed exhaustively against this buffer.

Plasma clearance and liver association

Male Wistar rats, weighing 225–325 g, were used. They were anaesthesized by intraperitoneal injection of 15–20 mg of sodium pentobarbital and the abdomen was opened. Radiolabelled lactosylated LDL or native LDL were injected via the penile vein at a dose of 50 μg of apolipoprotein/kg body wt. At the indicated times, blood samples of 0.2/0.3 ml were taken from the inferior vena cava and collected in heparinized tubes. The samples were centrifuged for 2 min at 16000 *g*. Duplicate samples of the plasma were assayed for radioactivity after precipitation of protein with 10% (w/v) trichloroacetic acid. The total amount of radioactivity in plasma was calculated by using the equation:

$$\text{Plasma volume (ml)} = [0.0219 \times \text{body wt. (g)}] + 2.66 \text{ [12]}$$

At the indicated times, liver lobules were tied off and excised, and at the end of the experiment the remainder of the liver was removed. The amount of liver tissue tied off successively did not exceed 15% of the total liver mass. Radioactivity in liver at each time point was calculated from the radioactivities and weights of the liver samples, and corrected for the radioactivity in plasma present in the tissue at the time of sampling (85 μl/g fresh wt. [13]).

Isolation of liver cells

Rats were anaesthesized and injected with ^{125}I-LDL or lactosylated ^{125}I-LDL as described in the previous section. At 10 min after injection, the vena porta was canulated and the liver was perfused with Ca^{2+}-free Hanks balanced salt solution containing 10 mM-Hepes, pH 7.4 (8 °C), at a flow rate of 14 ml/min. After 8 min, a lobule was tied off for determination of the total liver uptake. Then the liver was perfused with 0.05% (w/v) collagenase in Hanks medium containing 10 mM-Hepes, pH 7.4, and parenchymal and non-parenchymal cells were isolated as described previously [11]. The non-parenchymal cell preparation was further fractionated into endothelial and Kupffer cells by centrifugal elutriation as described in detail previously [14]. The contributions of the various cell types to the total liver uptake were calculated as described previously [14]. As found previously with other substrates [11,14,15], no significant amounts of radioactivity were lost from the cells during the isolation procedure. This was checked in each experiment by comparing the calculated liver uptake (i.e. the summation of the contributions of the various cell types) with the value actually measured in the liver lobule.

Determination of proteins

Protein concentrations in homogenates, cell suspensions and solutions of LDL were determined by the method of Lowry *et al.* [16], with BSA as standard. The values found for LDL were multiplied by a factor 0.82 to correct for the higher colour yield of apolipoprotein B [17].

RESULTS

Coupling of lactose to LDL

D-Galactosyl residues were coupled to LDL by incubating the lipoprotein with lactose (D-galactosyl-D-glucose) and sodium cyanoborohydride. The latter reduces the Schiff base between the

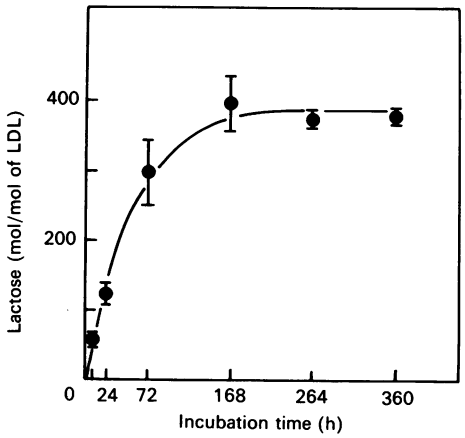

Fig. 1. Time course of coupling of [^{14}C]lactose to LDL

Portions (0.2 mg) of LDL were incubated at room temperature with 20 mg of [^{14}C]lactose (sp. radioactivity 0.1 μCi/mg) and 10 mg of sodium cyanoborohydride in 20 mM-sodium phosphate buffer, pH 7.0, containing 1 mM-EDTA (final volume 0.1 ml). At the indicated times, the mixtures were diluted 10-fold with cold phosphate-buffered saline and dialysed exhaustively. Incorporation of lactose was calculated from the amounts of radioactivity and protein in the dialysed sample, assuming the presence of one molecule of apolipoprotein B with an M_r of 512723 per LDL particle [29]. The results, expressed as mol of lactose/mol of LDL, are means ± S.E.M. for three separate experiments.

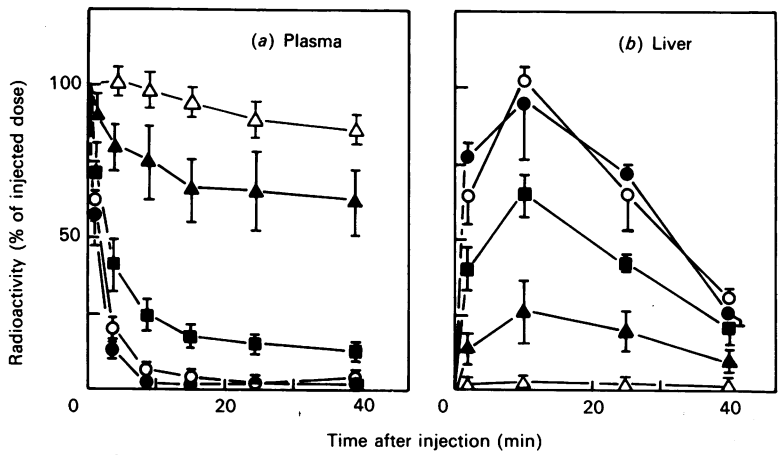

Fig. 2. Plasma clearance and liver uptake of lactosylated LDL

Rats were injected with preparations of ^{125}I-LDL that had been lactosylated for 8 (▲), 24 (■), 72 (○) or 168 (●) h. At the indicated times, the trichloroacetic acid-precipitable radioactivity in plasma (*a*) and the total radioactivity in liver (*b*) were determined. The clearance and uptake of native ^{125}I-LDL are shown by △. Values are means ± S.E.M. for three rats.

glucose moiety of lactose and amino groups on LDL, which results in covalent attachment of lactose to LDL [18]. The extent of coupling of lactose to LDL was studied by measuring radioactivity in the lipoprotein after incubation with [^{14}C]lactose and sodium cyanoborohydride. Fig. 1 shows that the association of lactose to LDL increases with time. After 8 h of incubation, 57±9 mol of lactose are bound/mol of LDL (means±S.E.M. of 3 experiments). The incorporation reaches a maximum of approx. 400 mol of lactose/mol of LDL after 168 h of incubation.

An LDL particle consists of an apolipoprotein and a lipid moiety. By extracting [^{14}C]lactosylated LDL with ethanol/ether, it was found that lactose associates predominantly (> 90%) with the apolipoprotein. Analysis by density-gradient centrifugation indicated that the coupling of lactose to LDL causes a small change in the density of the particle. The density of highly lactosylated LDL (peak density 1.053 g/ml) was found to be slightly higher than that of native LDL (peak density 1.038 g/ml). Both protein and cholesterol of lactosylated LDL were found at the same density, which indicates that lactosylation does not affect the integrity of the particle.

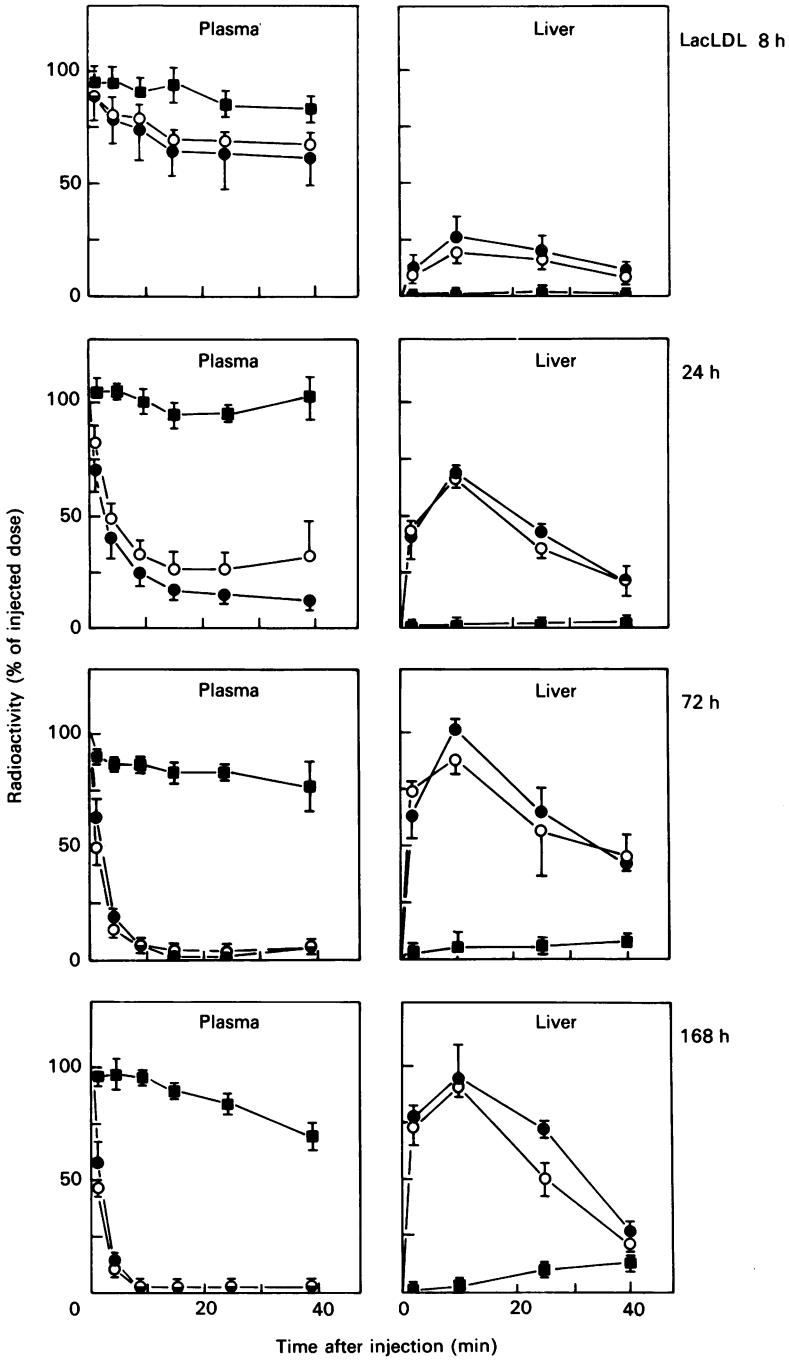

Fig. 3. Effects of *N*-acetylgalactosamine and *N*-acetylglucosamine on plasma clearance and liver association of lactosylated LDL

Rats were injected with ^{125}I-LDL that had been lactosylated (LacLDL) for 8, 24, 72 or 168 h. At 1 min before the injection of radiolabel, the animals received 400 mg of *N*-acetylgalactosamine/kg body wt. (■), 400 mg of *N*-acetylglucosamine/kg body wt. (○) or solvent (phosphate-buffered saline; ●). At the indicated times, the trichloroacetic acid-precipitable radioactivity in plasma and the total radioactivity in liver were determined. Values are means±S.E.M. for three rats.

Plasma clearance and liver association of lactosylated LDL

Native LDL disappears very slowly from the circulation of the rat (half-life of 5–10 h), and only 2.1 ± 0.1 % (mean ± S.E.M. of 8 animals) of the injected dose is found in liver at 10 min after injection of radiolabelled LDL. To study the effect of the coupling of lactose to LDL on its plasma clearance and liver uptake, rats were injected with preparations of ^{125}I-LDL that had been lactosylated for 8, 24, 72 or 168 h. Fig. 2 shows that all preparations of lactosylated LDL are cleared from the plasma at significantly higher rates than for native LDL. The rapidity of clearance is correlated with the length of the lactosylation period. At high degrees of lactosylation, obtained after > 72 h of incubation, virtually all of the injected material is cleared from the circulation within 10 min. The decrease in plasma radioactivity coincides with an increase in the radioactivity associated with liver. The radioactivity in liver is maximal at 10 min after injection, and subsequently declines. The decrease in liver radioactivity is accompanied by a rise in the trichloroacetic acid-soluble radioactivity in plasma. In rats injected with 168 h-lactosylated ^{125}I-LDL, for instance, the acid-soluble radioactivity in plasma increases from 0.1 ± 0.1 % of the dose at 1 min after injection to 4.1 ± 0.1 % at 40 min after injection (means ± S.E.M.; 3 rats). These results suggest that lactosylated LDL, once associated with the liver, is degraded to acid-soluble products that rapidly diffuse to the blood. Subcellular-fractionation studies, performed as described by De Duve *et al.* [19], indicate that lysosomes are involved in the intracellular processing of lactosylated LDL in the liver (results not shown).

The mechanism of the liver association of lactosylated LDL was determined in several ways. Fig. 3 shows that pre-injection with *N*-acetylgalactosamine (400 mg/kg body wt.) almost completely inhibits the plasma clearance and liver association of all the lactosylated LDL preparations, whereas preinjection with the same dose of *N*-acetylglucosamine has no effect at all. In further experiments, rats were injected with ^{125}I-LDL that had been incubated for 168 h with either sodium cyanoborohydride alone (50 mg/ml) or with sodium cyanoborohydride (50 mg/ml) in combination with cellobiose (100 mg/ml). At 10 min after injection, the livers of these animals contained only 3.8 ± 1.1 % and 6.4 ± 1.3 % of the injected dose respectively (means ± S.E.M.; 3 rats). These results indicate that the introduced terminal galactose residues of lactosylated LDL are responsible for the hepatic uptake of the particle, and that uptake is not due to other modifications (e.g. oxidation of lipids and/or the apoprotein).

Uptake of lactosylated LDL by liver cells

To study the effect of lactosylation of LDL on the recognition of the lipoprotein by the various cell types in the liver, rats were injected with ^{125}I-LDL that had been lactosylated for 0–168 h; 10 min later, the liver was perfused, and Kupffer, endothelial and parenchymal cells were isolated. The cell isolation procedure was performed at a low temperature (4–8 °C) to prevent degradation of the internalized material. Fig. 4 shows that the uptake by all the three liver cell types increases with the incubation time, i.e. with increasing degree of lactosylation. After 8 h of incubation, when approx. 60 molecules of lactose are bound per LDL particle, the association of radioactivity to the liver cells is 6–8

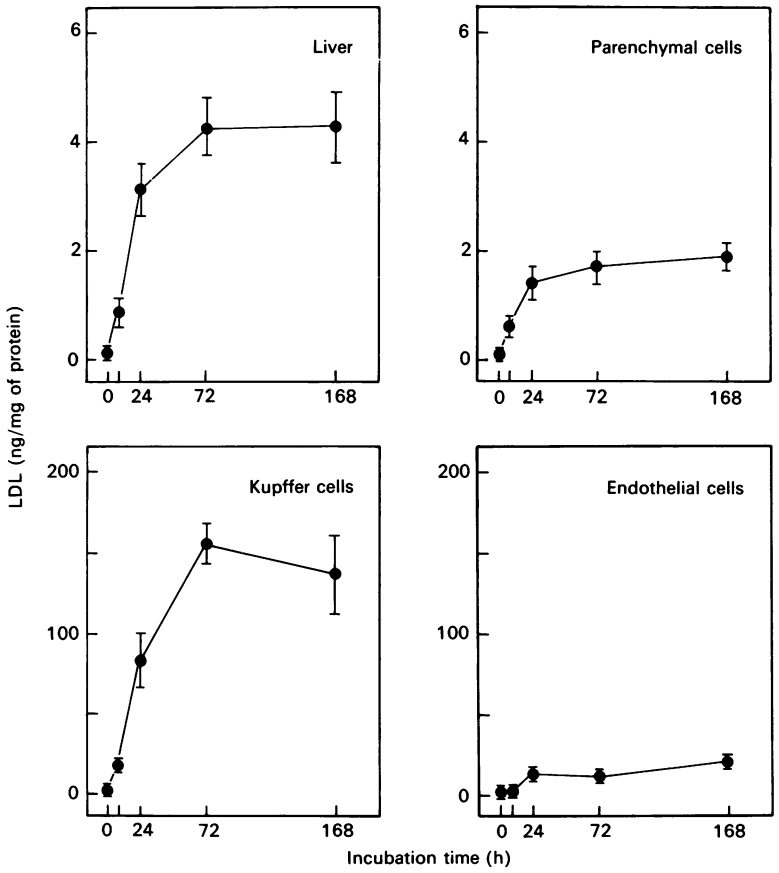

Fig. 4. Effect of lactosylation of LDL on its association with liver and various liver cell types

Rats were injected with ^{125}I-LDL that had been lactosylated for 0–168 h; 10 min after injection, parenchymal, Kupffer and endothelial cells were isolated, and the association of radioactivity with each cell type was determined. The results, expressed as ng of lactosylated ^{125}I-LDL/mg of cell protein, are means ± S.E.M. for three experiments.

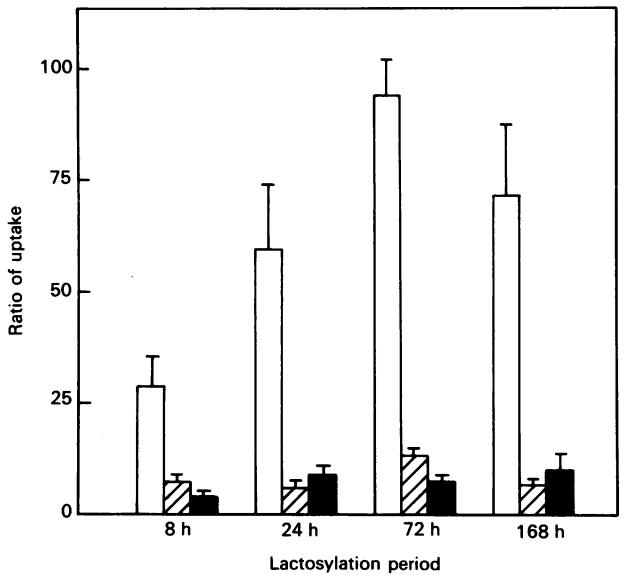

Fig. 5. Comparison of galactose-mediated uptake of lactosylated LDL by different liver cell types

Rats were injected with ^{125}I-LDL that had been lactosylated for 8, 24, 72 or 168 h; 10 min after injection, liver cells were isolated, and the association of lactosylated ^{125}I-LDL with each cell type (ng/mg of cell protein) was determined. The galactose-mediated uptake by the cells was calculated by correcting the association of lactosylated LDL for the association of native LDL (0.08 ± 0.01, 2.20 ± 0.12 and 0.45 ± 0.07 ng of LDL/mg of cell protein for parenchymal, Kupffer and endothelial cells respectively). The following ratios of galactose-mediated uptake are shown: Kupffer cells/parenchymal cells (□), Kupffer cells/endothelial cells (▨) and endothelial cells/parenchymal cells (■). All values are means ± S.E.M. for three experiments.

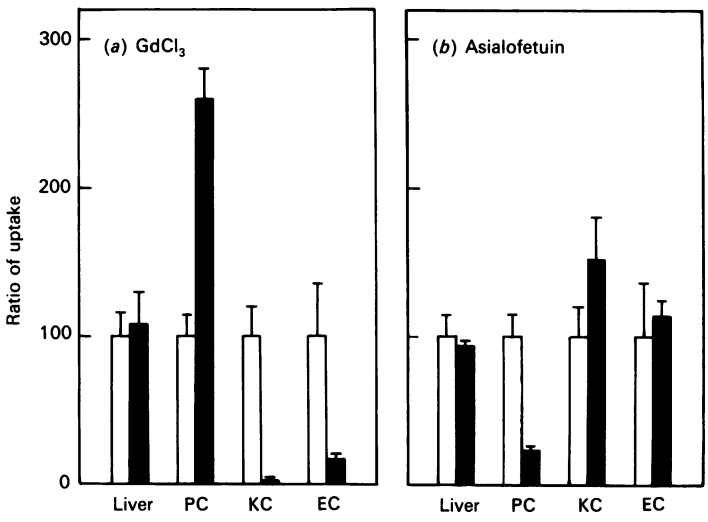

Fig. 6. Effects of $GdCl_3$ and asialofetuin on the association of 168 h-lactosylated LDL with liver and various liver cell types

Rats were injected with 168 h-lactosylated ^{125}I-LDL at a dose of 50 μg of apolipoprotein/kg body wt. The animals had been pre-injected with $GdCl_3$ (■, *a*; 20 μmol/kg body wt.) or asialofetuin (■, *b*; 50 mg/kg body wt.) at 24 h or 1 min respectively before injection of lactosylated LDL. Controls were not pretreated (□). Liver cells were isolated at 10 min after injection, and the association of radioactivity with each cell type was determined. The results are expressed as percentages of the uptake by liver cells of untreated controls. The control values for uptake by parenchymal cells (PC), Kupffer cells (KC), endothelial cells (EC) and total liver are 2.0 ± 0.3, 135.4 ± 29.6, 20.7 ± 7.3 and 4.3 ± 0.7 ng/mg of cell protein respectively. Values are means ± S.E.M. for three experiments.

times higher than the association of native ^{125}I-LDL. At this relatively low degree of lactosylation, the galactose-mediated association of lactosylated LDL to Kupffer cells (expressed as ng/mg of cell protein) is 28 and 4 times higher than to parenchymal and endothelial cells respectively (Fig. 5). Parenchymal cells are, nevertheless, the main site of hepatic uptake, since these cells contribute > 90% to the liver protein mass, and Kupffer and endothelial cells only a small percentage. Incubation for longer than 8 h results in the association of larger amounts of lactosylated LDL with the liver cells. However, the increase in uptake by Kupffer cells is relatively larger than that by parenchymal cells. Fig. 5 shows that, after injection of ^{125}I-LDL that has been lactosylated for 72 h or longer (> 300 lactose/LDL), the galactose-mediated association of radioactivity to Kupffer cells is 70–95 times the association to parenchymal cells. At these high ratios Kupffer cells, despite their small number and size, are responsible for the major part (approx. 60%) of the liver uptake.

Modulation of the intrahepatic distribution of lactosylated LDL by $GdCl_3$ and asialofetuin

The results of the previous section indicate that Kupffer cells are the main site of uptake of 168 h-lactosylated ^{125}I-LDL. Recently it was found that in rats receptor-mediated endocytosis by Kupffer cells is selectively inhibited by pretreating the animals with $GdCl_3$ [20]. Surprisingly, pretreatment of rats with $GdCl_3$ had no effect on the liver uptake of 168 h-lactosylated ^{125}I-LDL. It appears that the intrahepatic distribution of the internalized material is, however, dramatically altered (Fig. 6*a*). The uptake by Kupffer cells is virtually abolished. The capacity of the liver to remove 168 h-lactoslyated LDL from the circulation is, however, not seriously affected, since the impaired uptake by Kupffer cells in $GdCl_3$-pretreated animals is compensated by a much higher uptake by parenchymal cells. These cells then account for 95% of the total liver uptake.

Galactose-mediated uptake by parenchymal liver cells can be blocked specifically with asialofetuin. This galactose-terminated glycoprotein inhibits internalization via the galactose-specific receptors on parenchymal liver cells, but not galactose-mediated uptake by Kupffer cells [6]. Pre-injection of rats with asialofetuin (50 mg/kg body wt.) has no large effect on the liver uptake of 168 h-lactosylated ^{125}I-LDL (Fig. 6*b*). However, the pre-injection considerably changes the contributions of the various liver cell types to the total hepatic uptake. Internalization by parenchymal cells is much decreased. The inability of asialofetuin to decrease the total liver uptake is explained by the finding that the lower internalization by parenchymal cells is compensated by a higher uptake by Kupffer cells.

The effect of asialofetuin on the liver uptake of 8 h-lactosylated ^{125}I-LDL is different. Pre-injection with asialofetuin (50 mg/kg) decreases the hepatic uptake at 10 min after injection from 27.2 ± 11.3% of the dose to 4.5 ± 1.8% (means ± S.E.M.; 3 rats).

DISCUSSION

In the present study we show that incubation of LDL with lactose and sodium cyanoborohydride results in a reproducible time-dependent covalent coupling of lactose to the apolipoprotein. Maximally, about 400 molecules of lactose can be bound per LDL particle. After intravenous injection into rats, lactosylated LDL is rapidly cleared from the circulation, owing to galactose-specific uptake by the liver.

In an earlier study by Attie *et al.* [21], it was found that LDL containing 200–250 lactose residues per particle is cleared from plasma at a much lower rate (half-life approx. 45 min) than shown here. We do not have an explanation for the discrepancy between those earlier results and the present data. However, the short plasma half-lives of lactosylated LDL observed in our study comply with the rapid plasma clearance seen after lactosylation of other proteins, e.g. serum albumin, ferritin and RNA A [22–25].

Lactosylated LDL is taken up via hepatic galactose-specific receptors. Uptake of the particles via (extra)hepatic LDL receptors is probably low, since the lysine residues of the apoprotein, which are involved in the interaction with the LDL receptor [26,27], are modified by the lactosylation procedure. Furthermore, the galactose-receptor-mediated plasma clearance of lactosylated LDL is much faster than the LDL-receptor-mediated clearance of native heterologous or homologous LDL (plasma half-life several hours) [28].

In the liver, lactosylated LDL is mainly taken up by Kupffer and parenchymal cells. At a low degree of lactosylation (about 60 lactose/LDL; obtained after 8 h of incubation), the specific uptake by Kupffer cells, expressed as ng/mg of cell protein, is 28 times that by parenchymal cells. However, because of their smaller number and size, the contribution of Kupffer cells to the total liver uptake is lower than of parenchymal cells. At high degrees of lactosylation (> 300 lactose/LDL; obtained after > 72 h of incubation), the specific uptake by Kupffer cells is 70–95 times the specific uptake by parenchymal cells. Despite their smaller size and number, Kupffer cells contribute at these ratios more (about 60%) to the total liver uptake than do parenchymal cells (approx. 30%). Endothelial liver cells play only a minor role in the liver uptake of lactosylated LDL. At the various degrees of lactosylation these cells account for only 5–10% of the total liver uptake.

If receptor-mediated endocytosis by Kupffer cells is blocked with $GdCl_3$, the parenchymal cells compensate for the impaired internalization by Kupffer cells. As a result, 168 h-lactosylated LDL is almost exclusively taken up by parenchymal cells. If, on the other hand, the internalization of 168 h-lactosylated LDL by parenchymal cells is blocked with asialofetuin, the particle is directed to the Kupffer cells. Thus Kupffer cells and parenchymal cells are both able to internalize the 168 h-lactosylated LDL. The intraphepatic distribution of this particle depends on the relative activity of galactose-receptor systems on parenchymal and Kupffer cells. Under normal conditions, these two cell types compete for uptake of 168 h-lactosylated LDL. Our data indicate that the intrahepatic distribution can be shifted in favour of either cell type by specifically inhibiting uptake by the other cell type. However, if the uptake of 8 h-lactosylated LDL by parenchymal cells is blocked with asialofetuin, the total liver uptake decreases considerably. Thus the lower uptake by parenchymal cells is not compensated by a higher uptake by Kupffer cells. Apparently, Kupffer cells hardly interact with LDL that contains a relatively low amount of terminal galactose residues.

Kupffer cells do not internalize small galactose-terminated molecules such as asialofetuin [6]. Galactose-specific uptake by these cells can be induced by coating several molecules of asialofetuin on to gold particles [2,7]. This suggests that multiple occupation of binding sites is necessary to induce internalization via the galactose receptors on Kupffer cells. The present data support this view. An increase in the number of galactose residues on LDL was found to result in a substantial increase in uptake by Kupffer cells. The uptake by parenchymal cells was also increased, but to a much smaller extent than that by Kupffer cells. This suggests that multiple occupation of binding sites is important for internalization of particles via the galactose receptor on Kupffer cells, whereas this aspect is less important for internalization via the receptors on parenchymal cells.

In an earlier study it was concluded that only particles with a size of maximally 10 nm are internalized via the galactose receptors on parenchymal cells [7]. We have found, however, that lactosylated LDL, a particle of diameter 23 nm, can be taken up rapidly via these receptors. The earlier studies were performed with gold particles that were coated with asialofetuin. In the analysis of their data the authors [7] did not, however, take into account the size of the asialofetuin molecules (diameter approx. 4.5 nm) that were coated on to the particles. The sizes of the particles were therefore underestimated, which may explain, at least in part, the difference between the earlier work and the present data. The ability of parenchymal cells to internalize relatively large particles via their galactose-receptors is advantageous, as it implies that relatively large quantities of drugs may be introduced into parenchymal cells via this route.

What are the factors that determine the contributions of Kupffer and parenchymal cells to the hepatic uptake of galactose-containing particles? It has been suggested previously that the size of the particles determines the intrahepatic distribution [2,7]. In the present study we demonstrate that the number of galactose residues on a particle is also important. Furthermore, it appears that the mode of coupling of galactose residues to LDL is, for unknown reasons, important. We have shown previously that, if a galactose-terminated cholesterol derivative is incorporated into the lipid moiety of LDL, less than 10% of the liver uptake occurs by parenchymal cells [11]. We have shown here that, after coupling of galactose residues to the apolipoprotein of LDL, 30–60% of the liver uptake may be performed by parenchymal cells. Moreover, we have found recently that about 90% of the hepatic uptake of immune complexes of LDL with lactosylated anti-LDL antibodies is performed by parenchymal cells (M. K. Bijsterbosch, F. Bernini, H. F. Bakkeren & T. J. C. van Berkel, unpublished work). We conclude that a number of factors influence the uptake of galactose-bearing particles by Kupffer and parenchymal cells, and that size is certainly not the only feature that determines the intrahepatic distribution. Insight into the specificities of the galactose receptor on Kupffer and parenchymal cells is necessary for the design of particulate carriers for drugs or other compounds of interest to specific liver cell types. Among the potential carriers, LDL has been recognized recently [8] as a particularly promising vehicle. Being an endogenous carrier, it is not rapidly removed from the circulation by the cells of the reticuloendothelial system. Furthermore, LDL can accommodate lipophilic drugs in its non-aqueous oily core [8]. The knowledge obtained in our studies should allow us to design conditions whereby LDL can be directed specifically to galactose receptors on either parenchymal liver cells or Kupffer cells.

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