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Lactosylated Low Density Lipoprotein: A Potential Carrier for the Site-Specific Delivery of Drugs to Kupffer Cells

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SUMMARY

Low density lipoprotein (LDL) is a spherical particle with a diameter of 22 nm. It consists of an apolipoprotein and a lipid moiety, in which a variety of lipophilic drugs and prodrugs can be incorporated. In the present study, lactose was coupled to the apolipoprotein of LDL by reductive amination (398 ± 40 residues/LDL particle). After injection into rats, radioactively labeled lactosylated LDL was cleared rapidly from the plasma (half-life, <2 min). Ten minutes after injection, the liver contained about 90% of the dose, whereas only small amounts of radioactivity were found in other tissues. Preinjection of *N*-acetylgalactosamine completely blocked liver uptake, whereas *N*-acetylglucosamine was ineffective. This indicates that the hepatic recognition site is galactose specific. Subcellular fractionation of liver indicated that the recognition of lactosylated LDL is followed by internalization and degradation of the apolipoprotein in the lysosomes. In the liver, Kupffer cells are mainly responsible for uptake. At 10 min after injection, these cells contained a 70 and 7 times higher amount of lactosylated LDL per mg of cell protein than parenchymal and endothelial cells, respectively. After galactose-specific uptake in parenchymal cells was blocked with asialofetuin, the relative concentration in Kupffer cells was even higher. The hepatic uptake of the lipid moiety of lactosylated LDL, labeled with [^{3}H]cholesteryl oleoyl ether, was identical to that of the ^{125}I-labeled apolipoporotein, which indicates that the particle is taken up as a unit. Thus, lactosylated LDL is taken up rapidly and selectively by Kupffer cells, and it appears that it might be a very effective vehicle for the specific delivery of lipophilic drugs, e.g., immunomodulators, to these cells.

Recent advances in molecular and cell biology have led to a better understanding of the mechanisms of uptake and subsequent handling of molecules by cells. The newly attained knowledge can be used to increase the therapeutic indices and efficacies of drugs by developing methods for the specific delivery to their desired sites of action. For instance, cell surface receptors that are unique for a particular cell type could be used to specifically target drugs to this cell type.

In liver, both Kupffer and parenchymal cells possess receptors that specifically bind and internalize D-galactose-containing material. The receptor on parenchymal cells is the classical asialoglycoprotein receptor that was originally described by Ashwell and Morell (1). The receptor on Kupffer cells was characterized more recently (2). This receptor is different from the receptor on parenchymal cells (3), and it was found that it specifically recognizes particles larger than 10–15 nm that have exposed galactose residues (4). Recent evidence suggests that this so-called galactose-particle receptor might be identical to a circulating lectin, serum amyloid P (5). Because galactose-specific receptors are largely confined to Kupffer and liver parenchymal cells, they can be considered suitable targets for the specific delivery of drugs to these cells.

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In earlier studies, it was shown that liposomes with galactose-containing structures on their surfaces can be taken up via galactose-specific receptors on both liver cell types (6–9). In the present study, LDL was modified to direct it to hepatic galactose receptors. LDL is a spherical particle with a diameter of about 22 nm. It consists of an apolar core, which is composed of cholesteryl esters, surrounded by a phospholipid monolayer in which cholesterol and a specific apolipoprotein (apolipoprotein B-100) are embedded (10). Because a variety of highly lipophilic compounds can be easily incorporated in the hydrophobic core of LDL (11, 12), it may be used as a carrier for lipophilic drugs. The apolipoprotein can be used for modification, and in the present study we have coupled terminal galactose residues to the apolipoprotein by reductive amination.

The results presented here indicate that lactosylated LDL is rapidly and specifically internalized by the galactose-specific receptors on Kupffer cells. Lactosylated LDL seems, therefore, to be a suitable carrier for the specific delivery of lipophilic drugs to these cells.

Experimental Procedures

Reagents. Lactose was supplied by Merck (Darmstadt, FRG). Sodium cyanoborohydride was from Aldrich (Brussels, Belgium). [1α,2α-(N)-^{3}H]Cholesteryl oleoyl ether, ^{125}I (carrier free), and [D-*glucose-1*-

ABBREVIATIONS: LDL, low density lipoprotein; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

^{14}C]lactose were supplied by Amersham International (Amersham, Bucks, UK). Collagenase type I, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, bovine serum albumin (fraction V), agarose-bound neuraminidase (from *Clostridium perfringens*; type VI-A), and fetuin (type IV) were obtained from Sigma (St. Louis, MO). All other chemicals were analytical grade.

Fetuin was desialyated enzymatically by incubating the protein, which was dissolved in 0.1 M sodium acetate buffer, pH 5.5, with agarose-bound neuraminidase (20 milliunits/ml) for 4 hr at 37°. A minimum of 80% of the sialic acid residues, assayed as described earlier (13), were removed by this procedure.

Isolation and radiolabeling of LDL. Human LDL ($1.019 < d < 1.063$) was isolated by two repetitive centrifugations, as described earlier (14). The lipoprotein was labeled with ^{125}I as described previously (15). Less than 1% of the labeled material was trichloroacetic acid soluble. For some experiments, LDL was labeled with ^{125}I-tyramine-cellobiose. Synthesis and subsequent radioiodination of tyramine-cellobiose were carried out as described earlier (16). Coupling of ^{125}I-tyramine-cellobiose to LDL was done as follows. To 50 μl of 0.3 mM ^{125}I-tyramine-cellobiose were successively added 20 μl 0.75 mM cyanuric chloride in acetone and 10 μl of 3.0 mM NaOH. After 20 sec, 20 μl of 2.25 mM acetic acid were added. The resulting activated ligand was added to 1–2 mg of LDL in 1 ml of 20 mM sodium tetraborate buffer, pH 9.0, that contained 0.12 M NaCl and 1 mM EDTA. After 30 min at room temperature, the reaction was quenched by the addition of an equal volume of 0.2 M NH_4HCO_3. Unbound label was removed by exhaustive dialysis against phosphate-buffered saline (10 mM sodium phosphate buffer, pH 7.4, containing 150 mM NaCl and 1 mM EDTA). Less than 1% of the labeled material was trichloroacetic acid soluble.

Lactosylation of LDL. Human LDL (2 mg/ml) in 20 mM sodium phosphate buffer, pH 7.0, that contained 1 mM EDTA) was incubated at room temperature with lactose and sodium cyanoborohydride to final concentrations of 100 and 50 mg/ml, respectively. After 7 days, the reaction mixture was diluted 5-fold with phosphate-buffered saline and was dialyzed exhaustively against this buffer.

Extraction of lactosylated LDL. [^{14}C]Lactosylated LDL, which was dissolved in borate-buffered saline (10 mM sodium borate buffer, pH 7.0, containing 150 mM NaCl and 1 mM EDTA), was freeze dried. The lyophilized material was extracted with ethanol/diethyl ether (3:1) for 16 hr at 4° and was subsequently centrifuged for 5 min at 2000 × *g*. The supernatant was aspirated, and the pellet was subjected to two similar extractions for 4 hr each. The final pellet was washed with ether, dried, and dissolved in 0.1 M sodium dodecyl sulfate. The dissolved pellet and the combined supernatants of the extractions were assayed for protein, phosphate (17), and radioactivity.

Incorporation of [^{3}H]cholesterol oleoyl ether into lactosylated LDL. The incorporation was carried out essentially as described by Blomhoff *et al.* (18). Twenty-five microcuries of [^{3}H]cholesteryl oleoyl ether, which was dissolved in 200 μl of acetone, were mixed with 2 ml of lipoprotein-deficient human serum (obtained by density gradient centrifugation; $d > 1.21$ g/ml), and the acetone was evaporated under a stream of nitrogen. After 20 min at room temperature, the mixture was shaken for 10 min at 37°. Then, 1 ml of a lactosylated LDL solution (1–2 mg of apolipoprotein/ml in phosphate-buffered saline) was added and the mixture was incubated for 5 hr at 37°. The labeled lactosylated LDL (specific radioactivity, approximately 20×10^6 dpm/mg of apoprotein) was subsequently purified by density gradient centrifugation. The preparations contained some loosely associated radioactivity. They were, therefore, screened by injecting 0.5 mg of the modified lipoprotein into rats that had been pretreated with 400 mg of *N*-acetylgalactosamine/kg of body weight. Total serum was collected 10 min later, and was used after dialysis against phosphate-buffered saline.

***In vivo* serum clearance and liver association.** Male Wistar rats, weighing between 225 and 325 g, were used. The animals were anaesthesized by intraperitoneal injection of 15–20 mg of sodium pentobarbital and the abdomen was opened. Radiolabeled lactosylated LDL was injected via the vena penis at a dose of 50 μg of apolipoprotein/kg of body weight. At the indicated times, blood samples of 0.2–0.3 ml were taken from the inferior vena cava and were collected in heparinized tubes. The samples were centrifuged for 2 min at 16,000 × *g*. Duplicate samples of plasma were assayed for radioactivity after precipitation of protein with 10% (w/v) trichloroacetic acid. The total amount of radioactivity in plasma was calculated using the equation: plasma volume (ml) = [0.0219 × body weight (g)] + 2.66. This relation between plasma volume and body weight was determined as described previously (19), using 17 rats with body weights ranging from 140 to 340 g.

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 radioactivity and weights of the liver samples and was corrected for the radioactivity in plasma assumed to be present in the tissue at the time of sampling (85 μl/g fresh weight; Ref. 20).

Isolation of liver cells. Rats were anaesthesized and injected with lactosylated LDL as described in the previous section. Ten minutes later, the vena porta was canulated and the liver was perfused with Ca^{2+}-free Hanks' balanced salt solution that contained 10 mM HEPES, pH 7.4 (8°), 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' solution that contained 10 mM HEPES, pH 7.4, and parenchymal and nonparenchymal cells were isolated as described previously (15). The nonparenchymal cell preparation was further fractionated into endothelial and Kupffer cells by centrifugal elutriation, as described in detail earlier (21). The contributions of the various cell types to the total liver uptake was calculated as described previously (21). As found earlier with other substrates (15, 21, 22), 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, subcellular fractions, cell suspensions, and solutions of LDL were determined by the method of Lowry *et al.* (23), with bovine serum albumin as the standard. The values found for LDL were multiplied by a factor of 0.82 to correct for the higher color yield of apolipoprotein B (24).

Results

Incorporation of lactose into LDL. Terminal D-galactosyl residues were incorporated into LDL by incubation of the lipoprotein with 100 mg/ml lactose (D-galactosyl-D-glucose) and 50 mg/ml sodium cyanoborohydride. The latter reduces the Schiff base between the glucose moiety of lactose and amino groups on LDL, which results in covalent attachment of lactose to LDL (25). The extent of incorporation was studied by measuring radioactivity in the lipoprotein after incubation with [^{14}C]lactose and sodium cyanoborohydride. It was found that 398 ± 40 mol of lactose (mean ± SE; three experiments) were incorporated per mol of LDL. Incubation for longer periods did not result in a higher degree of substitution.

An LDL particle consists of an apolipoprotein and a lipid moiety. To determine the site of incorporation of lactose, the protein and lipid moieties of [^{14}C]lactosylated LDL were separated by extraction with ethanol/ether. The results, shown in Table 1, indicate that lactose was predominantly associated with the apolipoprotein. The lipid fraction contained only a small amount of radioactivity, presumably due to contamination with protein. Because apolipoprotein B contains 355 lysine residues (26) and one lysine residue can react with two lactose

TABLE 1

Distribution of lactose over the apolipoprotein and the lipid moiety of lactosylated LDL

One milligram of LDL was lactosylated with [^{14}C]lactose (specific radioactivity, 0.1 μCi/mg) as described in Experimental Procedures. The apolipoprotein and lipid moieties were separated by extraction with ethanol/ether. The lipid and apolipoprotein fractions were assayed for protein, total phosphorus, and radioactivity. Values given, expressed as percentage of recovered amounts, are means ± standard errors of three separate experiments. Recoveries were phosphate, 104 ± 16%; protein, 129 ± 13%; and radioactivity, 99 ± 5%.

	Protein fraction	Lipid fraction
	% of recovered amount	
Phosphate	7 ± 1	93 ± 1
Protein	87 ± 4	13 ± 4
Radioactivity	94 ± 2	6 ± 2

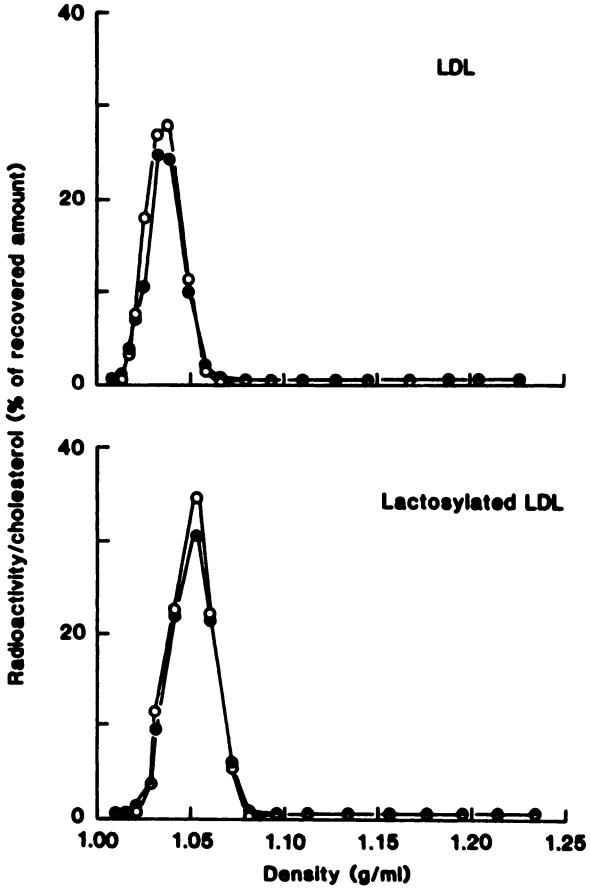

Fig. 1. Density gradient centrifugation of LDL and lactosylated LDL. Radioiodinated lactosylated LDL and native LDL (0.25 mg of apolipoprotein) were subjected to density gradient centrifugation as described by Redgrave *et al.* (14). The gradients (12 ml) were fractionated into fractions of approximately 0.7 ml. The fractions were assayed for radioactivity (●) and cholesterol (○), and their densities were measured. Recoveries of radioactivity and cholesterol were 94–105%.

molecules, it can be calculated that a minimum of 56% of the lysine residues of the apolipoprotein are substituted.

Lactosylated LDL was also analyzed by density gradient centrifugation. As shown in Fig. 1, the density of lactosylated LDL (peak density, 1.053 g/ml) was slightly different from that of unmodified LDL (peak density, 1.038 g/ml). Protein and cholesterol of lactosylated LDL were found at the same density, which indicates that lactosylation does not affect the integrity of the LDL particle.

Plasma clearance and liver uptake of lactosylated LDL. Native LDL disappears very slowly from the circulation of the rat (half-life, 5–10 hr), and only 2.1 ± 0.1% (mean ± SE of eight animals) of the injected dose is found in the liver at 10 min after injection of radiolabeled LDL. In sharp contrast, lactosylated ^{125}I-LDL was cleared from plasma very rapidly after intravenous injection into rats. Virtually all the injected material was cleared from the circulation within 10 min. The decrease in plasma radioactivity coincided with an increase in radioactivity associated with the liver. Radioactivity in liver was maximal at 10 min after injection and subsequently declined. The decrease in liver radioactivity was accompanied by an increase in the trichloroacetic acid-soluble radioactivity in liver and plasma (see Fig. 2 *insets*). These findings indicate that lactosylated LDL, once associated with the liver, is degraded to acid-soluble products that diffuse into the blood. The lag periods in the increase in acid-soluble radioactivity in plasma and liver suggest that lactosylated LDL has to be internalized before degradation takes place. To investigate the intracellular processing of lactosylated LDL, the liver was subjected to a subcellular fractionation (27). For these experiments, LDL was labeled with ^{125}I-tyramine-cellobiose, a label that after degradation of the protein is retained in cells (16). The distribution pattern of radioactivity closely resembles that of the lysosomal marker acid phosphatase (Fig. 3), whereas the microsomal marker glucose-6-phosphatase and the cytoplasmic marker lactate dehydrogenase show clearly different distributions.

The mechanism of liver association was determined by pretreating the animals with either *N*-acetylgalactosamine or *N*-acetylglucosamine. Pretreatment of rats with *N*-acetylgalactosamine (400 mg/kg of body weight) dramatically inhibited plasma clearance and liver uptake of lactosylated LDL, whereas pretreatment with the same dose of *N*-acetylglucosamine had no effect at all (see Fig. 4). This indicates that galactose-specific recognition sites in the liver are responsible for uptake.

Tissue distribution of lactosylated LDL. The results shown in the previous section point to a predominant role of the liver in the removal of lactosylated LDL from the circulation. To investigate possible specific uptake by other organs

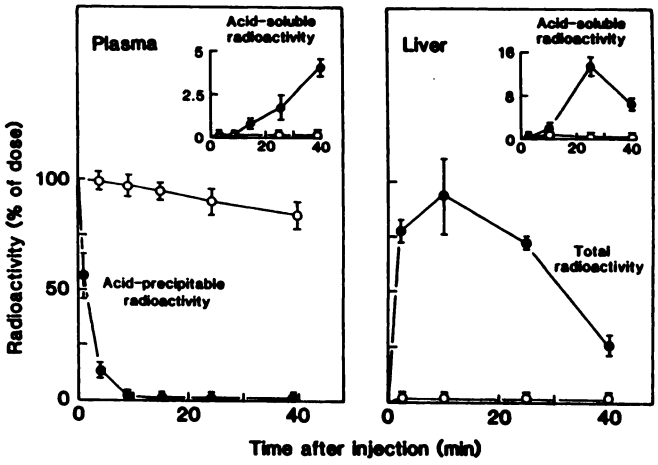

Fig. 2. Plasma clearance and liver uptake of lactosylated LDL. Rats were given injections of lactosylated ^{125}I-LDL (●) or native ^{125}I-LDL (○) at a dose of 50 μg of apolipoprotein/kg of body weight. At the indicated times, the amounts of radioactivity in plasma and liver were determined. *Left*, trichloroacetic acid-precipitable and acid-soluble (*inset*) radioactivities in plasma. *Right*, total and trichloroacetic acid-soluble (*inset*) radioactivities in liver. Values are means ± standard errors of three rats.

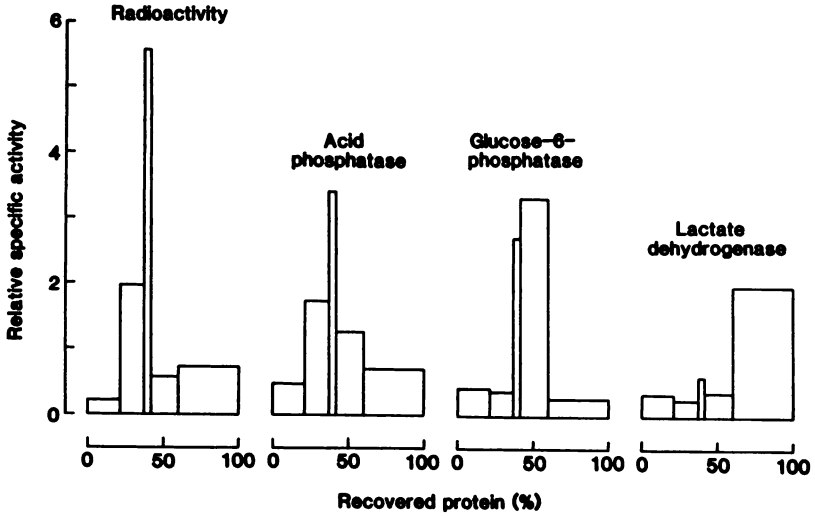

Fig. 3. Distribution patterns of radioactivity and marker enzymes over subcellular fractions of the liver after injection of radiolabeled lactosylated LDL. Rats were given injections of 50 μg of lactosylated LDL (labeled with ^{125}I-tyramine-cellobiose)/kg of body weight. Thirty minutes after injection, the liver was perfused with ice-cold 0.25 M sucrose, containing 10 mM Tris·HCl buffer, pH 7.5, and divided into subcellular fractions by differential centrifugation, as described earlier (27). The fractions were assayed for radioactivity, protein, and the activity of several marker enzymes (27); recoveries were 78–137%. *Bars* from *left* to *right* represent fractions in the order in which they were isolated: nuclear, mitochondrial, lysosomal, microsomal, and supernatant (cytosol) fractions. The relative protein concentration is given on the *abscissa*. The *ordinate* represents the relative specific activity (percentage of total recovered activity divided by percentage of total recovered protein).

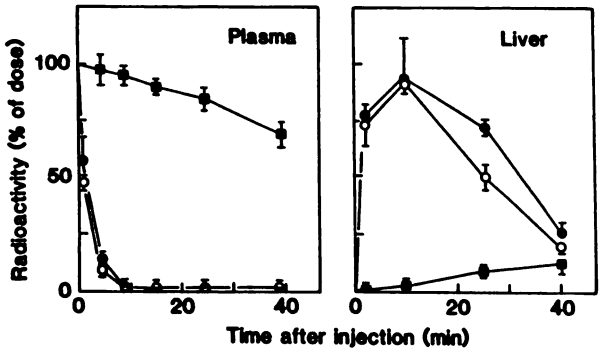

Fig. 4. Effects of *N*-acetylgalactosamine and *N*-acetylglucosamine on plasma clearance and liver uptake of lactosylated LDL. Rats were given injections of lactosylated ^{125}I-LDL (50 μg of apolipoprotein/kg of body weight). One minute before injection, the animals received 400 mg of *N*-acetylgalactosamine/kg of body weight (■), 400 mg of *N*-acetylglucosamine/kg of body weight (○), or solvent (phosphate-buffered saline) (●). At the indicated times, trichloroacetic acid-precipitable radioactivites in plasma and total radioactivities in liver were determined. Values are means ± standard errors of three rats.

and tissues, the distribution of lactosylated ^{125}I-LDL over a large number of tissues was determined at 10 min after injection. Table 2 shows that approximately 90% of the injected dose is recovered in the liver, with the remaining 10% evenly distributed throughout the body. The specific uptake by liver was found to be at least 1 order of magnitude higher than that in any other tissue.

Cellular distribution of lactosylated LDL in the liver. To identify the cell type(s) responsible for uptake in the liver, rats were injected with lactosylated ^{125}I-LDL and liver parenchymal, Kupffer, and endothelial cells were isolated from the liver 10 min later. The cell isolation procedure was performed at a low temperature (8°) to prevent degradation of the internalized ligand. The results are shown in Table 3. The Kupffer cells were by far the most active liver cells in the uptake of lactosylated LDL. The uptake by these cells, expressed per mg of cell protein, was approximately 70 and 7 times higher than uptake by parenchymal and endothelial cells, respectively. From the data on the uptake/mg of cell protein and the contributions of each cell type to the total liver protein, it was calculated that Kupffer cells, despite their small size and number, are responsible for most of the liver uptake.

In earlier studies it was shown that asialofetuin blocks uptake

TABLE 2

Tissue distribution of intravenously injected lactosylated LDL

Rats were given injections of lactosylated ^{125}I-LDL (50 μg of apolipoprotein/kg of body weight). At 10 min after injection, radioactivities in the indicated tissues and organs were determined (bone, skin, and muscle were dissolved in 10 M NaOH at 95°). The results are expressed as percentage of the recovered amount of radioactivity and as relative specific activity (percentage of recovered radioactivity divided by percentage of recovered weight). Recoveries of radioactivity and tissues were 107.6 ± 4.1% and 96.4 ± 0.6%, respectively. Values are means ± standard errors of three rats.

Tissue/organ	Radioactivity	Relative specific
	% of recovered dose	
Liver	89.0 ± 0.7	18.8 ± 1.1
Plasma, acid-soluble	<0.10	<0.10
Plasma, acid-precipitable	1.40 ± 0.20	0.41 ± 0.08
Adrenals	<0.10	0.94 ± 0.27
Bladder + urine	<0.10	<0.10
Blood cells	<0.10	<0.10
Bone	1.71 ± 0.16	<0.10
Bone marrow		0.12 ± 0.12
Brain	<0.10	<0.10
Fat		<0.10
Heart	<0.10	0.20 ± 0.04
Intestine, large	0.23 ± 0.01	<0.10
Intestine, small	0.70 ± 0.07	0.20 ± 0.01
Kidneys	0.43 ± 0.07	0.49 ± 0.06
Lungs	0.23 ± 0.03	0.54 ± 0.09
Lymph nodes		0.16 ± 0.06
Muscles	2.83 ± 0.16	<0.10
Pancreas	0.17 ± 0.06	0.40 ± 0.11
Penis (site of injection)	0.46 ± 0.28	4.18 ± 2.51
Reproductive organs	0.13 ± 0.02	<0.10
Skin	2.02 ± 0.43	0.10 ± 0.02
Spleen	0.12 ± 0.02	0.48 ± 0.07
Stomach + contents	0.39 ± 0.12	0.30 ± 0.06
Thyroid	<0.10	<0.10

via the galactose-specific receptors on parenchymal liver cells but not galactose-mediated uptake by Kupffer cells (28). In the experiments summarized in Fig. 5, we studied the effect of preinjection with asialofetuin on the intrahepatic distribution of lactosylated LDL. Preinjection with 50 mg of asialofetuin/kg of body weight only slightly reduced plasma clearance and liver uptake of lactosylated LDL. At 10 min after injection, liver and plasma contained 80.2 ± 3.0 and 13.8 ± 3.5% of the recovered dose, respectively (means ± SE of three rats). Cell isolation experiments indicated that the preinjection greatly reduces uptake by parenchymal cells. However, the lower uptake by parenchymal cells is compensated for by a higher uptake

TABLE 3

Uptake of lactosylated LDL by various liver cell types

Rats were given injections of lactosylated ^{125}I-LDL (50 μg of apolipoprotein/kg of body weight). Ten minutes later, parenchymal, endothelial, and Kupffer cells were isolated, and the association of radioactivity to each cell type was determined. Uptake by each cell type is expressed as ng of lactosylated LDL associated/mg of cell protein and as the relative contribution to the total liver uptake. The latter values were calculated from the uptake per mg of cell protein and the contribution of each cell type to the total liver protein (21). Values are means ± standard errors of three rats.

Cell type	Uptake of lactosylated LDL	
	ng/mg of cell protein	*% of total liver uptake*
Parenchymal cells	2.0 ± 0.3	31.8 ± 4.9
Kupffer cells	135.4 ± 29.6	57.1 ± 1.9
Endothelial cells	20.7 ± 7.3	11.1 ± 3.2

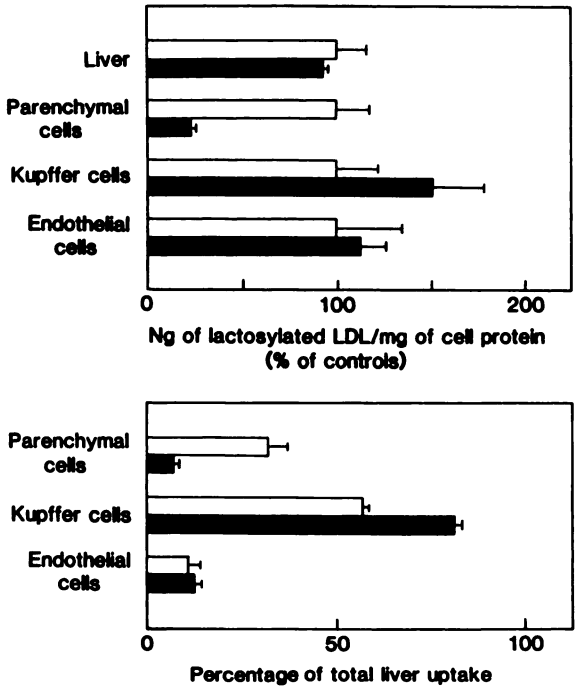

Fig. 5. Effect of asialofetuin on uptake of lactosylated LDL by various liver cell types. Rats were given injections of lactosylated ^{125}I-LDL at a dose of 50 μg of apolipoprotein/kg of body weight. One minute before injection, the animals received 50 mg of asialofetuin/kg of body weight (■). Controls (□) were not pretreated. Liver cells were isolated at 10 min after injection, and the association of radioactivity to each cell type was determined. *Upper*, the amounts of lactosylated LDL associated per mg of cell protein, as percentage of the controls. *Lower*, the contribution of each cell type to the total liver uptake (calculated as described in the legend to Table 3). Values are means ± standard error of three experiments.

by Kupffer cells, and under these conditions Kupffer cells contained about 80% of the total amount of radioactivity in the liver.

Plasma clearance and liver uptake of [^{3}H]cholesteryl oleoyl ether-labeled lactosylated LDL. In the previous sections, the fate of lactosylated LDL was followed by monitoring the radioactivity of the ^{125}I-labeled apolipoprotein. As outlined in the introduction, the lipid moiety of lactosylated LDL is crucial for its putative role as drug carrier. In order to follow the lipid core of LDL, we incorporated [^{3}H]cholesteryl oleolyl ether into the particle and monitored radioactivity after intravenous injection. The results, which are shown in Fig. 6, indicate that the radioactivity is rapidly cleared from plasma by the liver. Preinjection with 400 mg of *N*-acetylgalactosamine/kg of body weight completely blocked liver uptake, whereas the same dose of *N*-acetylglucosamine had no effect at all. The intrahepatic distribution of [^{3}H]cholesteryl oleoyl ether-labeled lactosylated LDL was studied at 10 min after injection. Kupffer, parenchymal, and endothelial cells contained 66.6 ± 4.0, 25.1 ± 7.6, and 8.2 ± 3.6% of the total liver radioactivity, respectively (means ± SE of three experiments). This distribution is very similar to the distribution found after injection of ^{125}I-labeled lactosylated LDL.

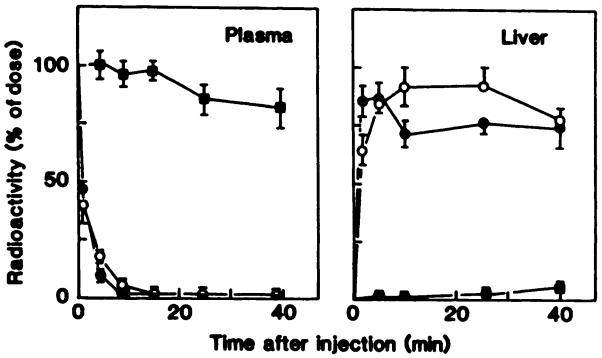

Fig. 6. Plasma clearance and liver uptake of [^{3}H]cholesteryl oleoyl ether-labeled lactosylated LDL: effects of *N*-acetylgalactosamine and *N*-acetylglucosamine. Rats were given injections of [^{3}H]cholesteryl oleoyl ether-labeled lactosylated LDL (50 μg of apolipoprotein/kg of body weight). One minute before injection, the animals received 400 mg of *N*-acetylgalactosamine/kg of body weight (■), 400 mg of *N*-acetylglucosamine/kg of body weight (○), or solvent (phosphate-buffered saline) (●). At the indicated times, radioactivities in plasma and liver were determined. Values are means ± standard errors of three rats.

Discussion

Our data clearly demonstrate that lactosylated LDL (398 ± 40 mol of lactose/mol of LDL) is extremely rapidly cleared from the circulation of the rat after intravenous injection. The rapid plasma clearance of lactosylated LDL was found to be due to galactose-specific uptake by the liver. This organ contained, at 10 min after injection, approximately 90% of the injected dose. The remaining 10% was evenly distributed over a variety of tissues.

The specific uptake by liver was 10–100 times higher than that by any other tissue. In the liver, Kupffer cells are mainly responsible for the uptake of lactosylated LDL. These cells internalize a 70- and 7-fold higher amount of lactosylated LDL, per mg of cell protein, than parenchymal and endothelial cells, respectively. After galactose-specific uptake in parenchymal liver cells was blocked by asialofetuin, the relative concentration of lactosylated LDL in Kupffer cells is even higher.

The protein and cholesterol of lactosylated LDL show identical density distributions after density gradient centrifugation. Clearance and hepatic uptake of the lipid moiety of lactosylated LDL, labeled with [^{3}H]cholesteryl oleoyl ether, were similar to those of the ^{125}I-labeled apolipoprotein. Thus, the integrity of the LDL particle is not affected by lactosylation, and it is taken up as a unit. This indicates that effective transport of core-associated drugs is assured.

In a previously reported study (15), galactose-specific uptake of LDL by Kupffer cells was induced by the incorporation of a galactose-containing cholesterol derivative (tris-galactose-cholesterol) into the lipid moiety of the lipoprotein. The complex of LDL with tris-galactose-cholesterol seems, nonetheless, to

be less suitable as a drug carrier. Only 60% of the injected dose could be directed in a galactose-specific way to the Kupffer cells, and 40% of the dose remained in the circulation. Furthermore, loading of the lipid moiety of LDL with tris-galactose-cholesterol might hamper the capacity of the lipid core to accommodate lipophilic drugs. In the case of lactosylated LDL, complete removal of the particle from the circulation is achieved. In addition, the galactose residues are attached to the apolipoprotein, which leaves the capacity of the core to incorporate drugs unaffected.

In earlier studies, Spanjer *et al.* (8, 9) incorporated galactose-containing compounds, like tris-galactose-cholesterol and lactosylceramide, into the phospholipid bilayer of small unilamellar liposomes (mean diameter, 25–35 nm). The incorporation of these galactose structures into the liposomes induced an increased uptake by Kupffer cells. However, the galactose-containing liposomes were cleared much more slowly (half-lives, >10 min) from the circulation than lactosylated LDL, and the galactose-specific uptake by Kupffer cells was lower. The shorter plasma half-life of lactosylated LDL is probably advantageous, because it forestalls possible leakage of incorporated drugs from the vehicle. On the other hand, the additional capacity of liposomes to transport hydrophilic drugs in their aqueous core potentially enables a more widespread use of these vesicles for the presently available drugs (29–31).

In conclusion, it appears that lactosylated LDL might be suitable for the specific delivery of drugs to Kupffer cells. Some highly lipophilic drugs incorporate spontaneously into LDL (11). Other more hydrophilic drugs should, however, be rendered more lipophilic by the coupling of fatty acyl chains (12). Lactosylated LDL could be particularly useful for the introduction into Kuppfer cells of drugs that modulate the immunological activity of these cells. For instance, activation of Kupffer cells to a tumoricidal state might prevent the development of liver metastases, a serious clinical problem (32). Lipophilic derivatives of the immunomodulator muramyl dipeptide (12, 33, 34) seem very suitable for this purpose. Preliminary experiments in our laboratory[2] indicate that such compounds do indeed incorporate into lactosylated LDL and render Kupffer cells tumoricidal.

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[2] Y. de Rijke *et al.*, unpublished results.