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Development of Lipoprotein-like Lipid Particles for Drug Targeting: Neo-High Density Lipoproteins

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SUMMARY

The possibility was explored of synthesizing, from commercially available lipids, high density lipoprotein (HDL)-like particles (neo-HDL) with the same physico-chemical and biological properties as native HDL. A preparation method involving egg yolk phosphatidylcholine, cholesterol, and apoproteins from HDL led to the formation of particles with a composition, size, electrophoretic mobility, and density similar to those of discoidal HDL. *In vitro* experiments with isolated parenchymal liver cells showed that unlabeled HDL and neo-HDL competed for the same high affinity binding sites as did radiolabeled neo-HDL, whereas an excess of unlabeled low density lipoprotein was ineffective. *In vivo* experiments with radio-labeled neo-HDL indicated that neo-HDL showed a slow decay upon injection into rats, whereas the liver uptake did not exceed >10% of the injected dose. The small additional liver uptake of radioactivity from neo-HDL, compared with HDL, was due to enhanced uptake by endothelial and Kupffer cells. Lactosylation of neo-HDL led to a markedly increased decay rate and a rapid uptake by rat liver (80% in 10 min). Parenchymal cells accounted for >90% of the total liver uptake of radiolabeled lactosylated neo-HDL. Because the liver uptake of lactosylated ^{125}I-neo-HDL could be blocked by preinjection of *N*-acetylgalactosamine, we conclude that the asialoglycoprotein receptor, which is specifically localized on parenchymal liver cells, is responsible for the avid liver uptake. With a fibroblast cell line transfected with the human asialoglycoprotein receptor, it was found that lactosylated neo-HDL binds with high affinity (K_d, 40 nM), in a galactose-specific way. It can be concluded that, with commercially available lipid components, HDL-like particles (neo-HDL) with virtually the same characteristics as found for native apolipoprotein E-free HDL can be reconstituted. Lactosylated neo-HDL, which is rapidly taken up by galactose-specific receptors on parenchymal liver cells, might be used to transport antiviral drugs specifically to parenchymal liver cells.

The therapeutic efficacy of drugs is often diminished by their inability to reach the site of action at an accurate dosage. Therefore, targeting of macromolecular drug conjugates to a specific cell type, leading to improvement of the therapeutic index, is an important challenge. A number of soluble molecules and particulate systems have been proposed as carriers (for review, see Ref. 1). An interesting recent development is the potential use of endogenous biological carrier systems, such as lipoproteins (2–4). These endogenous particles do not trigger immunological reactions, are completely biodegradable, and are not rapidly cleared by the reticuloendothelial system. Furthermore, the biological fate of lipoproteins has been investigated extensively. Until now the main emphasis has been on the potential use of LDL. Recently we suggested that HDL may also be used, because it can be specifically targeted to rat liver parenchymal cells after lactosylation of the protein moiety (5). This lactosylated HDL is recognized by galactose receptors present on parenchymal cells. The binding of a wide range of galactose-mediated ligands to isolated Kupffer and parenchymal cells was studied in our laboratory (6). It was found that for efficient uptake by Kupffer cells a minimum size of 12 nm is required, whereas parenchymal cells internalize ligands of all sizes. Lactosylated HDL appeared to be too small for uptake via the galactose receptors on Kupffer cells. It is, however, rapidly taken up by parenchymal cells in a galactose-specific manner. This rapid uptake of lactosylated HDL by galactose-specific receptors on parenchymal liver cells may be useful for transporting lipophilic drugs (i.e., antiviral drugs), in a pharmaceutical formulation, more specifically to parenchymal liver cells.

Possible large-scale pharmaceutical application of lipoproteins as drug carriers is hampered by their limited availability. It might be possible, however, to prepare particles with physicochemical properties similar to those of HDL by association of apolipoproteins with microemulsions of commercially available lipids, so called neo-HDL (7, 8).

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ABBREVIATIONS: LDL, low density lipoprotein; HDL, high density lipoprotein; DMEM, Dulbecco's modified Eagle medium; BSA, bovine serum albumin; apoE, apolipoprotein E.

In the present paper we evaluated the biological properties of these neo-HDL particles. Furthermore, the effect of lactosylation of the apolipoproteins on the fate of the neo-HDL was studied, to test whether selective interaction with the asialoglycoprotein receptors on liver parenchymal cells could be achieved.

Experimental Procedures

Materials. Lactose was supplied by Merck (Darmstadt, Germany), sodium cyanoborohydride was from Aldrich (Brussels, Belgium), and Nycodenz was obtained from Nycomed (Oslo, Norway). Collagenase type I and type IV, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, and BSA (fraction V) were purchased from Sigma Chemical Co. (St. Louis, MO). $Na^{125}I$ (carrier-free) in NaOH and ^{14}C-labeled egg yolk phosphatidylcholine were purchased from Amersham International (Amersham, Bucks, UK). Pronase and fetal calf serum were obtained from Boehringer (Mannheim, Germany), and DMEM was from Flow Laboratories (Irvin, Scotland). All other chemicals were analytical grade.

Preparation and characterization of neo-HDL. Neo-HDL was prepared according to the method described by Pittman *et al.* (7), with some modifications. Three milligrams of (^{14}C-labeled) egg yolk phosphatidylcholine and 0.9 mg of cholesterol, dissolved in $CHCl_3$, were mixed in a 20-ml glass scintillation counting vial and the solvent was evaporated under a stream of nitrogen. Ten milliliters of sonication buffer (10 mM Tris·HCl buffer, pH 8.0, containing 0.1 M KCl, 1 mM EDTA, and 0.025% NaN_3), degassed and saturated with nitrogen, were added and the contents of the vial were sonicated with a Macrotip (14-μm output) under a stream of nitrogen. The temperature was maintained at 49–52°. The sonication was stopped after 60 min, and the temperature was lowered to 42–44°. Sonication was continued and 20 mg of apolipoproteins-HDL, dissolved in 1 ml of 4 M urea, were added in 10 equal portions over a period of 10 min. After all protein was added, sonication was continued for 30 min. The sonication mixture was then centrifuged at 500 × *g* for 5 min to remove large particles. The preparation was then subjected to density gradient ultracentrifugation, as described by Redgrave *et al.* (9). Particles in the density range of 1.06–1.13 g/ml were pooled, concentrated, and applied to a Superose 6 column (60 × 1.8 cm), using the Pharmacia fast protein liquid chromatography system. Fractions were assayed for the presence of egg yolk phosphatidylcholine and protein by measuring radioactivity and absorbance at 280 nm, respectively. Fractions containing neo-HDL, indicated by the presence of both phospholipid and protein, were pooled and concentrated. The preparations were stored at 4° and used within 3 weeks. Neo-HDL was characterized by its chemical composition, electrophoretic mobility, and size.

Protein was measured by the method of Lowry *et al.* (10), using BSA as a standard. The electrophoretic mobility was determined by agarose gel electrophoresis, according to the method of Demacker (11).

The size of the preparations was determined by nondenaturing gradient gel electrophoresis (12) using calibration proteins with known particle sizes (High Molecular Weight calibration kit; Pharmacia) and by laser light-scattering techniques using a Malvern 4700C submicron particle
analyzer.

Preparation and labeling of lipoproteins and neo-lipoproteins. Human HDL and LDL were isolated from plasma by density gradient ultracentrifugation techniques (9). The HDL was passed over a Sepharose-heparin column (13) and the apoE-free fraction was used for experiments. HDL apoproteins were isolated by delipidation with diethylether/ethanol (1:3), dried, and stored under nitrogen at −20°. All ^{125}I-labeled preparations used in this study were prepared by the ^{125}ICl method of McFarlane (14), as modified by Bilheimer *et al.* (15).

In the HDL and neo-HDL preparations 96.6 ± 0.66% of the radioactivity was associated with the apolipoproteins, 1.6 ± 0.2% was trichloroacetic acid soluble, and 1.8 ± 0.2% of the radioactivity was present in lipids. The distribution of label over the apolipoproteins was determined after separation of the apolipoproteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, using 5–22.5% gels. The gels were stained, dried, cut into pieces, and counted for radioactivity. In native HDL the distribution of the ^{125}I-label between the apolipoproteins was 44 ± 2% in apolipoprotein A-I, 44 ± 3% in apolipoprotein A-II, and 6 ± 1% in apolipoproteins C. For neo-HDL these values were 34.7 ± 0.2%, 50.4 ± 1.5%, and 13.4 ± 3.7%, respectively.

Lactosylation of HDL and neo-HDL. HDL or neo-HDL (0.5 mg, dispersed in 1 ml of 20 mM sodium phosphate buffer, pH 7.0, containing 1 mM EDTA) was incubated at 37° with lactose and sodium cyanoborohydride, at final concentrations of 100 mg/ml and 50 mg/ml, respectively. After 48 hr, the reaction was stopped by the addition of 0.5 ml of 0.1 M NH_4HCO_3. Sodium cyanoborohydride and unbound lactose were removed by exhaustive dialysis against phosphate-buffered saline. The preparations were maximally substituted with galactose residues. The apoproteins in the preparations contained approximately 0.25 mg of lactose/mg of protein.

***In vivo* serum clearance and liver association.** Male Wistar rats (approximately 250 g) were anesthetized by intraperitoneal injection of approximately 30 mg of sodium pentobarbital. The abdomen was opened and 100 μg of radiolabeled ligand were injected into the inferior vena cava at the level of the renal veins. Blood sampling and excision of liver lobules were performed at the indicated times as described before (16).

Determination of association with liver cells. Rats were anesthetized and given injections of the radiolabeled ligand. Parenchymal, endothelial, and Kupffer cells were separated by collagenase perfusion (collagenase type I), followed by differential centrifugation and counterflow centrifugal elutriation at 4°, as described in detail elsewhere (17). The contributions of the different liver cell types to the total hepatic uptake of the injected ligands were calculated with the assumption that parenchymal, endothelial, and Kupffer cells account for 92.5, 3.3, and 2.5%, respectively, of the total liver protein mass (18).

***In vitro* studies with freshly isolated liver parenchymal cells.** For *in vitro* studies, parenchymal cells were isolated by perfusion of the liver with 0.05% collagenase (type IV) at 37°, as described previously (18). The parenchymal cells obtained were ≥95% viable, as judged by 0.2% trypan blue exclusion, and ≥99% pure, as judged by light microscopy. The cells were resuspended in DMEM supplemented with 2% BSA, pH 7.4. Aliquots of 0.5 ml of the cell suspensions, containing 1–2 mg of cell protein, were incubated with radiolabeled ligand and competitors at the indicated concentrations and for the indicated times. Incubations were carried out in plastic containers (8.5 ml; Kartell) with continuous shaking (150 rpm). Every 30 min, the air was saturated with oxygen by flushing with Carbogen (95% O_2/5% CO_2). After incubation, the cells were centrifuged at 50 × *g* for 1 min at 4° and washed two times with wash buffer (50 mM Tris·HCl, pH 7.4, containing 0.9% NaCl, 1 mM EDTA, 5 mM $CaCl_2$, and 0.2% BSA), followed by two washes with wash buffer without BSA. Finally, cells were lysed in 1 ml of 0.1 N NaOH and their radioactivity and protein content were determined.

***In vitro* studies with cultured 1-7-1 cells.** Transfected murine NIH 3T3 fibroblasts expressing the human asialoglycoprotein receptor (1-7-1 cell line) (19) were a kind gift of Dr. M. A. Shia, Boston University Medical Center, Boston, MA. The cells were cultured at 37° in 24-well dishes containing DMEM supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 3.5% glucose. Experiments were performed when the cells were confluent. Thirty minutes before the experiments were started, the medium was replaced with DMEM containing 1% BSA. Lactosylated HDL and lactosylated neo-HDL, dissolved in DMEM containing 1% BSA and 3.5% glucose, were added and binding of the ligands to the cell was then determined as described before for human parenchymal liver cells *in vitro* (20).

Results

Synthesis and characterization of neo-HDL. A preparation of lipid vesicles was obtained by co-sonication of cholesterol and phospholipids. The proportions of these components were based upon potential formation of discoidal HDL (21). During co-sonication of cholesterol and phospholipids, we monitored the size of the particles with laser light-scattering techniques. After 45 min, the particle size was decreased from an initial diameter of 180 nm to 23 nm. Further sonication had no effect on the particle size. After addition of the apolipoproteins, the sonication was continued for another 30 min. After this sonication the apolipoproteins were still intact and no degradation products of the apoproteins could be detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (data not shown).

The preparation was then subjected to density gradient ultracentrifugation (9). Particles in the density range of 1.06–1.13 g/ml were collected (70–80% of the phospholipids and cholesterol and 35% of the protein could be recovered in this density range), applied to a Superose 6 column (60 × 1.8 cm), and eluted with 0.15 M NaCl, pH 7.2, containing 0.02% NaN_3 and 0.01% EDTA. Elution of phospholipid was monitored by measuring the radioactivity of [^{14}C]phosphatidylcholine present in the preparation, and elution of proteins was monitored by measuring absorbance at 280 nm. The phosphatidylcholine tracer eluted with part of the apolipoprotein moiety, separate from free apoproteins.

The composition of the particle in the major peak (80–95 ml) eluted from the gel filtration column was 40.4 ± 8.2% phospholipids, 9.8 ± 4.6% cholesterol, and 49.8 ± 0.2% protein (w/w/w).

The size distribution of neo-HDL was determined by two unrelated methods. The nondenaturing gel electrophoresis method (12) showed neo-HDL particles with an apparent Stokes radius of 8.8 ± 0.7 nm, whereas apoE-free HDL particles possess an apparent Stokes radius of 9.5 ± 0.3 nm. Particle size determination by photon correlation spectroscopy indicated a particle size of 12.5 ± 1.5 nm for neo-HDL and of 9.5 ± 4.5 nm for apoE-free human HDL.

The electrophoretic mobility of neo-HDL was investigated using agarose gel electrophoresis. Fig. 1 shows that neo-HDL has a mobility comparable to that of apoE-free HDL and clearly distinct from that of LDL. The recovery for LDL, HDL, and neo-HDL was 80%, 95%, and 81%, respectively.

***In vivo* characteristics of ^{125}I-neo-HDL, compared with human apoE-free ^{125}I-HDL.** To investigate the *in vivo* fate of neo-HDL, rats were injected with ^{125}I-labeled neo-HDL. Fig. 2 shows that the clearance of radioactivity from the serum was relatively slow and at 60 min after injection 68 ± 8% of the injected dose was still present in the blood compartment. At 10 min after injection 9.0 ± 4.0% of the label was found to be associated with the liver. This amount decreased slowly and after 60 min 3.8 ± 1.0% of the radioactivity remained associated with the liver. For comparison, the decay and liver uptake of ^{125}I-HDL are also shown in Fig. 2 (from Ref. 22). Ten minutes after injection 2.3 ± 0.1% of the label was associated with the liver, and this percentage remained relatively constant up to 60 min after injection.

The tissue distribution of ^{125}I-neo-HDL was studied at 10 min after injection. Table 1 shows that 83.2 ± 5.1% of the injected dose was still present in serum. The liver contained

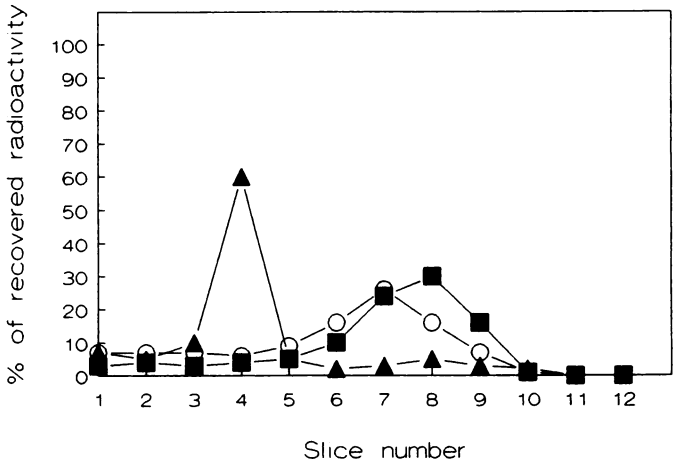

Fig. 1. Electrophoretic mobility of neo-HDL in comparison with the mobility of native HDL and LDL. To investigate the electrophoretic mobility of neo-HDL, ^{125}I-neo-HDL (○), ^{125}I-HDL (■), and ^{125}I-LDL (▲) were subjected to agarose gel electrophoresis. The gel was cut into slices of 1 cm, and the radioactivity was measured and plotted as a function of the distance from the starting point (slice 1).

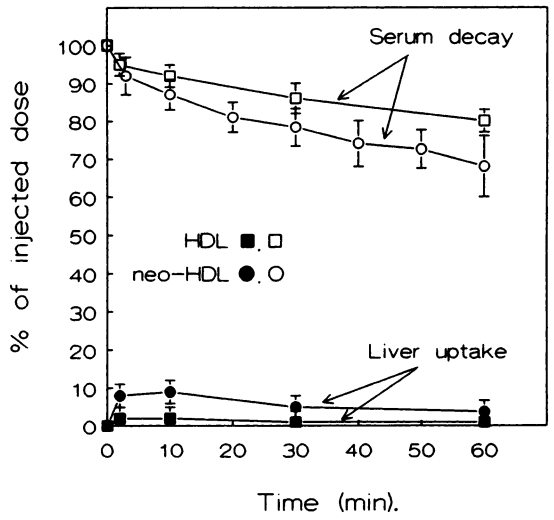

Fig. 2. Plasma clearance and liver uptake of ^{125}I-neo-HDL and ^{125}I-HDL. Rats were injected with radiolabeled neo-HDL (○, ●) or radiolabeled HDL (□, ■), both at a dose of 50 μg of protein/kg of body weight. At the indicated times, radioactivities in serum and liver were determined. The radioactivity in the liver was corrected for the activity present in the blood. Values are means ± standard errors for three rats.

9.0 ± 4.0% of the dose, and 4.7 ± 0.6% was recovered in the skin. Only small amounts of radioactivity were found in other tissues. These results are comparable to those found for ^{125}I-HDL.

To investigate the relative contributions of the different liver cell types to the liver association of neo-HDL, we isolated the various liver cell types at 10 min after injection of ^{125}I-neo-HDL. The cell isolation procedure was performed at low temperature (8°) to prevent degradation of the associated ligand. In Table 2, the radioactivity of ^{125}I-neo-HDL in the liver cell types is compared with that of ^{125}I-HDL. It appears that the Kupffer and endothelial cells are mainly responsible for the higher hepatic uptake of ^{125}I-neo-HDL (9.0 ± 4.0% of the dose versus 2.3 ± 0.1% for ^{125}I-HDL). To determine whether impurities or denatured proteins in the ^{125}I-neo-HDL preparation may influence the liver uptake and relative cellular distribution,

TABLE 1

Tissue distribution of ^{125}I-HDL and ^{125}I-neo-HDL at 10 min after injection into rats

Rats were injected with radiolabeled neo-HDL or radiolabeled HDL (50 μg of protein/kg of body weight). At 10 min after injection, radioactivities in the indicated tissues and organs were determined. The results are expressed as percentage of the recovered dose. Values are means ± standard errors for three rats or means for two rats.

Tissue/organ	Radioactivity	
	ApoE-free HDL	Neo-HDL
	% of dose	
Liver	2.3	9.0 ± 4.0
Serum	91.6	83.2 ± 5.1
Muscle	1.8	1.7 ± 0.9
Skin	3.1	4.7 ± 0.6
Small intestine	0.6	1.0 ± 0.3
Spleen	0.3	0.3 ± 0.1
Adrenal gland	0.3	0.1 ± 0.1

TABLE 2

Association of intravenously injected ^{125}I-neo-HDL and ^{125}I-HDL with liver cell types

Rats were injected with ^{125}I-HDL or ^{125}I-neo-HDL at a dose of 50 μg/kg of body weight. Ten minutes after injection, parenchymal, endothelial, and Kupffer cells were isolated, and the association of radioactivity with each cell type was determined. The association is expressed as percentage of the injected dose/liver cell type, and values are means ± standard errors of three determinations or means of two experiments.

Cell type	Association	
	Neo-HDL	ApoE-free HDL
	% of the injected dose/liver cell type	
Parenchymal cells	2.7	1.74 ± 0.09
Endothelial cells	4.2	0.29 ± 0.04
Kupffer cells	2.1	0.30 ± 0.01

we injected iodinated neo-HDL into rats and let it circulate for 10 min, isolated the serum, and injected it directly into a second rat. At 10 min after injection it was found that the distribution of this "biologically screened" ^{125}I-neo-HDL over the cell types in the liver was completely comparable to the liver cell distribution of ^{125}I-apoE-free HDL (data not shown).

***In vitro* association of ^{125}I-neo-HDL with rat liver parenchymal cells, competition by unlabeled lipoproteins and neo-lipoproteins, and effect of lysosomotropic agents.** To investigate the specificity of the interaction of neo-HDL with parenchymal liver cells, we studied *in vitro* the effects of various amounts of unlabeled neo-HDL, LDL, or HDL on the association of ^{125}I-labeled neo-HDL with parenchymal liver cells. The cells were incubated for 10 min at 37° with 5 μg/ml ^{125}I-neo-HDL in the presence or absence of competitors. A 10-min incubation period was chosen to minimize possible exchange of apolipoproteins. The experiment was performed at 37° instead of a low temperature because the interaction of HDL with cells is temperature dependent (23). In Fig. 3 it is shown that an excess of unlabeled neo-HDL as well as an excess of unlabeled HDL decreased the amount of cell-associated radioactivity by 80%, whereas an excess of unlabeled LDL did not inhibit cell association of labeled neo-HDL appreciably.

Chloroquine and NH_4Cl are unrelated compounds that can inhibit the lysosomal degradation of internalized proteins (24) by raising the pH inside the lysosomes. As with apoE-free ^{125}I-HDL, specific degradation and association of ^{125}I-neo-HDL were not significantly affected by these lysosomotropic agents (data not shown).

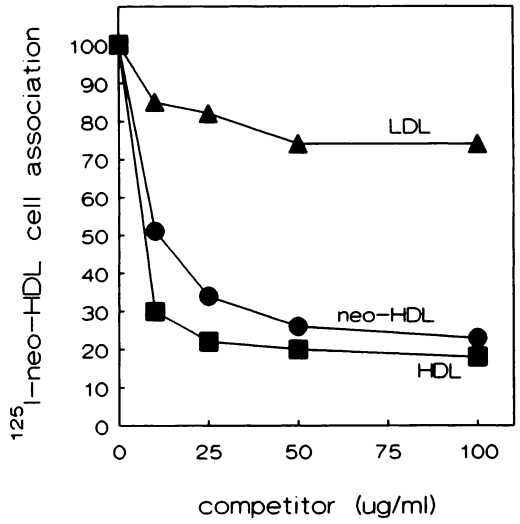

Fig. 3. Effects of unlabeled lipoproteins and neo-lipoproteins on the association of ^{125}I-neo-HDL with isolated rat liver parenchymal cells. Cells were incubated for 10 min at 37° with 5 μg/ml radiolabeled neo-HDL, in the presence of the indicated amounts of unlabeled neo-HDL (●), HDL (■), or LDL (▲). The association with the cell is expressed as a percentage of control (radioactivity associated in the absence of unlabeled lipoprotein or neo-lipoprotein). The values are given as the means of two experiments.

Serum decay and liver association of lactosylated neo-HDL. In a recent study, HDL was provided with terminal galactose residues by means of reductive lactosamination (5). The lactosylated HDL was very rapidly and highly specifically taken up by galactose receptors on parenchymal liver cells. To investigate whether neo-HDL could be directed to liver parenchymal cells, neo-HDL was reductively lactosaminated and then injected into rats. The serum decay of lactosylated ^{125}I-neo-HDL was very fast (Fig. 4A). Five minutes after injection only 13% of the radioactivity was still present in serum. Ten minutes after injection 63.9 ± 6.1% of the injected dose was associated with the liver (Fig. 4B). To investigate whether galactose-specific recognition sites are responsible for the uptake of ^{125}I-labeled lactosylated neo-HDL, rats were preinjected with 50 mg/kg *N*-acetylgalactosamine 1 min before ^{125}I-labeled lactosylated neo-HDL. Preinjection with *N*-acetylgalactosamine inhibited the hepatic association of lactosylated ^{125}I-neo-HDL considerably. At 10 min after injection only 25.2% of the injected dose was found in the liver. For lactosylated ^{125}I-HDL this value was 25.7 ± 0.7%. Controls that had been preinjected with phosphate-buffered saline or preinjected with the same dose of *N*-acetylglucosamine showed no significant effect on the serum decay or liver association of lactosylated ^{125}I-neo-HDL (data not shown).

Cellular distribution of lactosylated ^{125}I-neo-HDL in rat liver. To quantify the relative importance of the liver endothelial, Kupffer, and parenchymal cells in the hepatic association of lactosylated ^{125}I-neo-HDL, the various liver cell types were isolated at 10 min after injection and the association of radioactivity with each cell type was determined. Taking into account the contribution of each cell type to the total amount of protein in the liver, it was calculated that lactosylated ^{125}I-neo-HDL was predominantly (93.5%) associated with the parenchymal cells. Endothelial and Kupffer cells contained only small amounts of radioactivity. These values are very similar to those found for lactosylated ^{125}I-HDL (Table 3).

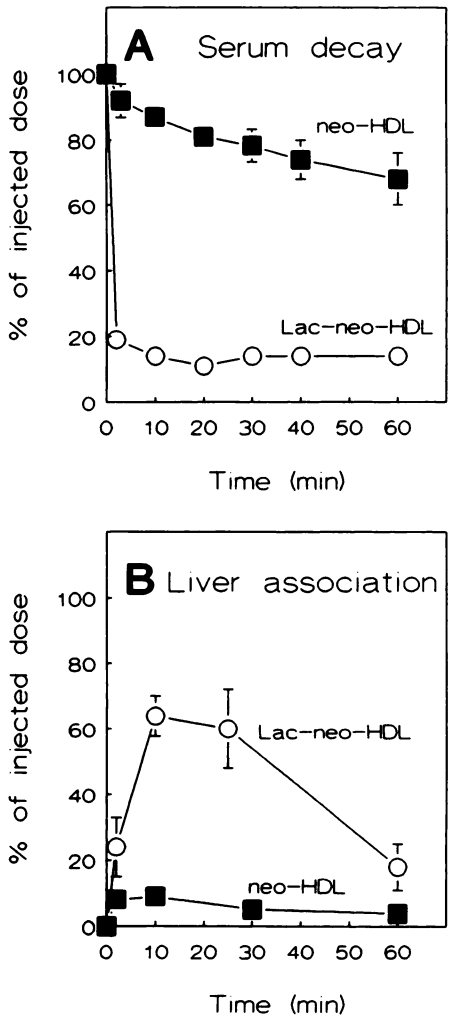

Fig. 4. Serum clearance and liver uptake of lactosylated ^{125}I-neo-HDL and ^{125}I-neo-HDL. Rats were injected with radiolabeled lactosylated neo-HDL or radiolabeled neo-HDL, both at a dose of 50 μg of protein/kg of body weight. At the indicated times, radioactivities in serum and liver were determined. A, Serum decay of ^{125}I-neo-HDL (■) and lactosylated ^{125}I-neo-HDL (○). B, Liver uptake, corrected for blood values, of ^{125}I-neo-HDL (■) and lactosylated ^{125}I-neo-HDL (○). Values are means ± standard errors for three rats.

TABLE 3

Uptake of intravenously injected lactosylated ^{125}I-neo-HDL and lactosylated ^{125}I-HDL by liver cell types

Rats were injected with lactosylated ^{125}I-HDL or lactosylated ^{125}I-neo-HDL at a dose of 50 μg/kg of body weight. Ten minutes after injection, parenchymal, endothelial, and Kupffer cells were isolated, and the association of radioactivity with each cell type was determined. The amounts of radioactivity per mg of cell protein in the isolated cell fractions were multiplied by the amount of protein that each cell type contributes to the total liver protein mass. The values are expressed as percentage of the total liver uptake of lactosylated ligand and are means ± standard errors of three experiments or means of two experiments.

Cell type	Liver uptake	
	Lactosylated neo-HDL	Lactosylated apoE-free HDL
	% of total	
Parenchymal cells	93.5	98.1 ± 0.6
Endothelial cells	5.2	0.9 ± 0.8
Kupffer cells	1.3	1.0 ± 0.2

Binding of lactosylated ^{125}I-neo-HDL to 1-7-1 cells and effects of specific competitors. The interaction of lactosylated ^{125}I-neo-HDL with the human asialoglycoprotein receptor was studied *in vitro*, using 1-7-1 cells (transfected murine NIH 3T3 fibroblasts expressing the human asialoglycoprotein receptor).

The amount of cell binding as a function of the concentration of lactosylated ^{125}I-neo-HDL is shown in Fig. 5. The cells were incubated at 4° for 2 hr. The nonspecific binding was determined in the presence of 100 mM *N*-acetylgalactosamine. Calculation of the binding parameters indicated that lactosylated neo-HDL binds to the human asialoglycoprotein receptor on murine fibroblasts with a high affinity (K_d, 40 nM; B_{max}, 548 fmol/mg of protein). The specificity of the interaction of lactosylated neo-HDL with 1-7-1 cells was studied by incubating the cells for 2 hr at 4° with 0.85 μg/ml ^{125}I-labeled lactosylated neo-HDL in the absence or presence of an excess of lactosylated HDL, *N*-acetylgalactosamine, or *N*-acetylglucosamine. It was found that lactosylated HDL and *N*-acetylgalactosamine inhibited the cell association of lactosylated ^{125}I-neo-HDL to 33 ± 6% and 21 ± 4% of the control value, respectively, whereas with *N*-acetylglucosamine no effect was observed.

Discussion

Recently, we demonstrated that intravenously injected lactosylated ^{125}I-HDL is extremely rapidly cleared from the circulation of the rat (5). The rapid plasma clearance was found to be due to galactose-specific uptake by the liver parenchymal cells. Because the lipid moiety of lipoproteins can accommodate a large variety of lipophilic compounds (25–28), lactosylated HDL was suggested to be an effective vehicle for the specific delivery of lipophilic drugs or prodrugs (2, 5). Such a specific delivery of drugs incorporated into lactosylated HDL to the parenchymal cells may be relevant for the treatment of metabolic diseases or viral infections like hepatitis B.

A major drawback to the use of lipoproteins for drug targeting

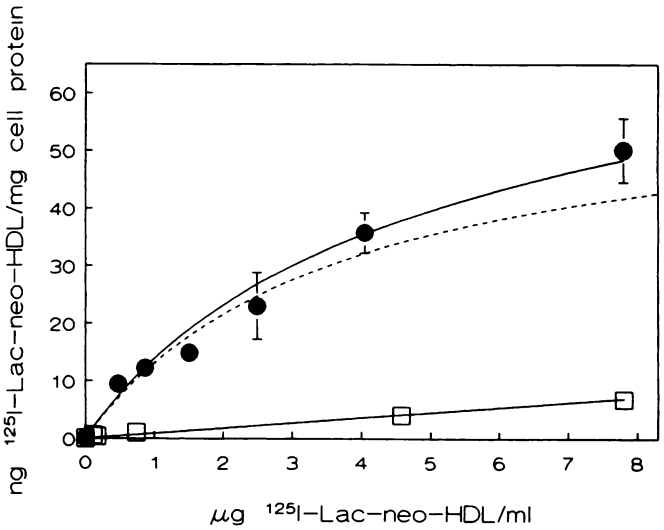

Fig. 5. Relation of the concentration of lactosylated ^{125}I-neo-HDL to the extent of binding by 1-7-1 cells. Cells were incubated for 2 hr at 4° with the indicated concentrations of lactosylated ^{125}I-neo-HDL. Total binding to the cells was determined (●). The nonspecific binding (□) was determined in the presence of 100 mM *N*-acetylgalactosamine. – – –, High affinity binding (corrected for nonspecific binding). The values are means ± standard errors of three experiments.

is their lack of availability. They must be isolated from blood, which limits their use on a large-scale pharmaceutical basis. It is therefore of great importance to devise artificial particles with properties similar to those of native lipoproteins.

In the present study, we investigated the possibility of synthesizing, from commercially available lipids, HDL-like particles (neo-HDL) with the same physico-chemical and biological properties as native HDL. Such a particle should, after lactosylation, be a suitable carrier for the selective delivery of lipophilic drugs or prodrugs to the asialoglycoprotein receptor on liver parenchymal cells.

Our results indicate that the composition, size, electrophoretic mobility, and density of the neo-HDL particles are similar to those found for discoidal HDL (21). *In vitro* experiments with isolated parenchymal liver cells showed that unlabeled HDL and neo-HDL competed for the same high affinity binding site as radiolabeled neo-HDL, whereas an excess of unlabeled LDL was ineffective. Like apoE-free HDL, neo-HDL does not follow a lysosomal pathway for its degradation, because chloroquine and NH_4Cl, two unrelated lysosomotrophic agents (24), did not significantly affect the degradation of neo-HDL. However, after injection of the neo-HDL particles into rats, a small additional uptake of radioactivity by the liver was obtained (maximally 7% of the injected dose). This additional uptake was found to be completely due to enhanced uptake by endothelial and Kupffer cells. After biological screening of the preparation for 10 min and subsequent injection into another rat, however, neo-HDL had the same biological behavior as apoE-free HDL.

We found that, after modification of the protein moiety by lactosylation, neo-HDL is rapidly taken up by rat liver. Within 10 min, 80% of the injected dose disappeared from the circulation and approximately 60% was recovered in the liver. Parenchymal cells accounted for >90% of the total liver uptake of radiolabeled lactosylated neo-HDL. Because the hepatic uptake could be blocked by preinjection of *N*-acetylgalactosamine, we conclude that this uptake is mediated by the asialoglycoprotein receptor, which is specifically localized on parenchymal liver cells.

The characteristics of the binding of lactosylated neo-HDL to the human asialoglycoprotein receptor were studied in transfected murine NIH 3T3 fibroblasts (1-7-1 cells; kindly provided by Dr. A. M. Shia, Boston University Medical Center, Boston, MA). Our experiments show that lactosylated ^{125}I-neo-HDL binds specifically to 1-7-1 cells with an affinity (K_d, 40 nM) that is comparable to the affinity of the binding of lactosylated HDL to rat parenchymal cells (K_i, 15 nM) (6). Competition studies indicate that, indeed, galactose groups on lactosylated neo-HDL serve as recognition markers. Thus, we conclude that our presently devised lactosylated neo-HDL system will be able to interact with the human asialoglycoprotein receptor, extending the relevance of our studies to humans.

Kupffer cells, the resident macrophages in liver, also possess high affinity galactose-specific receptors. In contrast to lactosylated HDL, lactosylation of LDL resulted in a rapid galactose-mediated uptake of these particles by Kupffer cells (29). The different fates of the lactosylated lipoproteins are probably due to their different sizes. Kupffer cells specifically recognize galactose-bearing particles larger than 10–15 nm (6). Our results indicate that, as has been found with lactosylated HDL, lactosylated neo-HDL is a particle with a sufficiently small size to escape recognition by galactose-specific receptors located on Kupffer cells. This allows an optimal interaction with the galactose-specific (asialoglycoprotein receptor) recognition site on parenchymal cells.

The results presented here indicate that, by using commercially available components (in the future probably including recombinant apoproteins) (30), HDL-like particles (neo-HDL) with virtually the same characteristics as found for native apoE-free HDL can be synthesized. The rapid uptake of lactosylated neo-HDL by galactose-specific receptors on parenchymal liver cells may be useful for transporting lipophilic drugs (e.g., antiviral drugs), in a pharmaceutical formulation, more specifically to parenchymal liver cells.

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