papers and notes on methodology

Functional characteristics of LDL particles derived from various LDL-apheresis techniques regarding LDL-drug-complex preparation

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Abstract Low density lipoproteins (LDL) have the potential to serve as cell specific drug carriers. The LDL may be derived in large quantities from LDL-apheresis procedures. Therefore, LDL particles isolated from the waste of three types of LDL-apheresis were investigated concerning their functional integrity in cell transport tests. LDL particles obtained from dextran sulfate-apheresis (DSA) and heparin extracorporeal lipoprotein precipitation (HELP)-LDL-apheresis are capable of specific internalization into HepG2 cells via the apoB receptor pathway. DSA-LDL-apoB appears to be split into two fragments as judged by SDS gel-polyacrylamide gel electrophoresis without changing transport behavior. Membrane differential filtration (MDF)- and HELP-derived LDL particles showed parallel transport behavior and electrophoretic mobility. Acetylated LDL particles obtained from MDF-LDL-apheresis and from blood donation plasma were transported into P_{388}-macrophages via the scavenger receptor pathway. The results confirm the use of LDL particles from LDL-apheresis as substrates for transformation into drug carriers. —**Schultis, H-W., H. v. Baeyer, H. Neitzel, and E. Riedel.** Functional characteristics of LDL particles derived from various LDL-apheresis techniques regarding LDL-drug-complex preparation. *J. Lipid Res.* 1990. **31:** 2277–2284.

Supplementary key words drug targeting • macrophages • LDL-derivatives • cell transport tests

Preparation of targetable drugs is a new field of pharmacological research. Antibodies, hormones, liposomes, and other macromolecules have been proposed as cell type-specific drug carriers (1). Along these lines, lipoproteins are currently being investigated for their possible utility to transport antimetabolites in chemotherapy of malignant diseases and HIV infection (2–5). The theoretical basis of this is the fact that lipoproteins are selectively transported into target cells via binding and internalization through specific receptor pathways. The fate of the internalized lipoproteins is lysosomal degradation. Hence, any compound evading lysosomal degradation can be presented to the cytosol compartment for further pharmacological action. Many attempts to utilize the apoB receptor pathway for administration of cytotoxic chemotherapeutics were published since the first suggestion of Gal et al. in 1980 (2), who referred to the reconstitution method of Krieger et al. (6). However, the specificity of this administration route is not very strict, since other apoB receptor-carrying cells (i.e., hepatocytes) may compete with the targeted tumor cells. This problem is avoided when scavenger receptor pathways are considered as a transport route. Scavenger receptors are known to be confined to the mononuclear-phagocyte cell system. HIV-infected macrophages provide the virus reservoir in the natural course of AIDS (7–10). Thus, targeting of antiviral drugs to this cell system is reasonable in order to avoid undesired side effects on other cell systems, such as hematopoesis, and the nervous system.

Validation of this concept requires preparation of large quantities of LDL particles with functional integrity in terms of binding, internalization, and degradation in the targeted cell population.

This report deals with cell transport tests with LDL particles derived from blood donation plasma and LDL particles obtained from the "waste" of three LDL-apheresis procedures, i.e., dextran sulfate adsorption with LA40 columns (Kaneka, Japan), heparin extracorporeal lipoprotein precipitation (HELP, Braun Melsungen, FRG), and membrane differential filtration (MDF, Fresenius AG, own design). The tests were performed in two cell lines, one representing the apoB receptor pathway (HepG2) and the other representing the scavenger receptor pathway (murine macrophages (P_{388})) of LDL uptake.

Abbreviations: MDF, membrane differential filtration; HELP, heparin extracorporeal lipoprotein precipitation; DSA, dextran sulfate LDL-apheresis; TC, total cholesterol; CE, cholesteryl ester; FC, free cholesterol; PL, phospholipids; TG, triglycerides; Prot, protein; LMW, low molecular weight kit; HMW, high molecular weight kit; LDL, low density lipoprotein; LPDS, lipoprotein-deficient serum.

The results of the cell transport experiments were compared with the electrophoretic mobility and the lipid composition data of the LDL particle preparations.

The data indicate that LDL particles obtained from dextran sulfate adsorption LDL-apheresis and, with some limitations, those obtained from membrane-differential-filtration LDL-apheresis maintain functional integrity. Thus, this material can be used for further biochemical derivatization to form drug carriers. LDL particles derived from HELP-LDL apheresis are equally suitable; however, the LDL particle yield is smaller as with other LDL-apheresis procedures.

METHODS

Isolation of LDL particles from plasma and LDL-apheresis waste

LDL particles were obtained from patients with familial hypercholesterolemia by three LDL-apheresis procedures. Anticoagulation was accomplished by an initial i.v. administration of 2000 IE heparin (Liquemine; Hoffman La Roche) and continuous admixture of ACD-solution 1:16 to the processed blood. Immediately after LDL-apheresis, the LDL-containing modules were eluted as follows.

Dextran sulfate LDL-apheresis (DSA). After separation of corpuscular blood components, LDL particles are extracted from patient's plasma by affinity chromatography with dextran sulfate columns (Kaneka Co., Osaka, Japan) (11). The bound LDL particles are eluted from the columns with 4% NaCl solution.

Heparin extracorporeal lipoprotein precipitation (HELP). After separation of corpuscular blood components, LDL particles are precipitated in the presence of heparin in acidified plasma (pH 5.5) by filtration. The LDL particles are eluted from the filtration module with 500 mM Tris-HCl buffer (pH 8.0).

Membrane-differential-filtration (MDF). After separation of corpuscular blood components, the plasma is filtered through a membrane filter with an apparent cut off of 600 kD (13). The LDL-containing waste solution is eluted with phosphate-buffered isotonic NaCl (0.5 mM phosphate, pH 7.4).

Citrated plasma of healthy blood donors was obtained as a byproduct of red blood cell donation (courtesy Dr. Eckstein).

The LDL particle-containing solutions of all sources were purified by immediate ultracentrifugation after the separation procedure. The LDL fraction 1.019–1.063 g/ml and the lipoprotein-deficient serum (LPDS), d > 1.21 g/ml were prepared by sequential ultracentrifugation, applying the KBr standard method to citrated plasma (14). The isolated LDL fractions were dialyzed for 36 h against 1000 volumes of standard buffer (0.15 M NaCl, 0.5 mM EDTA, pH 7.4, 4°C). The defibrination of LPDS was performed by incubation with thrombin (20 NIH U/ml) at 24°C for 10 min and subsequent centrifugation at 30,000 *g* for 90 min. The purified fractions were filtered (for sterility) over a 0.22-μm Millex filter (Millipore Co.) and thereafter stored at 4°C.

Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according the method of Cardin et al. (15). An acrylamide gradient of 3–20% was used in the presence of 6 M urea. Samples were prepared as follows. Lyophylized protein dialyzate (1 mg/100 μl) was incubated at 60°C for 20 min. Ten μl of buffer (100 μg protein) was laid on the gel. After a run of 3 h at 16 mA (gel: 150 × 100 × 1.7 mm) and 25°C, the gel was immersed in a fixing bath of isopropanol–acetic acid-water 25:10:65 for 60 min. Subsequently, the gel was stained with Coomassie Brilliant Blue. The calibration of molecular masses was performed with the low molecular weight kit (LMW: 94, 67, 43, 30, 20.1 and 14.4 kD) and the high molecular weight kit (HMW: 330, 220, 67, 36 and 18.5 kD) of Pharmacia. Agarose lipoprotein electrophoresis was conducted with the Paragon Lipo Kit of Beckman according to the instructions.

Lipid composition analysis

Total cholesterol (TC) was determined using Chod-PAP, Monotest (Boehringer Mannheim, FRG); free cholesterol (FC) was determined using Chod-PAP, Test Combination for Free Cholesterol (Boehringer Mannheim, FRG); triglycerides (TG) were determined using GPO-PAP, Test Combination Triglycerides (Boehringer Mannheim, FRG); and phospholipids (PL)-phosphorus were analyzed by the method of Bartlett (16), using a multiplication factor of 25. Protein (Prot) was determined by the method of Lowry et al. (17).

Iodination of LDL

Isolated LDL was iodinated using the ICl method of Karlin et al. (18). The ^{125}I-labeled LDL was purified over Sephadex G75 columns (20 × 1 cm) and subsequently dialyzed four times against 1 liter of standard buffer. The trichloracetic acid precipitates contained 97–99% of the radioactivity. The specific activity was 200–600 cpm/ng protein.

Acetylation of LDL

LDL particles were acetylated according to the method of Basu et al. (19) with acetic anhydride at 4°C. The acetylated LDL particles were dialyzed for 36 h at 4°C against standard buffer (0.15 M NaCl, 0.5 mM EDTA, pH 7.4). Occasionally appearing turbidity was removed by centrifugation for 20 min at 12,000 *g*. Sterile filtration was conducted as described above.

Cell transport experiments

HepG2 cells were from American Type Culture Collection (ATCC). The cells were routinely screened for mycoplasma infection. Culturing was performed with Eagles minimum essential medium containing 10% fetal calf serum (v/v) at 37°C and 5% CO_2. For the transport experiments, the cells were trypsinized (0.05% trypsin, 0.05% EDTA) and transferred with culture medium into dishes (60 × 15 mm). After the third day, the culture medium was replaced by LPDS-containing medium (10% v/v) for 12 to 18 h. The cell protein concentration determined at day 4 was 1–2 mg/dish. Binding, incorporation, and degradation were determined according to the method of Goldstein and Brown (20) with ^{125}I-labeled LDL particle fractions.

Murine macrophages (P_{388} ATCC) were cultured in RPMI medium with addition of nonessential amino acids and 10% fetal calf serum (v/v). For the transport experiments, the cells were carefully suspended and transferred into dishes (35 × 15 mm) with culture medium. After 1 h, when the surviving cells became adherent, the medium was exchanged. The LDL uptake was determined by measuring intracellular cholesterol concentration using an HPTLC method (21).

RESULTS

Lipid composition data

Two hundred fifty ml of citrated plasma (blood donor plasma) yielded 50–100 mg of LDL (protein). With the same preparation technique, 1 to 2 g of LDL (protein) can be isolated from MDF or DSA LDL-apheresis waste material obtained in one session of LDL-apheresis in patients with familial hypercholesterolemia. With HELP LDL-apheresis, the resolution of adsorbed LDL-heparin-aggregates from the filtration membrane is the limiting factor for the yield. Usually 250–500 mg of LDL (protein) can be obtained from each filtration membrane.

The lipid composition of the LDL particles from LDL-apheresis waste materials and from blood donor plasma (d 1.019–1.063 g/ml) is shown in **Table 1.**

There were minor differences between LDL-apheresis waste materials and plasma-LDL. For PL, FC, and TG this difference was less than ± 10%; for TC (CE) the maximum deviation was + 15%.

Electrophoretic data

The LDL preparations obtained from the LDL-apheresis procedures and from plasma showed the following characteristics when analyzed by SDS-PAGE and agarose electrophoresis.

Plasma LDL. By SDS-PAGE, plasma LDL showed a single homogeneous band (lanes B and G, **Fig. 1**) with an apparent molecular mass of 500 kD; by agarose electrophoresis, plasma LDL also showed a single band (lanes B and G, **Fig. 2**). Both techniques demonstrate the purity of the plasma LDL preparation. Thus, this preparation was further used as a standard of comparison.

Heparin extracorpeal lipoprotein precipitation LDL-apheresis (HELP). The LDL obtained from this procedure appeared as undenatured, native LDL by SDS-PAGE (Fig. 1, lane E) and agarose lipoprotein electrophoresis (Fig. 2, lane H).

Dextran sulfate adsorption LDL-apheresis (DSA). The LDL preparation obtained from dextran sulfate adsorption showed by SDS-PAGE a proteolytic hydrolysis of apoB-100 into two major fragments with apparent molecular masses of 200 and 400 kD (Fig. 1, lane C). This finding was supported by Byrne and Scanu (22), who have shown that coincubation of LDL and dextran sulfate results in measurable quantities of apoB hydrolysis products. Agarose lipoprotein electrophoresis showed no change in the surface charge of these preparations in comparison to native plasma LDL (Fig. 2, lane C).

Membrane-differential-filtration LDL-apheresis (MDF). Agarose lipoprotein electrophoresis (Fig. 2, lanes E, I–K) showed a negative shift of surface charge between various preparations. By SDS-PAGE, apoB-100 of this LDL preparation had an apparent molecular mass of 500 kD, as a single protein band corresponding to native plasma LDL preparations (Fig. 1, lane D).

TABLE 1. Lipid composition data of LDL particles from various sources

Parameter	Source			
	HELP (n = 3)	DSA (n = 2)	MDF (n = 3)	Plasma (n = 5)
	mg/mg			
PL/Prot	0.74 ± 0.11	0.84 ± 0.09	0.77 ± 0.09	0.69 ± 0.05
TC/Prot	1.39 ± 0.20	1.44 ± 0.25	1.51 ± 0.05	1.30 ± 0.08
FC/Prot	0.37 ± 0.07	0.37 ± 0.08	0.37 ± 0.05	0.36 ± 0.04
CE/Prot	1.02 ± 0.20	1.07 ± 0.25	1.14 ± 0.05	0.94 ± 0.08
TG/Prot	0.32 ± 0.06	0.37 ± 0.06	0.29 ± 0.07	0.25 ± 0.04

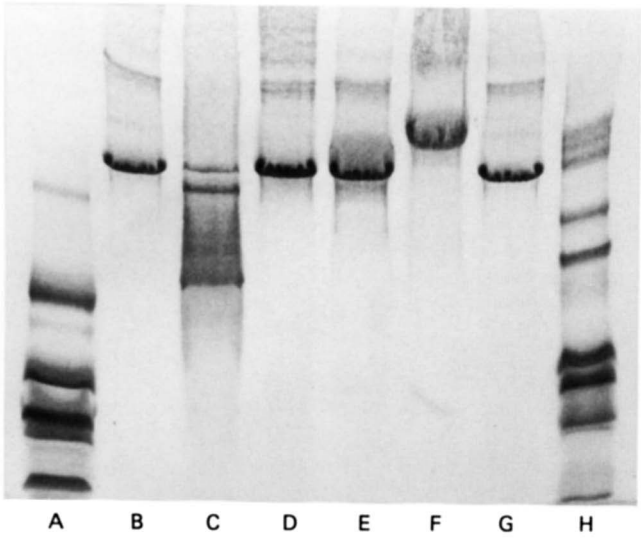

Fig. 1. SDS-PAGE (3–20% acrylamide, 6 M urea). Lane A: LMW; lanes B and G: LDL from healthy blood donors; lane C: LDL from dextran sulfate LDL-apheresis (DSA); lane D: LDL from membrane-differential-filtration (MDF); lane E: LDL from heparin extracorporeal lipoprotein precipitation (HELP); lane F: acetylated LDL; Lane H: HMW. DSA-LDL consistently shows proteolytic splitting of apoB. The other preparations of LDL particles (MDF-LDL, HELP-LDL) show the anticipated electrophoretic mobility indicating intact apoB structure. Acetylated LDL is found at a higher apparent molecular mass than anticipated. This is due to the change of surface charge (more negative).

Cell culture experiments

Macrophages (murine macrophages P388).

Fig. 3 demonstrates the results of transport experiments with murine macrophages (P_{388} cells). These cells internalize acetylated (therefore surface-modified LDL) leading to intracellular enrichment of cholesteryl esters. Native plasma LDL, on the other hand, was not internalized and hence no enrichment of intracellular cholesteryl esters was observed. The increase of cholesteryl esters after 4 h of incubation depended on the medium LDL concentration for various preparations of LDL as illustrated in Fig. 3. The following results for various LDL preparations were obtained.

Heparin extracorporeal lipoprotein precipitation LDL-apheresis (HELP). Since no electrophoretic differences between plasma LDL and HELP-LDL were demonstrated (Figs. 1, 2) and, furthermore, since no detectable accumulation of cholesteryl ester from plasma LDL occurred in macrophages (Fig. 3), experiments with HELP-LDL were omitted.

Dextran sulfate adsorption LDL-apheresis (DSA). The native cationic charge of DSA-LDL, as demonstrated by agarose electrophoresis, was consistent with lack of internalization via the scavenger receptor pathway in P_{388}-macrophages (Fig. 3).

Membrane-differential-filtration LDL-apheresis (MDF). The negative shift of cationic LDL charge corresponded to a small but detectable internalization into P_{388} cells (Fig. 3).

MDF waste LDL exhibited varying electrophoretic mobility among several LDL-apheresis procedures. For example, in Fig. 3 material E (agarose electrophoresis, Fig. 2) was used; in contrast, material K (Fig. 2) behaved exactly like plasma LDL (not shown). Thus, the degree of spontaneous modification of the surface charge of MDF-LDL during LDL-apheresis corresponds with the ability of internalization via scavenger receptor pathway.

Acetylated MDF-LDL and acetylated plasma-LDL showed an equal uptake in macrophages (not shown). Their electrophoretic mobilities were identical, as shown in Fig. 2 (lanes D, F).

Hepatocytes (human hepatocytes HepG2)

Fig. 4 demonstrates the results of transport experiments with human hepatocytes (HepG2). These cells internalize LDL with cationic surface charge via a specific apoB/E receptor. In the experiments, degradation of previously internalized LDL was determined by measuring free ^{125}I-labeled tyrosine in the medium. In contrast, acetylated ^{125}I-labeled LDL was not degraded by hepatocytes, as shown.

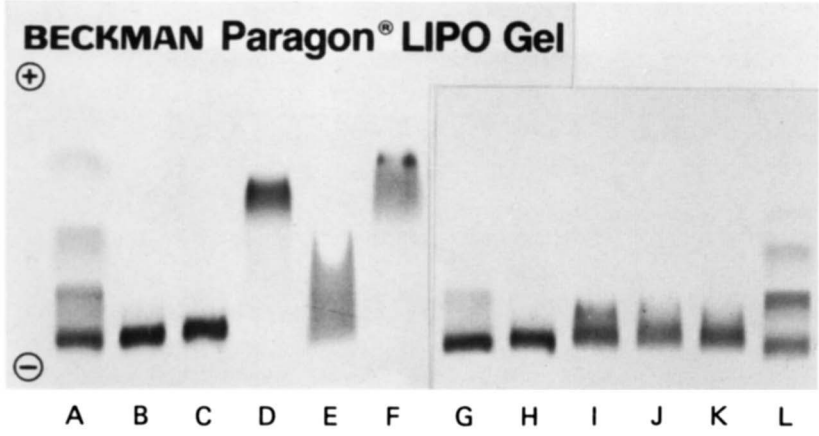

Fig. 2. Agarose lipoprotein electorphoresis: lanes A and L: plasma from health blood donors: α-, pre-β-, and β-lipoprotein; lanes B and G: LDL isolated from healthy blood donors (plasma LDL); lane C: LDL from dextran sulfate LDL-apheresis (DSA); lane D: acetylated plasma LDL; lanes E, I, J, K: LDL from membrane-differential-filtration (MDF); lane F: acetylated MDF-LDL; lane H; LDL from heparin extracorporeal lipoprotein precipitation (HELP). HELP-LDL and DSA-LDL do not show any variation of surface charge. MDF-LDL exhibits various degrees of increased negative charge from preparation to preparation. Acetylated LDL migrates towards the anode because of its negative surface charge.

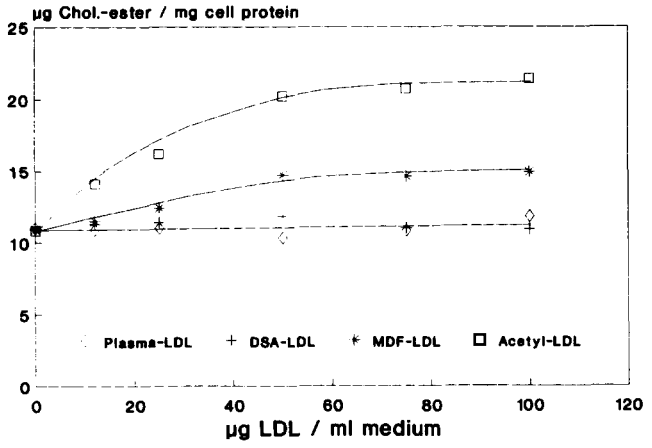

Fig. 3. Transport of LDL particles into P_{388}-macrophages as determined by intracellular cholesteryl ester measurements (ng/mg cell protein/P_{388}). Effect of LDL concentration in the medium. Upper curve: acetylated LDL; middle curve: MDF-LDL (material E, Fig. 2); lower curve: plasma- and DSA-LDL. Incubation: 4 h, 37°C, 5% CO_2. All experiments were performed two times; each point represents an average of two values.

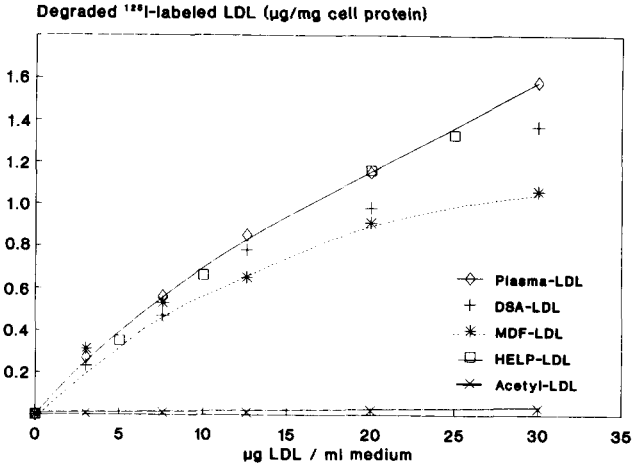

Fig. 4. Transport of iodinated LDL particles into hepatocytes (HepG2) as determined by measuring externalized ^{125}I-labeled tyrosine (degradation). Effect of LDL concentration in the medium. Upper curve: plasma- and HELP-LDL; middle curve: DSA- and MDF-LDL; lower curve: acetylated LDL. Incubation: 4 h, 37°C, 5% CO_2.

Heparin extracorporeal lipoprotein precipitation LDL-apheresis (HELP). The LDL obtained by this procedure showed by either SDS-PAGE (Fig. 1, lane E) or agarose lipoprotein electrophoresis (Fig. 2, lane H) undenatured, native LDL. Binding, incorporation (not shown), and degradation (Fig. 4) corresponded to those observed with native plasma LDL preparations.

Dextran sulfate adsorption LDL-apheresis (DSA). In spite of the unequivocal proteolytic splitting of apoB-100 as shown by SDS-PAGE, the biological activity of the DSA-LDL at the apoB receptor on HepG2 cells was maintained. Binding, incorporation (not shown), as well as degradation (Fig. 4) of these LDL fractions corresponded to turnover rates of native plasma-LDL preparations in this cell line. The nonspecific, nonreceptor-mediated uptake of acetylated LDL is shown for comparison.

Membrane-differential-filtration LDL-apheresis (MDF). The affinity of these preparations for the apoB receptor on hepatocytes (HepG2) remained unaltered in spite of the change of the surface charge when compared with native plasma LDL particles (Fig. 4). Only at higher LDL concentrations in the medium was the degree of degradation of MDF-LDL with a modified cationic surface charge somewhat smaller in comparison to native plasma LDL preparations (material E, Fig. 2). At the same LDL medium concentration (6.7 µg/ml) the MDF-LDL showed, in comparison with plasma LDL, a normal binding, incorporation, and degradation over a period of 6 h in transport tests with hepatocytes (**Fig. 5A, B**).

Inhibition studies

Inhibition of LDL degradation was demonstrated in two sets of experiments. **Fig. 6** illustrates the inhibitory effect of unlabeled LDL obtained from LDL-apheresis

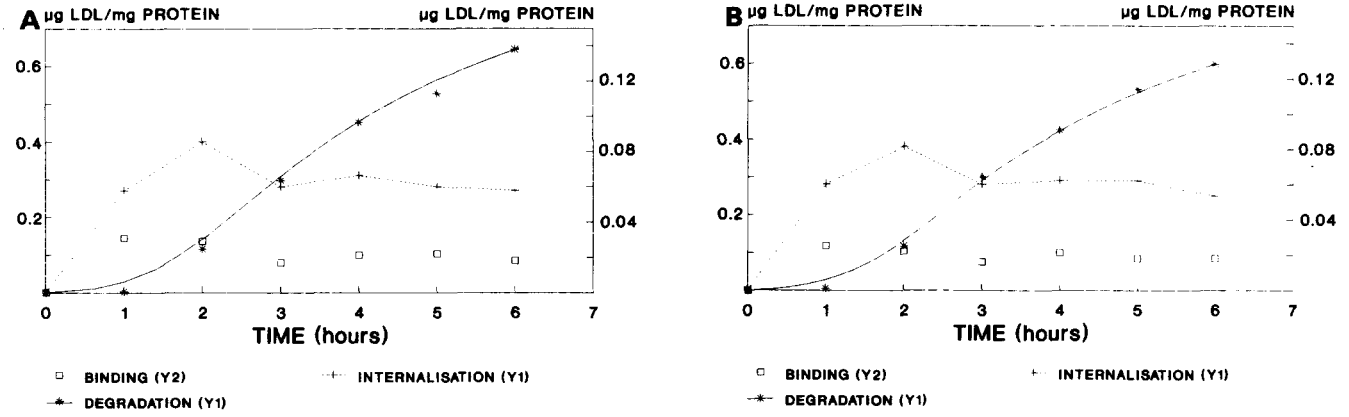

Fig. 5. Reaction velocity of binding, internalization, and degradation of plasma LDL particles (A) or MDF-LDL particles (B) as a function of time determined in hepatocytes (HepG2). Incubation 37°C and 5% CO_2. The curves show the characteristic catabolic cycle of internalized LDL. Binding is complete after the first hour; internalization has a maximum after 2 h; degradation starts after the first hour. Internalization returns after the 2 h peak to a steady state, while diffusion of ^{125}I-labeled tyrosine keeps increasing, indicating complete intracellular proteolysis of apoB-100.

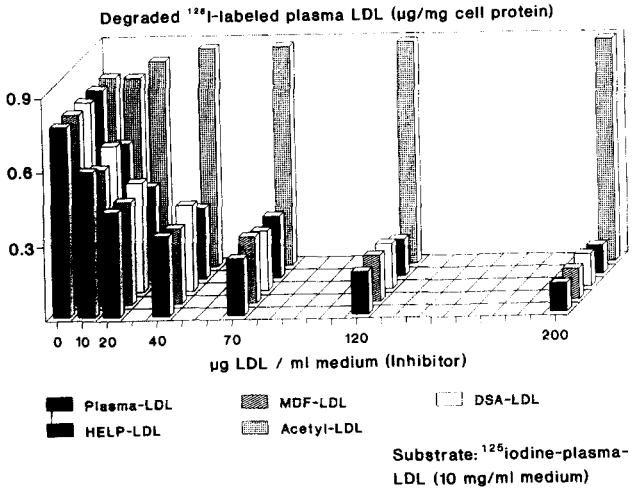

Fig. 6. Inhibition experiments. Degradation of ^{125}I-labeled plasma LDL as function of unlabeled LDL concentration (inhibitor) at constant substrate concentration (^{125}I-labeled plasma LDL). After a 4-h incubation (37°C, 5% CO_2, HepG2 cells), LDL degradation was determined by quantification of externalized ^{125}I-labeled tyrosine. All three LDL-apheresis materials (HELP, DSA, MDF) show the same potential for competitive inhibition. In contrast, acetyl-LDL does not exert an inhibitory effect.

waste materials and from plasma LDL on the degradation of ^{125}I-labeled plasma LDL. By comparison, the degradation of ^{125}I-labeled plasma LDL in response to increasing amounts of unlabeled acetylated LDL was measured. In these experiments, there was an unexpected increase of ^{125}I-labeled plasma LDL degradation. This phenomenon was reproducible, however, as yet unexplained.

Fig. 7 shows the reverse experiments, in which substrate and inhibitor were exchanged. The data demonstrate equal inhibition of degradation of LDL-apheresis materials by increasing amounts of unlabeled plasma LDL.

In the same experiments binding and internalization were determined. The same type of results were demonstrated (data not shown). Both experiments support the specific handling of LDL-apheresis materials via the apoB receptor pathway in hepatocytes. Besides this finding, the specificity of LDL receptor binding the internalization was clearly demonstrated by the lack of competitive inhibition by acetylated LDL, and by almost absent degradation of all acetylated LDL preparations in this cell type.

DISCUSSION

The aim of the study was to investigate the biological activity of LDL particles derived from various LDL-apheresis procedures. Knowledge of this is the basis for the decision as to which material can best serve as substrate for drug conjugation in order to design a targetable delivery system. The test methods were transport and transport inhibition experiments in hepatocytes and macrophages, which were studied in conjunction with electrophoretic mobility studies and lipid composition data.

Preparation techniques consisting of ultracentrifugation and SDS-PAGE identified the waste-derived material as apoB-100-containing lipoprotein particles in the density range 1.019–1.063 g/ml without detectable contamination by other lipoproteins. Furthermore, the lipid composition data of the LDL-apheresis materials showed no major deviation from the lipid composition of plasma LDL with respect to PL, FC, and TG. Total cholesterol seems to be somewhat enriched in the LDL-apheresis materials, probably due to the fact that the LDL was from patients with familial hypercholesterolemia. Hence, LDL-apheresis-derived LDL particles are comparable with those from native, plasma-derived LDL particles in terms of lipid composition.

LDL obtained by dextran sulfate adsorption LDL-apheresis (DSA) showed, by SDS-PAGE, proteolytic splitting of apoB-100 into two major fragments at apparent molecular masses of 200 and 400 kD. The biological activity of the DSA-derived LDL particles as determined by binding, incorporation, and degradation in HepG2 cells, as well as demonstration of competitive inhibition of these functions, corresponds to those of native plasma LDL particles. Thus, proteolytic splitting of the apoB had no impact on the ligand function of the lysine-enriched binding site of the LDL particle at the apoB/E receptor. On the other hand, DSA-derived LDL particles are not internalized via the scavenger receptor pathway on P_{388}-

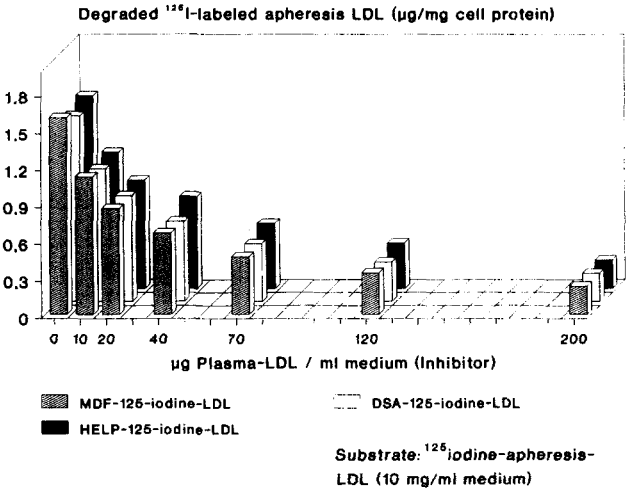

Fig. 7. Inhibition experiments. Degradation of ^{125}I-labeled apheresis-LDL as a function of unlabeled plasma LDL concentration (inhibitor) at constant substrate concentration (^{125}I-labeled apheresis-LDL). After a 6-h incubation (37°C, 5% CO_2, HepG2 cells), LDL degradation was determined by quantification of externalized ^{125}I-labeled tyrosine. Degradation of all three LDL-apheresis materials (HELP, DSA, MDF) can be competitively inhibited by plasma LDL.

macrophages. This is consistent with the demonstration of an unchanged cationic surface charge. With a yield of 2 g of LDL protein per preparation, this LDL-apheresis method ranks first for preparation of LDL with full biological integrity as judged by studies using the two cell lines.

LDL obtained by membrane-differential-filtration LDL-apheresis (MDF) demonstrates a more or less pronounced charge (more negative) of the primarily cationic surface charge. This causes significant, however incomplete, internalization into the P_{388}-macrophages. In addition, this LDL preparation showed a slightly reduced degradation by HepG2 cells at higher LDL concentrations in the medium in comparison to native plasma LDL preparations. Other batches of MDF-LDL particles, however, with regular agarose electrophoresis behavior, showed the same transport behavior as plasma LDL particles in macrophages and hepatocytes. The reason for the modification of surface charge could not be identified. However, one may speculate that some kind of artificial membrane/lipoprotein interaction was responsible. Inhibition experiments supported specificity of transport of MDF-derived LDL in hepatocytes. With a maximum yield of 2 g LDL protein per preparation, the membrane-differential-filtration can be utilized to prepare biologically active LDL particles, with some limitations: the loss of selectivity of internalization via the apoB receptor pathway in hepatocytes (HepG2 cells) and on the scavenger receptor pathway in P_{388}-macrophages when there is a shift to a less positive charge on the LDL surface. In contrast, acetylated MDF-derived LDL particles were exclusively internalized by P_{388}-macrophages. In this case, their transport behavior was identical with that of acetylated LDL particles derived from blood donor plasma.

LDL particles derived from heparin extracorporeal lipoprotein precipitation (HELP) exhibited the same behavior as plasma-derived LDL particles. This was true with respect to electrophoretic mobility and to cell transport tests including competition experiments. Thus, the heparin-LDL-complex formation was fully reversible by back titration with Tris-HCl. However, the relatively small yield of 250–500 mg LDL protein per preparation puts HELP LDL-apheresis-derived materials behind those derived from citric plasma of healthy blood donors. This is due to the fact that resolubilization of the heparin-LDL-complexes from the filtration membrane is a more elaborate procedure.

Competition experiments with acetylated LDL particles in HepG2 cells revealed a surprising phenomenon: binding, internalization, and degradation were significantly stimulated in the presence of acetylated LDL. This finding is as yet unexplained.

The experiments have demonstrated that LDL-apheresis-derived LDL particles, which can be obtained in large amounts, are suitable for further processing, because they maintain their functional properties. DSA-derived LDL particles seem to be the most useful material for further utilization. The apparent splitting of apoB, as judged by SDS-PAGE, had no functional impact on the transport behavior of the entire particle. MDF-LDL is evidently altered during the filtration process. ApoB receptor interaction was diminished and some scavenger pathway activities were observed. Acetylation, however, leads to a fully functioning product, which was targetable to macrophages.

With respect to the envisioned aim of targetable delivery of therapeutic agents, one should remember that modified LDL particles have been used successfully for diagnostic purposes in order to identify the receptor-independent degradation pathway (23, 24). Thus, at the present time, there is no evidence that excludes administration of modified LDL particles in man. Acetylation of LDL particles was not exclusively mandatory for scavenger pathway uptake (25). Coupling of therapeutic agents with the lysyl residues of apoB may suffice to selectivity target macrophages and to exclude hepatic and myeloic uptake. However, this remains to be demonstrated in experimental animals.

Investigation of antiviral drug-coupled LDL particles targeted to the mononuclear-phagocyte system seems to be a promising research field of anti-HIV therapy in the future.

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