Studies on the Role of Specific Cell Surface Receptors in the Removal of Lipoprotein (a) in Man

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ABSTRACT The binding of ^{125}I-lipoprotein (a) [Lp(a)] to cell surface receptors was studied on cultured human fibroblasts. The results were compared with corresponding data obtained with ^{125}I-low density lipoproteins (LDL). Equilibrium binding studies showed that Lp(a) is bound with high affinity by the cell surface receptors. The maximum binding capacity for Lp(a) was 37% lower than for LDL. For Lp(a) and LDL, the Scatchard plots displayed linearity, indicating a single category of binding sites. Half-maximal saturation occurred at a concentration of 9.52±1.04 nM for Lp(a) and 7.76±1.29 nM for LDL. Competition binding experiments revealed that Lp(a) and LDL are nearly equally potent in competing each other for the binding sites. Binding of Lp(a) and LDL were followed by suppression of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. Cyclohexanedione treatment of Lp(a) and LDL completely abolished receptor binding. Neither Lp(a) nor LDL were specifically bound by fibroblasts obtained from a patient with homozygous familial hypercholesterolemia (FH).

The removal mechanisms for Lp(a) and LDL were further compared by in vivo studies. Radioiodinated Lp(a) and LDL were injected intravenously into 12 normolipemic individuals to measure kinetic parameters of these two lipoproteins simultaneously in each subject. Mean fractional catabolic rate (FCR) of Lp(a) was 0.260±0.060 and mean FCR of LDL was 0.377±0.077 (mean±SD). In each subject, FCR of Lp(a) was lower than the FCR of LDL; the mean difference was 31%. The absolute synthetic rate of Lp(a) was significantly lower than the corresponding value of LDL. In each individual, the percentage of total Lp(a) that was contained in the intravascular space was higher than the corresponding value of LDL; the mean difference was 19%. A highly significant positive correlation was found between FCR of LDL and FCR of Lp(a) ($r = 0.853$, $P < 0.01$). No relationship was found between the serum concentration of LDL-apolipoprotein B and Lp(a). The serum level of Lp(a) was positively related to the absolute rate of Lp(a) synthesis ($r = 0.979$, $P < 0.01$). The serum level of LDL-apolipoprotein B was inversely related to FCR of LDL ($r = 0.613$, $P < 0.05$). In a patient with homozygous FH, FCR of LDL was 0.205 and FCR of Lp(a) was 0.210.

The results of these studies show that Lp(a) is specifically bound with high affinity to the same receptors of human fibroblasts as LDL. The affinity and maximum binding capacity are slightly lower for Lp(a) than for LDL. The results of the turnover studies are consistent with the assumption that Lp(a) is removed from the plasma by similar mechanisms as LDL.

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INTRODUCTION

Lipoprotein (a) [Lp(a)][1] is a lipoprotein which originally was believed to represent a genetic variant of low density lipoprotein (LDL) or better, lipoprotein B (Lp B) (1). In later studies, Lp(a) was found to be a separate lipoprotein that can be demonstrated with sensitive immunological methods in the serum of all individuals containing apolipoprotein B (2). Lp(a) has gained great clinical interest since many studies have shown a relationship between the serum level of Lp(a) and coronary vascular disease (1, 3–5).

Lp(a) has many physicochemical properties in common with LDL (d = 1.006–1.063 g/ml). Most of the plasma Lp(a) is found in the density range of 1.055 to 1.100 g/ml. The lipid composition of Lp(a) and

[1] *Abbreviations used in this paper:* FCR, fractional catabolic rate; FH, familial hypercholesterolemia; HDL, high density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low density lipoprotein; Lp(a), lipoprotein (a); VLDL, very low density lipoprotein.

LDL are nearly identical (6–8). The hexose, hexosamin, and sialic acid content, however, are significantly higher in Lp(a) (9). The main protein constituent of both lipoproteins is apolipoprotein B. Lp(a) has an additional apoprotein that has been termed "specific Lp(a) antigen" (9–11). On agarose gel or cellulose acetate Lp(a) migrates somewhat faster than LDL and has therefore also been described as "pre-β_1-lipoprotein" (3, 4, 12).

In spite of these remarkable similarities between Lp(a) and LDL, Lp(a) is not a metabolic product of very low density lipoproteins (VLDL), LDL, or chylomicrons, but seems to be synthesized as a separate lipoprotein (13). Furthermore, serum Lp(a) is not converted to other serum lipoproteins (14). Recently, some kinetic parameters of Lp(a) have been reported (14). Again some similarities between Lp(a) and LDL exist: the mean fractional catabolic rate (FCR) of Lp(a) and the average percentage of the total Lp(a) pool that is in the circulation (percent intravascular) were equal or only slightly different from the corresponding values of LDL reported by others (15–18).

Goldstein and Brown (19) discovered specific receptors with high affinity for LDL on the cell surface of human fibroblasts. These receptors, which also exist on other cell types including smooth muscle cells (20), lymphocytes (21), and endothelial cells (22), play a major role in the removal and catabolism of LDL (23). Binding of LDL to the specific surface receptors is the first and rate limiting step in the LDL pathway, followed by endocytosis of the lipoprotein, utilization of its cholesterol and suppression of endogenous cholesterol synthesis within the cell (24). It is well established that the recognition site for binding of the LDL particle to the receptor resides with apoprotein B (25).

To date, the mechanism of Lp(a) catabolism has not been clarified. Since the main apolipoprotein of Lp(a) is apoprotein B, binding of Lp(a) to the cell surface receptors could play a similar role in its catabolism as demonstrated for LDL. With this respect, the reports in the literature are contradictory and do not answer this question. Maartman-Moe and Berg (26) concluded from their studies that the LDL receptor is of minor, if any, importance for Lp(a) cell surface binding and uptake. Havekes et al. (27) found that a lipoprotein fraction with an electrophoretic mobility on agarose between β- and pre-β-lipoproteins is bound, internalized, and degraded by cultured human fibroblasts. In this study, however, the isolated lipoprotein fraction was not identified as Lp(a) by immunological methods and no quantitative binding data were presented. Floren et al. (28) found that Lp(a) is able to stimulate cholesterol esterification and to increase cholesterol content in cultured human fibroblasts. The authors speculate that Lp(a) enters fibroblasts via the LDL receptor pathway since no effect on cholesterol content was observed when the study was performed in LDL receptor negative cells. Here again, no binding data were presented.

The following studies have been undertaken in an attempt to define binding characteristics of Lp(a) in comparison with LDL. Furthermore, the catabolic rate of Lp(a) was compared with the catabolic rate of LDL in vivo by simultaneous turnover studies of both lipoproteins in the same individuals.

METHODS

Binding studies

Isolation of Lp(a) and LDL. The details of the method for isolation of Lp(a) and LDL were described recently (14). Plasma was obtained by plasmapheresis from healthy volunteers who had high levels of Lp(a) as checked by double-immunodiffusion using monospecific anti-Lp(a) antibodies (14). The plasma was subjected to sequential ultracentrifugation to obtain density fractions from 1.006 to 1.055 g/ml and from 1.055 to 1.110 g/ml. The densities were adjusted by addition of solid NaCl and checked with a density meter (Anton Paar K. G., Graz). All centrifugal procedures were performed in a Beckman L8-70 centrifuge using a Ti 50.2 rotor (Beckman Instruments, Inc., Fullerton, CA). The supernatants, containing the lipoproteins of the *d* 1.006–1.055 g/ml and 1.055–1.110 g/ml, were collected by tube slicing. These fractions were concentrated to a volume of ~5 ml by dialysis against polyethyleneglycol and then applied to an agarose column (Bio Gel A-5m, Bio-Rad Laboratories, Richmond, CA). Elution of the lipoproteins was performed with 0.15 M NaCl, pH adjusted to 8.5 by addition of NH_4OH. During all steps of the isolation procedure, Na_2EDTA and NaN_3 were present in a concentration of 1 mg/ml. The elution profile and the characterization of the eluted Lp(a) peak have been described previously (14).

Iodination of Lp(a) and LDL. Fractions containing the isolated Lp(a) or LDL were dialyzed against 0.1 M glycine buffer, pH 10, and then iodinated with ^{125}I according to the McFarlane method (29) as modified by Bilheimer et al. (30). Free iodine was removed by filtration on Sephadex G-25 and subsequent dialysis against 0.15 M NaCl with several bath changes. The whole procedure of the isolation and iodination took 4 d. Labeled Lp(a) and LDL were characterized by the same methods as described recently (14). Most of the radioactivity was bound to the apoprotein; only 0.5–3% was found in the lipid moiety after extraction with chloroform-methanol (2:1).

3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase assay. HMG-CoA reductase activity was determined as described by Brown et al. (31) with some modifications (32).

Chemical modification of Lp(a) and LDL with 1,2-cyclohexanedione (25). 5 mg of Lp(a) or LDL in 1 ml 0.15 M NaCl containing 0.01% Na_2EDTA were mixed with 2 ml 0.15 M 1,2-cyclohexanedione in 0.2 M sodium borate buffer, pH 8.1, and incubated at 37°C for 2 h. The samples were then dialyzed for 22 h against 0.15 M NaCl at 4°C with several bath changes.

Preparation of an apolipoprotein B-free Lp(a)-antigen/lipid complex. The Lp(a) antigen was purified as described earlier (8). 5 mg of this apoprotein, solubilized in 1 ml of 0.1 M Tris–HCl buffer (pH 8.5) containing 6 mol urea/liter,

was mixed with a lipid dispersion. This dispersion was prepared by mixing 10 mg of high density lipoprotein lipids from normal serum with 1 ml 6 M urea solution in 0.1 M Tris-HCl buffer and ultrasonification for 2 min at 45°C. After incubation for 1 h at 37°C, the mixture was passed over a column packed with Sephadex G-25 in 0.1 M Tris-HCl buffer to remove the urea. The material eluting at the void volume was adjusted to a density of 1.15 g/ml by adding solid NaBr, ultracentrifuged for 24 h at 150,000 *g*, and dialyzed against 0.1 M Tris-HCl buffer. This reconstituted complex had the following characteristics: chemical composition: 32.6% protein, 37.7% phospholipids, 11.6% free cholesterol, 13.7% cholesterol ester, 4.3% glycerides (by weight). On agarose gel electrophoresis it migrated as a broad band between slow-β and pre-β_1 lipoproteins. With monospecific antisera against the Lp(a) antigen it gave an immunochemical reaction identical to that of native Lp(a), indicating that the reconstituted lipoprotein had the antigenic determinants exposed at the surface similarly to native Lp(a).

Determination of lipoprotein concentrations. All lipoprotein stock solutions used to prepare incubation media were dialyzed under vigorous shaking against 0.15 M NaCl (pH adjusted to 7.5) for 24 h at 4°C. Defined volumes of the lipoprotein solutions and buffers were pipetted into glass vials, evaporated to dryness, and heated overnight to 45°C under high vacuum. The residual weight of the lipoproteins was calculated by subtracting the weight of background salt from the total weight of the dried lipoprotein plus salt.

Cell culture. The normal human fibroblast strains used in these experiments were established from skin samples of a 6-yr-old boy and a 10-yr-old girl, removed at the time of surgery because of herniotomia. Receptor negative fibroblasts were cultured from a skin sample that was obtained from a patient with homozygous familial hypercholesterolemia. This patient was a 7-yr-old boy (brother of subject 13 in Table II). The clinical data are presented below.

Stock cultures were maintained for up to 2 wk in 250-ml plastic culture flasks containing 12 ml growth medium consisting of Eagle's minimal essential medium (MEM) supplemented with penicillin (100 U/ml), streptomycin (1 μg/ml), 1% nonessential amino acids and 10% (vol/vol) fetal calf serum. The nutrient medium was renewed every 3–4 d and the cells were maintained at 37°C in a humidified 95% air–5% CO_2 atmosphere.

Experiments were carried out between the 10th and 25th passage. Cells from stock flasks were dissociated with trypsin-EDTA (0.05%) at 37°C and seeded (day 0) at a concentration of $\sim 1 \times 10^5$ cells/2 ml of media in each well of FB6-TC multi-dish trays. On day 3, the medium was replaced with 2 ml of fresh standard growth medium. At day 5, the monolayers were washed with phosphate-buffered saline, after which 2 ml of fresh Eagle's MEM containing 5% human lipoprotein-deficient serum was added. All experiments were initiated after the cells had been incubated with lipoprotein-deficient serum for 48 h.

Binding of ^{125}I-Lp(a) and ^{125}I-LDL by intact fibroblast monolayers. Monolayers, prechilled for 1 h in a cold room, were incubated with Eagle's MEM supplemented with 5% lipoprotein-deficient serum for 2 h at 4°C with indicated concentrations (Figs. 2 and 3) of radioligand alone or radioligand plus binding competitors. To terminate binding reactions, the medium was removed and each monolayer was rapidly washed four times with ice-cold 0.05 M Tris-HCl buffer (pH 7.4) containing 0.15 M NaCl and 0.2% bovine serum albumin. The cells were then washed two additional times with the same buffer without albumin, then dissociated with 2 ml 0.05% trypsin-EDTA at 37°C and finally quantitatively transferred to test tubes for the assay of radioactive iodine.

Specific binding of ^{125}I-Lp(a) or ^{125}I-LDL was defined as total binding minus (nonspecific) binding seen in the presence of either unlabeled Lp(a) or LDL, at 500-μg/ml concentrations. Binding activity is expressed as moles of radioligand bound to cells per culture dish, assuming a molecular weight of 5.6×10^6 for Lp(a) (11) and 3×10^6 for LDL (33). Variation of protein content per dish within individual experiments was <3%. Between different experiments protein contents were in the range of 300 to 450 μg/culture dish. Protein concentrations were measured according to Lowry et al. (34).

Turnover studies

Subjects. The turnover studies were performed on 12 male individuals who did not suffer from hepatic, renal, or thyroid dysfunction or diabetes mellitus. None of them received any treatment known to influence lipid metabolism. Their height, weight, age, and relevant lipid concentrations are given in Table II. Subject 13 was a 16-yr old boy who suffered from homozygous FH. A deficiency of specific LDL receptors had already been established previously. This boy showed extensive tuberous xanthomas. Coronary angiography revealed an occlusion of the left anterior descending and a severe stenosis of the circumflex artery. This patient had a 7-yr-old brother with some tuberous xanthomas and a cholesterol level of 460 mg/100 ml. Both parents of these two boys had elevated plasma cholesterol levels in the range between 385 and 430 mg/100 ml. In all subjects, body weight and the concentration of serum triglycerides, cholesterol, and Lp(a) were constant throughout the study. All were hospitalized throughout the study and received a "normal" hospital diet containing 45–50% of total calories as carbohydrate, 30–35% as fat, and 15–20% as protein. Potassium iodide (3×60 mg daily) was administered to each individual beginning 3 d before the study and continuing throughout the entire experiment. Informed consent to the study was obtained from each subject.

Study protocol. Lp(a) and LDL were separated and iodinated as described above. Since both lipoproteins were injected simultaneously to the individuals, ^{125}I and ^{131}I were used for iodination. Both isotopes were used alternately for labeling Lp(a) or LDL.

50 μCi of labeled Lp(a) and labeled LDL were injected into each subject after an overnight fast. Prior to the injection, the labeled lipoproteins were sterilized by passage through a Millipore filter (0.45 nm, Millipore Corp., Bedford, MA). Venous blood was drawn 10 min, 4 h, and 8 h after the injection of the labeled lipoproteins and then daily for up to 14 d. The radioactivity of ^{125}I and ^{131}I was measured in the serum using a Packard Autogamma Scintillation Spectrometer 5160 (Packard Instrument Co., Inc., Downers Grove, IL).

Calculations. It is well established that the apoproteins of LDL (35) and Lp(a) (14) are not removed from the lipoprotein particle within the plasma. Since >96% of the radioactivity of the labeled Lp(a) and LDL was bound to the apolipoprotein, the turnover parameters of Lp(a) and LDL could be calculated directly from the die-away curves of ^{125}I and ^{131}I in the serum. In each subject, the serum decay curves of Lp(a) and LDL could be resolved into two exponential components indicating a two-compartment model as already demonstrated for these two lipoproteins (14, 15). Fig. 1 shows

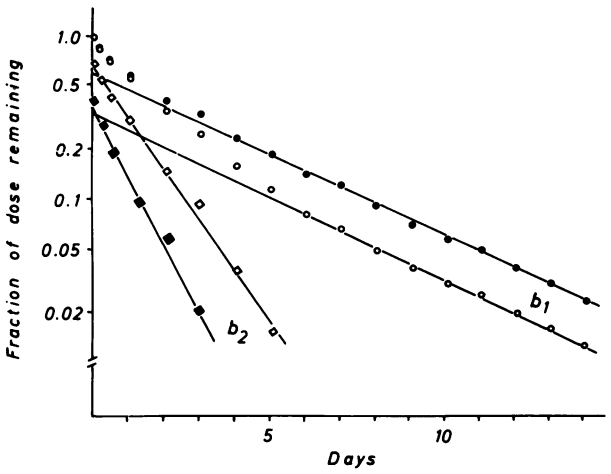

FIGURE 1 Serum die-away curves of subject 1 (Table II) after intravenous injection of ^{125}I-Lp(a) (●) and ^{131}I-LDL (○). The fraction of injected radioactivity remaining in the serum is plotted semilogarithmically against time. The radioactivity decay curves can be resolved into two exponential components. The first exponential (b_1) was calculated from the terminal linear portion of the decay curve. The second exponential [b_2 for Lp(a) ◆, b_2 for LDL ◇] was obtained by subtraction of the first exponential from the serum die-away curve.

representative serum die-away curves for the two lipoproteins in one subject. The fractional catabolic rate (FCR, i.e. the fraction of the intravascular pool catabolized per day) and the distribution of the label between the intra- and extravascular pool (percent intravascular) were calculated according to Matthews (36). The absolute rate of synthesis or catabolism was calculated as the product of FCR times plasma volume times plasma concentration of Lp(a) or apolipoprotein B of LDL. The plasma volume was estimated using Edelman's formula (37).

Statistical calculations. Statistical calculations were performed according to standard methods (38) using the BMDP 1981 program (39).

Chemical and immunological analyses. Serum triglycerides were estimated according to Eggstein and Kreutz (40). Total cholesterol was measured in the whole serum and in the $d > 1.006$-g/ml fraction by the Lieberman Burchard kit from Boehringer Mannheim GmbH, Mannheim, West Germany. HDL-cholesterol was measured after polyanion precipitation with phosphotungstate and $MgCl_2$ (41). In the $d > 1.006$-g/ml fraction, the concentration of Lp(a) and apolipoprotein B was measured. The concentration of Lp(a) was estimated by Laurell's electroimmunoassay as described recently (14). Apolipoprotein B concentration was measured by radial immunodiffusion (42). LDL-apolipoprotein B was obtained by subtraction of Lp(a)-apolipoprotein B, assuming that Lp(a) contains 25% apolipoprotein B by mass (8).

Materials. Eagle's MEM, streptomycin solution, trypsin-EDTA solution, and nonessential amino acid solution were purchased from Gibco Bio-Cult, Glasgow, Scotland. Penicillin was a product of Biochemie, Kundl, Austria. In all experiments serum of the same lot (Seromed, München, West Germany) was used. Tissue culture flasks and plates were purchased from Falcon Plastics, Div. of BioQuest, Oxnard, CA. [^{125}I]Sodium iodide and [^{131}I]sodium iodide were obtained from The Radiochemical Centre, Amersham, England, and Bio-Gel A-5m from Bio-Rad Laboratories, Richmond, CA. DL-3-hydroxy-3-methyl[3-^{14}C]glutaryl-CoA was obtained from New England Nuclear (Boston, MA), DL-mevalonic acid lactone from Sigma Chemical Co. (St. Louis, MO). All other enzymes and substrates for the HMG-CoA reductase assay were purchased from Boehringer Mannheim GmbH.

RESULTS

Equilibrium binding studies. Fibroblast monolayers were allowed to bind ^{125}I-Lp(a) and ^{125}I-LDL for 2 h at 4°C under equilibrium conditions for both ligands. Under these conditions no internalization and degradation occurs. Specific binding of ^{125}I-Lp(a) as well as ^{125}I-LDL to cultured human fibroblasts was saturable indicating a finite number of binding sites (Fig. 2). The maximum binding capacities for Lp(a) and LDL were calculated from the Scatchard plots (43) of six independent experiments using fibroblasts of two different cell lines. Fig. 2 also shows the Scatchard plots of one typical experiment. In all experiments the Scatchard plots of Lp(a) and LDL displayed linearity indicating a single category of binding sites without site-to-site interactions. Half maximal saturation occurred at a mean concentration of 9.52±1.04 nM for Lp(a) and 7.76±1.29 nM for LDL. These values are the dissociation constants (K_d) of the ligands for the binding sites. The single values are given in Table I.

Competition binding studies. The specificity of the binding of ^{125}I-Lp(a) to intact human fibroblasts was probed by competition studies utilizing unlabeled Lp(a) and LDL. The relative affinity of the unlabeled ligands is reflected by their relative potency in competing with ^{125}I-Lp(a) for these binding sites (Fig. 3). Specificity of ^{125}I-LDL binding sites was assessed for control purposes (Fig. 3).

When HDL were used to compete with ^{125}I-Lp(a) for the binding sites, ~100 times as much HDL (by weight) was required for 50% inhibition of ^{125}I-Lp(a) binding.

In another set of experiments a binding study with a Lp(a)-antigen–lipid complex was performed. Absolutely no specific binding of this particle to human fibroblasts could be observed.

Effect of cyclohexanedione. Cyclohexanedione treatment of Lp(a) and LDL completely abolished specific binding of these two lipoproteins to fibroblasts.

HMG-CoA reductase activity. HMG-CoA reductase activity of cultured human fibroblasts was measured after incubation with different concentrations of Lp(a) or LDL. In the absence of lipoproteins, HMG-CoA reductase activity was 50 pmol/min per mg protein and was suppressed to 16.9 pmol/min per mg protein by 4.5 nM Lp(a). This effect was similar to the

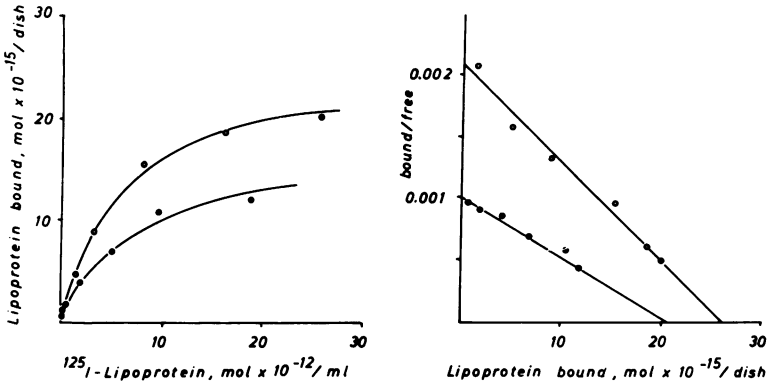

FIGURE 2 ^{125}I-Lp(a) and ^{125}I-LDL binding to fibroblast monolayers as a function of lipoprotein concentration. Fibroblast monolayers (340 μg protein/dish) were incubated with increasing concentrations (1–100 μg/ml) of ^{125}I-Lp(a) (●) or ^{125}I-LDL (○) at 4°C for 2 h. Left panel: The amount of lipoprotein bound specifically to surface receptors is plotted against the lipoprotein concentration in the medium. The specific binding refers to the difference in binding under the two conditions described in the method section. Each value represents the mean of two or three determinations in a typical experiment. Right panel: Scatchard plots of ^{125}I-Lp(a) (●) and ^{125}I-LDL (○) binding. The data from the left panel have been replotted. The linear regression analysis bound/free vs. bound yielded intercepts on the abscissa (maximal binding capacity) of 20.94 × 10^{-15} mol/dish for Lp(a) and of 25.91 × 10^{-15} mol/dish for LDL. The equilibrium dissociation constants, estimated from the slopes of the regression lines, were 9.32 × 10^{-9} M for Lp(a) and 5.65 × 10^{-9} M for LDL.

suppression of HMG-CoA reductase activity to 11.5 pmol/min per mg protein by 5.2 nM LDL. The decrease in HMG-CoA reductase activity by different concentrations of Lp(a) and LDL is shown in Fig. 4. If the percent suppression of HMG-CoA reductase activity is brought in relation to lipoprotein cholesterol, then Lp(a) is less effective, since Lp(a) has an approximately twofold higher molecular weight, but only a slightly lower cholesterol content than LDL.

Binding to fibroblasts in homozygous FH. In cultured fibroblasts from the patient with homozygous FH no specific binding of either Lp(a) or LDL could be demonstrated.

TABLE I
Dissociation Constants (K_d) and Maximum Binding Capacities (B_{max}) for Lp(a) and LDL

	Lp(a)		LDL	
	K_d	B_{max}	K_d	B_{max}
	nM	*fmol/dish*	*nM*	*fmol/dish*
	9.3	20.9	5.6	25.9
	11.8	17.2	8.9	28.7
	10.9	14.1	8.8	22.6
	9.2	28.7	7.9	55.8
	8.6	13.4	8.5	21.7
	7.3	17.7	6.9	23.4
Mean	9.52	18.66	7.76	29.68
SD	1.04	5.60	1.29	13.04

Turnover studies in normal individuals. The single values, means, standard deviations, medians, and quartiles of the kinetic parameters are shown in Table II. In each individual the FCR of Lp(a) was lower than the FCR of LDL. On the average, FCR of Lp(a) was 31% lower than the corresponding value of LDL. Since the distributions of the single values are not normal, nonparametric methods were used for statistical calculations. The difference between the mean FCR of Lp(a) and LDL was statistically significant (Wilcoxon signed-rank test. Median of the differences: 0.108, $P < 0.01$). Since there is no unanimous agreement concerning the apolipoprotein B content of Lp(a), the absolute synthetic rate of Lp(a) has been calculated in terms of total lipoprotein mass instead of apolipoprotein B mass as usually done for LDL. The mean absolute synthetic rate of Lp(a) was >10 times lower than the corresponding value of LDL (assuming that the apoprotein B content of LDL is ~20%). There was no difference in the half-life of the terminal linear part of the radioactivity decay curve between Lp(a) and LDL.

In each individual, the percentage of the Lp(a) pool that was found in the intravascular compartment (percent intravascular) was higher than the corresponding value of LDL. On the average, the percent intravascular of Lp(a) was 19% higher than that of LDL. The

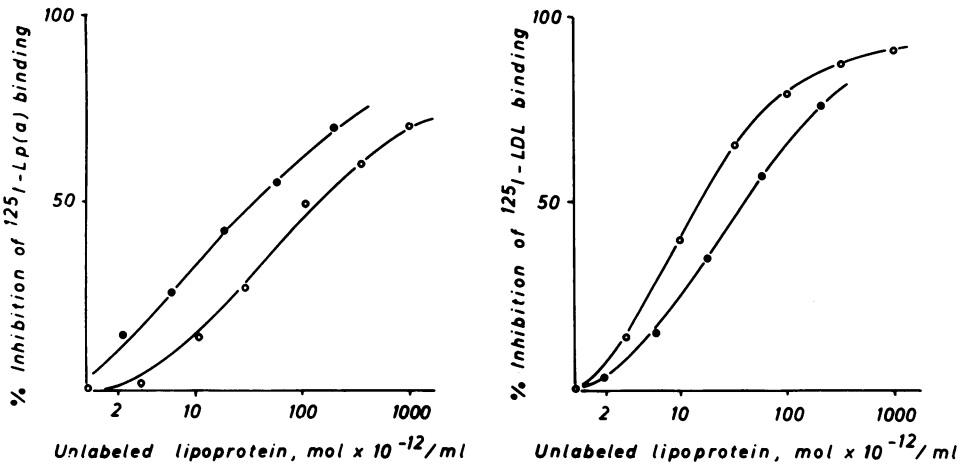

FIGURE 3 Competition binding studies: various concentrations of Lp(a) (●) and LDL (○) were incubated with intact fibroblasts with ^{125}I-Lp(a) (left panel) or ^{125}I-LDL (right panel) for 2 h at 4°C. Specifically bound counts were then determined as described in the method section. The data shown are mean values of three determinations.

difference between percent intravascular of Lp(a) and LDL was highly significant (median of the differences: 10.75, $P < 0.01$).

A highly significant positive correlation was found between FCR of LDL and FCR of Lp(a) (Spearman rank correlation: $r = 0.853$, $P < 0.01$) (Fig. 5). No relationship could be demonstrated between the serum concentration of Lp(a) and LDL-apolipoprotein B ($r = 0.311$). In agreement with previous results (14), a highly significant positive relationship between the absolute synthetic rate and the serum concentration of Lp(a) ($r = 0.979$, $P < 0.01$) was obtained. A negative, but statistically insignificant correlation was found between the FCR and the serum level of Lp(a) ($r = -0.489$, $P > 0.05$). In contrast to Lp(a), for LDL a negative correlation between the FCR and the serum concentration of LDL-apoprotein B was obtained ($r = -0.613$, $P < 0.05$) (Fig. 6). No correlation between the synthetic rate of LDL-apolipoprotein B and the serum level of LDL-apolipoprotein B was found ($r = 0.089$).

Turnover study in the patient with homozygous FH. The kinetic parameters of the patient with homozygous FH are presented in Table II. FCR of LDL was lower and the rate of LDL-apolipoprotein B synthesis was increased when compared with the corresponding values of the normals. FCR of Lp(a) was low, but within the standard deviation of the normal in-

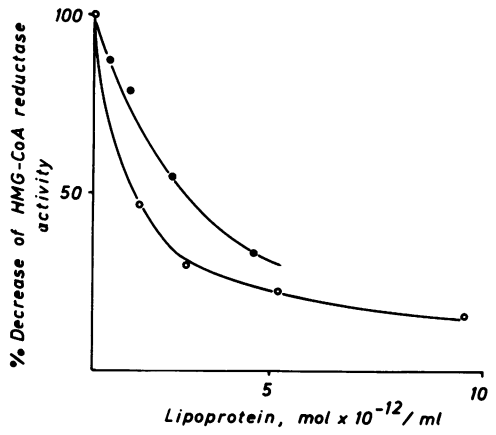

FIGURE 4 Suppression of HMG-CoA reductase activity in human fibroblasts after incubation with different concentrations of Lp(a) (●) and LDL (○).

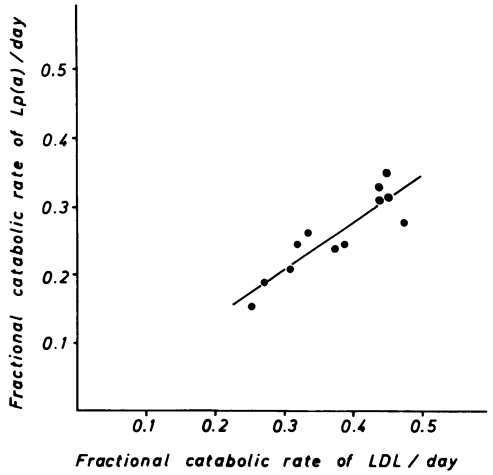

FIGURE 5 Relationship between FCR of LDL and FCR of Lp(a) in normolipemic subjects ($r = 0.853$, $P < 0.01$).

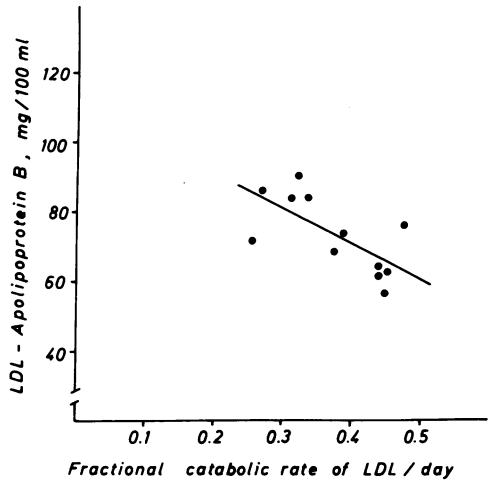

FIGURE 6 Relationship between FCR of LDL and the concentration of LDL-apolipoprotein B in the serum of normolipemic subjects ($r = -0.613$, $P < 0.05$).

dividuals. In contrast to LDL, the synthetic rate of Lp(a) was low. This explains the relatively low Lp(a) serum concentration in this FH patient.

DISCUSSION

The specific receptor-mediated catabolic pathway of LDL has been shown to play a major role in the regulation of the LDL metabolism (19, 20, 23). Goldstein and Brown (23) calculated from their own experiments and from kinetic data of others that in normolipemic subjects ~30% of the plasma pool is daily cleared by the receptor pathway, and ~15% of the plasma LDL is daily removed and catabolized via a nonreceptor route ("scavenger pathway").

In view of the similarities between Lp(a) and LDL with regard to their physicochemical properties, characteristics of Lp(a) binding sites were assessed by means of direct binding studies with ^{125}I-Lp(a). Saturable, high-affinity binding sites for ^{125}I-Lp(a) were identified in intact cultured human fibroblasts. The receptors studied are most likely a single receptor population as concluded from the linearity of the Scatchard plots. Scatchard analysis of binding equilibrium yielded an ~20% lower affinity for ^{125}I-Lp(a) binding as compared to ^{125}I-LDL binding. The number of ^{125}I-Lp(a) binding sites was constantly found to be lower than for LDL.

Studies of the relative potency of unlabeled Lp(a) and LDL in competing for ^{125}I-Lp(a) binding sites yielded similar results for both ligands. Thus, it is unlikely that binding of ^{125}I-Lp(a) occurs to an own specific Lp(a) receptor population distinct from the LDL receptors. This assumption is further supported by the finding that LDL receptor-deficient fibroblasts from a patient with homozygous FH did not bind specifically Lp(a). Mahley et al. (25) showed that modification of the arginyl residues of apolipoprotein B by cyclohexanedione abolished binding of LDL to high affinity cell surface receptors of human fibroblasts. In our experiments, cyclohexanedione treatment of Lp(a) and LDL resulted in a complete loss of specific binding of both lipoproteins. From this result it is suggested that similar recognition sites on both lipoproteins are responsible for specific binding to the receptor. The binding experiments with apolipoprotein B-free Lp(a)-antigen/lipid particles indicate that the specific Lp(a) antigen is not bound by the receptor. Therefore, similarly to LDL, the apolipoprotein B moiety of Lp(a) seems to be responsible for the recognition by the receptor.

Under the assumption that Lp(a) and LDL bind to the same type of receptor, the differences in affinity and binding capacity could be due to the larger molecular weight of the Lp(a) particles, which is approximately twice as high as LDL (11, 33). On the other hand, the specific Lp(a) antigen could possibly mask some of the apolipoprotein B recognition sites leading to altered steric effects of binding.

The inhibition of HMG-CoA reductase activity after binding of Lp(a) to the fibroblasts indicates that Lp(a) is taken up into the cells and regulates intracellular cholesterol metabolism in a similar way as LDL. Lp(a) seems to be less effective than LDL in suppressing HMG-CoA reductase activity. This could be a consequence of the lower affinity to the receptor.

There is, however, one major drawback to the hypothesis of a simple monocomponent binding system for LDL and Lp(a). If only one type of receptor exists with high affinity to LDL and a somewhat lower affinity to Lp(a), one would expect that unlabeled LDL is more potent than Lp(a) in competing for ^{125}I-Lp(a) binding sites. No significant difference between these two lipoproteins in competing for the binding sites, however, was found. Therefore the results of this study do not definitely rule out the possibility of a multicomponent receptor system. One could, for instance, speculate that the receptor-protein exists in two different conformations for Lp(a) and LDL. The theoretical possibility that the LDL receptor is a multicomponent molecule that possesses at least two discrete active sites, has been mentioned by Brown and Goldstein (44) in another context.

In a previous study turnover parameters of Lp(a) (14) were found to be similar to those of LDL as reported by others (15–18). In the present study the turnover of Lp(a) and LDL were measured simultaneously in normolipemic individuals. The FCR of Lp(a) was ~30% lower than FCR of LDL in each subject. A

TABLE II

Concentrations of Serum Lipids and Kinetic Parameters of Lp(a) and LDL

Subject	Age	Height	Weight	Serum cholesterol	Serum triglycerides	LDL-apolipoprotein B	Serum Lp(a)	$t_{\frac{1}{2}}$* of LDL
No.	*yr*	*cm*	*kg*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*d.*
1	64	173	71	188	209	56	12	3.01
2	61	185	92	182	88	61	18	4.25
3	76	155	50	203	124	76	10	3.71
4	52	168	67	187	88	64	45	3.05
5	82	170	66	245	143	86	50	4.38
6	50	168	55	158	80	63	4	3.79
7	74	170	74	180	104	72	43	4.65
8	44	164	70	284	150	86	84	4.62
9	66	170	69	191	73	84	91	3.59
10	80	156	57	202	190	91	9	2.85
11	51	174	61	198	87	68	41	4.25
12	78	170	64	191	114	74	92	3.94
Mean						73.4	41.6	3.84
SD						11.4	32.7	0.62
Median						73.0	42.0	3.87
Q_1, Q_2§						63.5, 85.0	11.0, 67.0	3.32, 4.32
13‖	16	160	46	521	65	253	17	5.17

* Half-life of the terminal linear part of the serum radioactivity time cure from day 2–14.

‡ Units for Lp(a) synthesis refer to the entire Lp(a) molecule [because of lack of an accurate estimation of apolipoprotein B in Lp(a)].

§ First and third quartile.

‖ Patient with homozygous familial hypercholesterolemia.

highly significant positive correlation between the FCR of Lp(a) and LDL was obtained. This result is consistent with the hypothesis that in normolipemic subjects the specific LDL receptors play a role also for the removal of Lp(a).

Although a close correlation between the FCR of Lp(a) and LDL was obtained, no relationship was found between the serum levels of these two lipoproteins. The lack of correlation between Lp(a) and LDL serum levels has also been observed by others (12). For Lp(a), no relationship between the FCR and the serum level, but a highly significant correlation between the synthetic rate and the serum concentration was found. This is in agreement with our previous finding that the Lp(a) serum concentration is mainly regulated by the synthetic rate of this lipoprotein (14). The synthetic rate of Lp(a) (Table II) is expressed in terms of total lipoprotein mass because of lack of an accurate estimation of the apolipoprotein B content of Lp(a). The reported values of the protein content of Lp(a) vary from 30 to 38% (8–11, 45), and it is not clear which portion of the Lp(a) apolipoproteins comprises apolipoprotein B. Assuming from the literature that the apolipoprotein B content is ~25% of the total Lp(a) mass, the values given in Table II may be divided by four in order to obtain the synthetic rate of apolipoprotein B in Lp(a). In contrast to Lp(a), no correlation between synthetic rate and serum level could be demonstrated for LDL, but a weak, yet statistically significant negative correlation between FCR and serum level of LDL was obtained. Therefore, apparently different mechanisms regulate the serum concentrations of these two lipoproteins. This might explain why the serum levels of Lp(a) and LDL are not related to each other.

In the patient with homozygous FH, the FCR of LDL was markedly lower than in any of the normolipemic subjects. This value was of the same magnitude as reported for other homozygous patients (16). FCR of Lp(a) was also decreased, but not to the same extent as that of LDL. This result has to be discussed with regard to the quantitative role of the LDL receptor in the removal and catabolism of Lp(a). The turnover and binding studies in normal subjects seem to indicate

TABLE II (*Continued*)

Fractional catabolic rate of LDL	Rate of LDL-apoprotein B synthesis	Distribution of LDL	$t_{\frac{1}{2}}$* of Lp(a)	FCR of Lp(a)	Rate of Lp(a)‡ synthesis	Distribution of Lp(a)
Fraction of i.v. pool/d	*mg/kg/d*	*% intravascular*	*d*	*Fraction of i.v. pool/d*	*mg/kg/d*	*% intravascular*
0.452	12.04	69.5	3.02	0.351	2.00	72.1
0.441	12.81	50.5	3.57	0.329	2.81	68.9
0.475	17.18	57.1	3.55	0.275	1.31	75.6
0.440	12.10	64.1	3.36	0.310	5.99	73.6
0.271	11.09	66.8	5.37	0.188	4.47	74.7
0.455	12.32	54.8	3.52	0.316	0.54	69.7
0.256	8.62	65.8	5.46	0.153	3.13	86.6
0.310	11.19	66.5	4.08	0.211	7.62	77.6
0.334	13.35	70.2	3.59	0.263	11.39	80.6
0.322	13.95	85.6	2.95	0.246	1.05	93.2
0.373	10.90	58.3	4.50	0.235	4.12	65.4
0.391	13.77	61.5	4.20	0.243	10.64	74.8
0.377	12.44	64.2	3.93	0.260	4.60	76.1
0.077	2.09	9.1	0.83	0.060	3.64	7.7
0.382	12.21	64.95	3.58	0.255	3.63	74.8
0.316, 0.447	11.14, 13.56	57.7, 68.2	3.44, 4.35	0.223, 0.313	1.66, 6.81	70.9, 79.1
0.205	23.44	69.4	4.81	0.210	1.61	71.6

that Lp(a) is removed by the same mechanisms as LDL. The result of the turnover experiment in the homozygous FH patient, however, could be an argument against a major role of the LDL receptor in the removal of Lp(a), since in this patient the FCR for Lp(a) was 81% of the mean FCR for Lp(a) in the normal subjects, whereas the FCR for LDL was decreased to 54% of normal. In the homozygous FH patient neither Lp(a) nor LDL were bound by specific cell surface receptors. Therefore, these lipoproteins have to be removed by a nonreceptor pathway. The finding that in the absence of specific LDL receptors the FCR of Lp(a) is less reduced than that of LDL indicates that: at least in this homozygous FH patient the nonreceptor pathway is more effective in removing Lp(a) than LDL. The conditions that influence the amount of LDL and Lp(a) that is catabolized via the nonreceptor pathway are poorly understood. Until now, no studies on the catabolism of Lp(a) via the nonreceptor pathway have been performed. Our studies only show that under normal conditions Lp(a) is bound and degraded by the receptor system, but they do not explain which percentage of the Lp(a) pool is cleared by the receptor or by the nonreceptor pathway under various conditions.

Although it has to be assumed that in this patient neither Lp(a) nor LDL can be removed by the receptor pathway, only the level of LDL was increased in the serum. In our FH patient the high serum concentration of LDL was the consequence of the decreased FCR and the increased synthetic rate of LDL. An increased rate of LDL synthesis in FH has also been reported by others (16, 18). The synthetic rate of Lp(a), however, was low in our FH patient. This might explain why the Lp(a) serum level was not increased in contrast to the LDL level.

It has been shown by others that cell surface receptors are able to bind with high affinity not only LDL, but also VLDL (19, 46) as well as HDL_c, an apolipoprotein E containing subfraction of HDL (47, 48). This study gives evidence that Lp(a) is an additional lipoprotein that is specifically bound to cell surface receptors. The binding of Lp(a) to these receptors seems to play a role in the regulation of the intracel-

lular cholesterol metabolism and in the removal of Lp(a) from the plasma.

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