Binding and Degradation of Lipoprotein(a) and LDL by Primary Cultures of Human Hepatocytes

Comparison With Cultured Human Monocyte-Macrophages and Fibroblasts

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Abstract

Although lipoprotein(a) (Lp[a]) has structural similarities to low-density lipoprotein (LDL) that include the presence of apolipoprotein B\(_{\text{apo}}\), there is some disagreement over the strength of its interaction with the LDL receptor and its cellular catabolism by the LDL receptor-mediated pathway. To clarify this subject we evaluated LDL receptor-mediated binding and degradation of Lp(a) and LDL in three human cell lines. The binding of 50 nmol/L Lp(a) at 37°C to the LDL receptor of primary hepatocytes, macrophages, and fibroblasts was only 10%, 29%, and 29% of the respective value obtained with 50 nmol/L LDL. Analysis of 4°C binding curves indicated that Lp(a) and LDL had equal affinities for the LDL receptor of fibroblasts, whereas maximal binding of Lp(a) was remarkably lower than that of LDL. LDL receptor-mediated degradation of 50 nmol/L Lp(a) in hepatocytes, macrophages, and fibroblasts was only 17%, 22%, and 26%, respectively, of the value obtained with 50 nmol/L LDL and varied greatly among the cells in that it was lowest in hepatocytes, an order of magnitude greater in macrophages, and two orders of magnitude greater in fibroblasts. In contrast, the nonspecific degradation rate of Lp(a) was similar to that of LDL in each of the three tested cell lines. However, the proportion of the degradation of Lp(a) that was nonspecific varied greatly, being 76%, 58%, and 33% in hepatocytes, macrophages, and fibroblasts, respectively. These studies indicate that not only is Lp(a) recognized by the LDL receptor but also that, in fibroblasts, Lp(a) and LDL have equal affinities for the LDL receptor, although Lp(a) has a much lower receptor occupancy than LDL. Additionally, they show that there are great cellular differences in the LDL receptor-mediated degradation of Lp(a). If these results can be extrapolated in vivo, where normal LDL levels are 40- to 50-fold higher than those of Lp(a), it would be unlikely that the hepatic LDL receptor is significantly involved in the degradation of Lp(a). (Arterioscler Thromb. 1994;14:770-779.)

Key Words: • dissociation constant • competitive inhibition • catabolism • steric hindrance

Studies on the cellular catabolism of lipoprotein(a) (Lp[a]) have focused on the role of the low-density lipoprotein (LDL) receptor because of its importance in the clearance of LDL from circulation and the fact that apolipoprotein (apo) B is an integral component of this LDL-like lipoprotein particle. Fibroblasts have been the cell line of choice in many of these investigations,\textsuperscript{1-12} while other cell lines have received less attention.\textsuperscript{12-16} Because of the importance of the hepatic LDL receptor in the clearance of LDL, we examined Lp(a) catabolism in primary cultures of human hepatocytes. Recent advances in medium formulation, culture technique, and in understanding hepatotropic growth factors have made it possible to maintain human hepatocytes in culture with continued capacity for proliferation and survival for 2 months or longer.\textsuperscript{17-19} This model system more closely approximates native human hepatocyte physiology than neoplastic cell lines such as Hep G2 or Hep 3B, as was concluded recently by De Water et al\textsuperscript{10} on discovering significant differences between the interaction of \(\beta\)-very-low-density lipoprotein with Hep G2 cells and with normal human hepatocytes.

Drawing on our experience with long-term cultures of primate hepatocytes,\textsuperscript{17,19} we have examined the binding and degradation of human Lp(a) and LDL in primary hepatocytes prepared from liver samples. We have further compared these findings with those obtained in cultured human monocyte-derived macrophages (HMDMs) and fibroblasts. HMDMs are an important component of atheromatous lesions and are known to possess receptors of high specificity for LDL. Fibroblasts were chosen because binding and degradation of LDL are almost completely mediated by the LDL receptor. We anticipated that the interaction of Lp(a) with fibroblasts might be similar and thus would not be confounded by interactions with other binding sites. Although past studies with fibroblasts have not yielded a consensus regarding the binding of Lp(a) by the LDL receptor, the most recent evidence suggests that Lp(a) binds, but with reduced affinity.\textsuperscript{5-8} Lp(a) also binds to the LDL receptor of HMDMs at 4°C but with an affinity nearly equal to that of LDL, although degradation of Lp(a) by the LDL-receptor pathway was much reduced in comparison with LDL.\textsuperscript{13} Some variability in the reported results with fibroblasts may have been caused by comparing Lp(a) and LDL on an equal protein, immunologically determined apoB, or cholesterol basis. Another source of variation may have been Lp(a) heterogeneity, as there is some evidence suggesting that different Lp(a) species...
are degraded differently by the LDL-receptor pathway of macrophages.14 To eliminate such sources of ambiguity, we tested only Lp(a) preparations having the same apo(a) isoform with all three cell types and compared our results with those of autologous LDL on a molar basis.

Methods

Materials

Sodium [125I]iodide was purchased from Amersham Corp., "Williams’ medium E (WME); minimum essential medium (MEM) with Earl’s salts and without L-glutamine; Dulbecco’s modified Eagle’s medium (DMEM) containing 25 mmol/L N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES), L-glutamine, and 4500 mg/L glucose; HEPES; L-glutamine; phosphate-buffered saline minus calcium and magnesium (PBS-CMF); and the antibiotic/antimycotic solution containing penicillin, streptomycin, and amphotericin B were from Gibco Laboratories. Fetal bovine serum (FBS) was from Hazelton Biologics; low-heat was from Merck Sharp & Dohme. MEM nonessential amino acid solution, collagenate types IV, heparin, 3-mono-L-iodotyrosine, growth factors, hormones, and other additives were obtained from Sigma Chemical Co. Plastic 12-well tissue culture dishes (4.5 cm² per well) were from Flow Laboratories.

Preparation of Lp(a) and LDL

Autologous Lp(a) and LDL were isolated from the plasma of two subjects with a fast apo(a) polymorphism (M.L.S., 281 000) as previously described.15 Before donation, both subjects gave informed consent. Lp(a) and LDL preparations were carried out for purity by sodium dodecyl sulfate (SDS)—gradient gel electrophoresis on 2.5% to 16% acrylamide gels (Isolab). Briefly, the Lp(a) and LDL (15 µg per sample) were incubated with 1% SDS for 5 minutes at 95°C. The gels were stained with Coomassie blue R-250. Both lipoproteins were stored after filter sterilization (0.45 µm) in Sarstedt vials filled to allow no airspace in 0.15 mol/L NaCl, pH 7.4, containing 0.01% Na₂EDTA and NaN₃.

Radioiodination of Lp(a) and LDL

Lp(a) and LDL were radioiodinated by the iodine monochloride method of McFarlane21 as modified by Bilheimer et al.22 The specific radioactivity for both lipoproteins averaged 2500–5000 cpm/µg apoB. The integrity of the radiolabeled Lp(a) and LDL was determined by 2.5% to 16% SDS—gradient gel electrophoresis. Approximately 10 000 cpm of [125I]-labeled Lp(a) and LDL each was transferred to nitrocellulose and then exposed to Kodak XAR-5 film. Further evidence supporting the notion that radioiodination does not denature the lipoprotein preparations came from previous work in which we showed that neither 131I-LDL nor 125I-Lp(a) bound to the scavenger receptor of human monocyte-macrophages.14 The radiolabeled lipoproteins were stored after filter sterilization (0.45 µm) and used within 2 weeks of preparation.

Isolation and Culture of Hepatocytes

The use of human liver in these studies was approved by the Institutional Review Board of The University of Chicago Division of the Biological Sciences. Tissue was obtained through the liver tissue procurement and distribution system. As required by federal statutes guiding human organ procurement, family members of organ donors provided written permission for tissue to be used for research purposes. The livers had been obtained from heart-beating cadaver donors for the purpose of segmental liver transplantation.23 During procurement, in situ perfusion with ice-cold 0.9% NaCl was followed by ex vivo perfusion with ice-cold University of Wisconsin preservation solution24 and immersion in preservation solution at 4°C. After transport to The University of Chicago, ex vivo segmentectomy was performed to obtain a graft for transplantation as described. The remainder of the liver was immediately transported to the laboratory, where under sterile conditions a 40- to 60-g portion was excised from an intact edge so that capsule covered all but the cut surface. Hepatocytes were then isolated from wedge segments and maintained in tissue culture by the procedure described by Lanford et al17 as adapted for human liver samples by Black et al.18 Briefly, the liver segment was initially perfused for 10 minutes at 50 mL/min with the calcium- and magnesium-free buffer of Seglen25 supplemented with 0.5 mmol/L EDTA, followed by perfusion for an additional 20 minutes at 50 mL/min with WME supplemented with 10 mmol/L HEPES (pH 7.4) and 200 U/mL collagenase type IV. Both perfusion buffers were kept saturated with bubbled oxygen at 37°C. After perfusion, the liver capsule was removed with sterile forceps, and the hepatocytes were dislodged and further dispersed in the collagenase solution using a stainless steel dog comb. The cell suspension was filtered through several layers of sterile gauze and mixed with an equal volume of WME containing 10% FBS. Cells were sedimented by centrifugation at 50g for 5 minutes, resuspended in WME containing 5% FBS, and reseeded and resuspended in the same manner an additional two to three times until the supernatant was clear.

The final cell suspension was plated at a density of 2.2×10⁶ cells/cm² into 12-well tissue culture dishes, and the cells were incubated at 37°C in 10% CO₂ for 16 to 24 hours to permit attachment. The cells were then washed with PBS(CMF) to remove nonadherent cells and overlaid with the serum-free medium III formulation (SFM III) described by Lanford et al.17 except that penicillin, streptomycin, and amphotericin B were substituted for gentamicin. The medium was changed every 72 hours thereafter. Within 10 to 14 days after plating, cells proliferated to a state of near confluency. By light microscopy, hepatocytes made up more than 98% of the cell population. Electrophoretic analysis of conditioned medium from both pulse-labeled and nonlabeled hepatocyte cultures confirmed their viability because of the presence of lipoprotein particles, including Lp(a) and HDL, in addition to apoB100, apoE, apoA-I, apoCs, plasminogen, fibrinogen, and albumin19 (M.L.S., G.M.F., this study, unpublished results).

Isolation and Culture of HMDM

Isolation and culture of human monocytes from donors who gave informed consent were carried out as previously described.13 The HMDMs were classified, and their viability was determined as described by Fogelman et al.26 The cells were greater than 99% monocyte-macrophages by the time the experiments were performed, and there was no change in cell viability after either binding or degradation experiments.

Culture of Human Fibroblasts

 Cultures of normal human fibroblasts (GM03348C) were obtained from the National Institute of General Medical Sciences Human Genetic Mutant Cell Repository and grown essentially according to procedures of Goldstein et al.27 On day 0, the fibroblasts were seeded at a density of 3×10⁴ cells/cm² in MEM with Earle’s salts supplemented with 1% MEM nonessential amino acid solution, 2 mmol/L L-glutamine, and 10% FBS into 12-well tissue culture dishes. The medium was replaced on day 3, and on day 5 the fibroblasts were switched into MEM containing 10% lipoprotein-deficient serum. All experiments were performed on day 7 when the cells had reached 80% confluence.

4°C Binding

Lipoprotein binding assays at 4°C were carried out essentially according to Innerarity et al28 using either 8-day-old HMDMs, 15- to 23-day-old hepatocytes, or 7-day-old fibroblasts. Briefly, the cell monolayers were cooled to 4°C, the medium was then removed, and medium containing 125I-Lp(a)
labeled lipoprotein with and without a 50-fold molar excess of unlabeled LDL that had been previously cooled to 4°C was added. Dishes were incubated for 4 hours with rocking. The cells were then washed four times with 1.0 mL per well of ice-cold PBS(CMF) (0.2% bovine serum albumin) and once with 1.0 mL per well of ice-cold PBS(CMF); the first wash was allowed to sit 10 minutes before proceeding with the remaining four washes in rapid succession as described by Haberland et al.29 The cell monolayer was then dissolved with two additions of 0.5 mL of 0.1N NaOH and counted for radioactivity, and an aliquot was removed to determine cellular protein by the method of Lowry et al30 as modified by Markwell et al11 using bovine serum albumin as a standard.

4°C Competitive Binding

Competitive binding assays at 4°C were carried out on 7-day-old fibroblasts. Briefly, the cell monolayers were chilled to 4°C, and the medium was removed and replaced with medium containing either 10 nmol/L 125I-Lp(a) or 125I-LDL alone and with increasing amounts of either unlabeled Lp(a) or LDL that had been previously chilled. Radiolabeled and native lipoproteins were mixed together before addition to the cells. Dishes were incubated for 4 hours at 4°C with rocking and washed, and the amount of radioactivity bound was determined as described above. In calculating the percentage of inhibition, the difference in binding obtained between 500 nmol/L unlabeled lipoprotein and no unlabeled lipoprotein was taken as 100% inhibition. Binding in the absence of unlabeled lipoprotein was taken as 0% inhibition.

37°C Binding and Degradation

Binding and degradation experiments were performed essentially as stated by Goldstein et al12 on either 8-day-old HMDMs, 15- to 23-day-old hepatocytes, or 7-day-old fibroblasts. Briefly, medium containing varying concentrations of either 125I-Lp(a) or 125I-LDL with and without a 50-fold molar excess of unlabeled LDL was added to the cells, and the cells were returned to the 37°C incubator for 5 hours. Proteolytic degradation of 125I-labeled lipoprotein was measured by assaying the amount of 125I-labeled trichloroacetic acid-soluble (noniodide) material formed by the cells and excreted into the medium. After 5 hours, the cell monolayers were cooled to 4°C, washed as described above, and then incubated for 1 hour with 10 mg/mL heparin in 50 mmol/L NaCl and 10 mmol/L HEPES, pH 7.4. The heparin wash was collected and counted for radioactivity to determine the heparin-releasable fraction. The cell monolayers were washed again, lysed, and counted for radioactivity to determine the heparin-resistant fraction, and protein was determined as described for the 4°C binding assays.

Analysis of Binding Data

The binding data were analyzed according to procedures outlined by Munson and Rodbard.32 We assumed that the interaction of the lipoprotein with the LDL receptor could be described by a single-site model according to the following equation:

\[\frac{[Lp]/[R]}{K_d + [Lp]/[R]}\]

where \([Lp]\) is the free lipoprotein, \([R]\) the unoccupied receptor, and \([Lp-R]\) the lipoprotein receptor complex. At apparent equilibrium where the forward and reverse reactions are equal, the law of mass action states that \(K_d = [Lp]/[R][Lp-R]\) where \(K_d\) (the dissociation constant) = \(K_a/K_i\). If the total receptor concentration is \(B_{max}\), then

\[K_d = [Lp]/(B_{max} - [Lp-R])/[Lp-R]\]

and

\([Lp-R] = B_{max} - [Lp-R]/[Lp-R]\)

Experimental data were fitted to this equation using the general curve-fitting routines of the KALEIDA-GRAPH graphics software.

Statistics

Statistical significance of differences between means was estimated using either the paired or unpaired Student’s t test. Analyses were performed using the software program STATVIEW.

Results

Cellular Binding of Lp(a) and LDL at 4°C

The binding of Lp(a) and LDL to primary human hepatocytes held at 4°C is shown in Fig 1A and 1B. For comparison, binding curves obtained with HMDMs (Fig 1C and 1D) and normal human fibroblasts (GM03348C; Fig 1E and 1F) are also included. Total binding of Lp(a)
to human hepatocytes was linear, and competition with a 50-fold molar excess of LDL depressed binding of Lp(a) to only a small extent, indicating a minimal involvement of LDL-specific binding sites. Although the total binding curve obtained for LDL was curvilinear and appeared to be reaching saturation, there was a substantial nonspecific component. Analysis of the LDL-specific binding curve for LDL indicated that the LDL receptor of human hepatocytes exhibited a high affinity for LDL, with a dissociation constant of 22 nmol/L and saturation at 50 nmol/L lipoprotein. However, because LDL-specific binding of Lp(a) at 4°C was such a small fraction of total binding, we were unable to accurately determine a dissociation constant for the interaction of Lp(a) with the hepatocyte LDL receptor.

In contrast, both fibroblasts and HMDMs bound a much larger proportion of Lp(a) through LDL-specific binding sites. However, as we observed previously,13 the binding of both Lp(a) and LDL to the LDL receptor of HMDMs was of low affinity (0.80 μmol/L for Lp(a) and 0.22 μmol/L for LDL) and required greater than 1 μmol/L lipoprotein to saturate LDL-specific binding sites on HMDM. On the other hand, binding of both Lp(a) and LDL by the LDL receptor of fibroblasts was saturable at much lower concentrations (20 to 30 nmol/L) and was of sufficient magnitude to permit accurate analyses of their respective binding curves. These results are shown in Table 1 and indicate that the affinity of Lp(a) (8.0 nmol/L) for the LDL receptor of fibroblasts was equal to that of LDL (11.4 nmol/L) within the experimental error of the methods. However, the maximal amount of Lp(a) bound to the LDL receptor (Bmax) was only 41% of the value obtained with LDL.

The LDL-specific nature of Lp(a) binding to fibroblasts was also confirmed by competitive binding assays performed at 4°C. As shown in Fig 2, unlabeled LDL competed equally effectively for both radioiodinated LDL and Lp(a), in that 14 nmol/L cold LDL was sufficient to inhibit 50% of specific binding. Unlabeled Lp(a), on the other hand, was less effective in competing for 125I-Lp(a) and had an IC50 value of 40 nmol/L. The higher value is probably the result of binding to sites specific for the apo(a) moiety of Lp(a) in addition to its interaction with the LDL receptor. In competition with 125I-LDL, unlabeled Lp(a) was less effective than unlabeled LDL, in that 57 nmol/L LDL was needed to inhibit 50% of specific binding. Nonspecific binding was 26%, 46%, 60%, and 66% of total binding at 50-fold molar excess of unlabeled lipoprotein when 125I-LDL competed with LDL and Lp(a) and when 125I-Lp(a) competed with Lp(a) and LDL, respectively.

### Table 1. Lp(a) and LDL 4°C-Binding Parameters of the Fibroblast LDL Receptor*†

<table>
<thead>
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<th>Kn (nmol/L)</th>
<th>Bmax (pmol/mg Cell Protein)</th>
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<tbody>
<tr>
<td>Lp(a)</td>
<td>8.0±1.1</td>
<td>0.12±0.03</td>
</tr>
<tr>
<td>LDL</td>
<td>11.4±4.5</td>
<td>0.29±0.06</td>
</tr>
</tbody>
</table>

Lp(a) indicates lipoprotein(a); LDL, low-density lipoprotein; Kn, dissociation constant; and Bmax, total receptor concentration.
*Values are the mean±SD of three experiments.
†Equilibrium dissociation constants determined assuming a one-site model.

### Cellular Degradation of Lp(a) and LDL

The degradation of Lp(a) and LDL as a function of lipoprotein concentration is illustrated in Fig 3. Overall, the degradation curves for Lp(a) and LDL were similar in appearance to the 4°C binding curves shown in Fig 1, indicating that the degradation of Lp(a) by hepatocytes and HMDMs also may occur by way of a nonsaturable process. Fibroblasts, on the other hand, degraded Lp(a) and LDL mainly via the LDL-receptor pathway, since competition with a 50-fold molar excess of cold LDL revealed only a small nonspecific component. In contrast, degradation of LDL in hepatocytes was almost equally specific and nonspecific. Although degradation of LDL in HMDMs was mainly LDL-receptor specific, it differed from the other two cell lines, in that the uptake of LDL occurred by way of a low-affinity process. The main difference among the three different cell types was that the degradation rate for both Lp(a) and LDL in hepatocytes was much lower than in HMDMs and especially fibroblasts.

### Comparison of the Binding and Degradation of Lp(a) and LDL by the Three Cell Types

To compare the binding and degradation of Lp(a) and LDL by the three cell types, we chose a lipoprotein concentration of 50 nmol/L that was slightly higher than the mean Lp(a) level (25 to 35 nmol/L) of a white population.33 (The molar concentration of Lp[a] is variable because of size polymorphism in apo[a] and depends on the molecular weight of the protein moiety of the Lp[a] standard used in the assay. The higher concentration correlates with the small apo[a]-isofrom Lp[a] used in this study). As mentioned above, the LDL receptors of both hepatocytes and fibroblasts appeared to be saturated at...
this concentration. It was not feasible to study LDL-specific binding and degradation of Lp(a) at saturation in HMDMs because of the low affinity of Lp(a) for the LDL receptor. Representative binding and degradation data are given in Table 2. Total 4°C binding of either Lp(a) or LDL was lowest in HMDMs and somewhat higher in primary hepatocytes and fibroblasts. The variation in binding of both Lp(a) and LDL by the three cell types ranged 2- to 4-fold. Total heparin-releasable binding of Lp(a) and LDL of hepatocytes held at 37°C was 35% and 22% of the respective 4°C binding values. Corresponding total heparin-releasable values obtained with HMDMs and fibroblasts were closer in magnitude (70% to 90%) to total binding at 4°C, with that of LDL in fibroblasts actually being 33% higher. The magnitude of total heparin-releasable binding was comparable in primary hepatocytes and HMDMs, but in fibroblasts it was higher for both Lp(a) and LDL. In contrast to the cellular binding, there were much larger differences in the total degradation rate of Lp(a) and LDL among the three cell types. The rate was lowest in hepatocytes, 9- and 7-fold higher in HMDMs (significantly different for both Lp(a) and LDL at \( P < .01 \) using unpaired Student's t test), and 51- and 47-fold higher in fibroblasts (significantly different for Lp(a) at \( P < .05 \) and LDL at \( P < .0005 \)), respectively. Even larger cellular differences were observed with LDL-specific degradation of Lp(a) and LDL that were 16- and 9-fold greater in HMDMs (\( P < .025 \) for both Lp(a) and LDL) and 141- and 65-fold greater in fibroblasts (\( P < .05 \) for Lp(a) and \( P < .0005 \) for LDL), respectively.

| TABLE 2. Binding and Degradation of Lp(a) and LDL at 50 nmol/L Lipoprotein in Three Cell Types* |
|-----------------------------------------------|-----------------------------------------------|
|                                              | Hepatocytes                                       | HMDM                                      | Fibroblasts                                      |
|                                              | Lp(a)                              | LDL                                      | Lp(a)                              | LDL                                      | Lp(a)                              | LDL                                      |
| 4°C Binding†                                 | 0.246±0.151§ | 0.186±0.153 | 0.067±0.023§ | 0.088±0.029 | 0.171±0.040§ | 0.319±0.063 | \( n \) | 4 | 4 | 5 | 5 | 3 | 3 |
| Specific                                     | 0.033±0.020 | 0.100±0.090 | 0.049±0.018§ | 0.069±0.023 | 0.097±0.022§ | 0.245±0.054 | \( n \) | 4 | 4 | 5 | 5 | 3 | 3 |
| 37°C Heparin-releasable binding†             | 0.085±0.051 | 0.040±0.012 | 0.056±0.030 | 0.078±0.045 | 0.139±0.051§ | 0.424±0.111 | \( n \) | 4 | 4 | 5 | 5 | 3 | 3 |
| Specific                                     | 0.003±0.002§ | 0.026±0.013 | 0.023±0.018§ | 0.061±0.040 | 0.111±0.046§ | 0.387±0.102 | \( n \) | 4 | 4 | 5 | 5 | 3 | 3 |
| 37°C Degradation‡                            | 0.053±0.007| 0.161±0.055 | 0.458±0.235 | 1.193±0.688 | 2.72±1.33% | 7.62±1.76 | \( n \) | 5 | 5 | 5 | 5 | 3 | 3 |
| Specific                                     | 0.013±0.002§ | 0.103±0.067 | 0.203±0.137 | 0.916±0.600 | 1.83±1.26% | 6.72±1.28 | \( n \) | 5 | 5 | 5 | 5 | 3 | 3 |

Lp(a) indicates lipoprotein(a); LDL, low-density lipoprotein; and HMDM, human monocyte-derived macrophages.
*Values are the mean±SD of the indicated number of experiments.
† \( ^{125} \)I-lipoprotein bound in units of pmol/mg cell protein.
‡ \( ^{125} \)I-lipoprotein degraded in units of pmol/5 hours per milligram cell protein.
Lp(a) significantly different from LDL at \( ^{125}I < .05; ^{125}I < .025; ^{125}I < .01; ^{125}I < .005 \) by paired t test.
 LDL receptor-mediated binding and degradation of Lp(a) and LDL in the three cell types is shown in Table 3 in relation to total binding and degradation. In general, binding and degradation of Lp(a) and LDL were most highly LDL receptor-specific in fibroblasts, followed by HMDMs, and least so in hepatocytes. In each cell type, the percentage of binding and degradation that was LDL receptor-mediated was significantly lower for Lp(a) than for LDL. This probably reflects the fact that Lp(a) can bind to cells not only through apoB but also by way of its apo(a) moiety and that a variable fraction of Lp(a) is degraded by a receptor pathway(s) that is specific to apo(a). LDL receptor-mediated binding and degradation of Lp(a) were especially low in hepatocytes, indicating that 76% of Lp(a) is degraded by a combination of nonspecific and putatively apo(a)-specific pathways.

 LDL receptor-mediated binding and degradation of Lp(a) relative to LDL are shown in Table 4. Less Lp(a) than LDL bound to the LDL receptor at 4°C in hepatocytes it was 33% and in fibroblasts 40%. This difference was much less pronounced in HMDMs, being 72% of the value obtained with LDL (significantly different from either hepatocytes or fibroblasts at P<.0005 by unpaired Student’s t-test). For each cell type, the percentage of Lp(a) compared with LDL that was bound by the LDL receptor was higher at 4°C than at 37°C. LDL receptor-mediated heparin-releasable binding and degradation of Lp(a) was 22% to 29% of the value obtained with LDL in fibroblasts and HMDMs and even lower in hepatocytes. In contrast, the nonspecific degradation rates of LDL and Lp(a), which are the differences between the total and specific degradation rates (see Table 2), did not significantly differ from each other in each of the three cell lines. The proportion of Lp(a) to LDL bound by the LDL receptor at 37°C and the proportion degraded by the LDL-receptor pathway were similar. Thus, once bound, both Lp(a) and LDL are degraded with similar efficiency by the LDL-receptor pathway, indicating that there are not major differences in the way Lp(a) and LDL are degraded intracellularly.

### Potential Factors Affecting Degradation of Lp(a) and LDL by Hepatocytes

As previously noted by Edge et al.\(^3\) when compared with LDL that was cell associated, the quantity of degraded LDL was much higher in fibroblasts than in hepatocytes. These authors postulated that the difference in the degradation rate was partially related to cell size. We therefore examined several other factors that could potentially affect degradation in hepatocytes. We first investigated the effect of cell density, since it is known that there is an inverse relation between LDL uptake and this parameter in rat hepatocyte monolayers.\(^3\) We observed a similar effect with human primary hepatocytes (see Fig 4), although between 140 and 400 \(\mu\)g cell protein per well, which was the range used in this study, the LDL-specific degradation rates for LDL and Lp(a) did not differ greatly. When cell protein was less than 50 \(\mu\)g per well, the degradation rate was substantially higher than in wells with greater cell density; however, in such wells the hepatocytes were sparse and confluent. We also examined whether the age of the cells had an effect on the degradation rate of LDL and Lp(a) but found no correlation in cultures that ranged in age between 2 and 23 days.

Additionally, we entertained the possibility that the low degradation rate observed in hepatocytes was related to the composition of the serum-free medium used to maintain the cells. Therefore, some hepatocytes were switched 48 hours preceding the experiment into MEM containing 10% lipoprotein-depleted serum (a medium used to upregulate LDL-receptor levels in fibroblasts) to compare the degradation rate of LDL and Lp(a) to that of hepatocytes maintained in SFM III medium. As shown in Fig 5A, neither total nor LDL-specific degradation of Lp(a) was affected by the change in medium.

### Table 3. Percentage of LDL Receptor-Mediated Binding and Degradation of Lp(a) and LDL*

<table>
<thead>
<tr>
<th></th>
<th>Hepatocytes</th>
<th>HMDM</th>
<th>Fibroblasts</th>
</tr>
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<tbody>
<tr>
<td><strong>4°C Binding</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lp(a)</td>
<td>14.3±6.6‡</td>
<td>50.1±9.9</td>
<td>78.1±6.4</td>
</tr>
<tr>
<td>LDL</td>
<td>72.1±6.9†</td>
<td>78.1±6.4</td>
<td>78.1±6.4</td>
</tr>
<tr>
<td><strong>37°C Heparin-releasable binding</strong></td>
<td>4.0±3.7‡</td>
<td>66.1±4.7</td>
<td>77.9±4.4</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>38.6±17.9‡</td>
<td>79.3±1.4§</td>
<td>79.3±1.4§</td>
</tr>
<tr>
<td>LDL</td>
<td>79.3±1.4§</td>
<td>91.2±1.0</td>
<td>91.2±1.0</td>
</tr>
<tr>
<td><strong>37°C Degradation</strong></td>
<td>23.8±3.7‡</td>
<td>60.0±12.6</td>
<td>74.1±6.2</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>42.2±12.1‡</td>
<td>66.5±1.1t</td>
<td>66.5±1.1t</td>
</tr>
<tr>
<td>LDL</td>
<td>74.1±6.2</td>
<td>89.0±3.4</td>
<td>89.0±3.4</td>
</tr>
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</table>

*Values are the mean±SD and were calculated from the data used to derive Table 2.

### Table 4. Comparison of the LDL Receptor-Mediated Binding and Degradation of Lp(a) to LDL*

<table>
<thead>
<tr>
<th></th>
<th>Hepatocytes</th>
<th>HMDM, %</th>
<th>Fibroblasts</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>4°C Binding</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lp(a)</td>
<td>33.0±11.7</td>
<td>72.1±16.4</td>
<td>39.6±7.7</td>
</tr>
<tr>
<td>LDL</td>
<td>72.1±16.4</td>
<td>72.1±16.4</td>
<td>72.1±16.4</td>
</tr>
<tr>
<td><strong>37°C Heparin-releasable binding</strong></td>
<td>11.5±10.8</td>
<td>29.2±6.9</td>
<td>28.7±3.1</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>29.2±6.9</td>
<td>28.7±3.1</td>
<td>28.7±3.1</td>
</tr>
<tr>
<td>LDL</td>
<td>28.7±3.1</td>
<td>28.7±3.1</td>
<td>28.7±3.1</td>
</tr>
<tr>
<td><strong>37°C Degradation</strong></td>
<td>12.6±5.6</td>
<td>21.7±6.0</td>
<td>27.2±12.5</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>21.7±6.0</td>
<td>27.2±12.5</td>
<td>27.2±12.5</td>
</tr>
<tr>
<td>LDL</td>
<td>27.2±12.5</td>
<td>27.2±12.5</td>
<td>27.2±12.5</td>
</tr>
</tbody>
</table>

*Values are the mean±SD and were calculated from the data used to derive Table 2.

LDL indicates low-density lipoprotein; Lp(a), lipoprotein(a); and HMDM, human monocyte-derived macrophages.
although there was a small increase in both total and specific degradation of LDL (Fig 5B). However, this increase was not large enough to explain the observed differences in degradation rate among the three cell types.

Low LDL receptor-mediated degradation of LDL might be indicative of suboptimal LDL-receptor expression. However, this is an unlikely possibility because the hepatocytes were maintained in serum-free, cholesterol-free medium, and LDL receptors should be maximally expressed. Nonetheless, to determine whether hepatocytes could be further upregulated, we treated some cells with lovastatin, an inhibitor of cholesterol synthesis. Our results indicated that at a concentration of 10 μg/mL lovastatin, we were able to increase the LDL-specific degradation of both lipoproteins only two-fold, indicating that a suboptimal expression of LDL receptors probably did not account for the observed low degradation rates.

A low degradation rate may also be caused by a deiodinase activity toward iodotyrosine, which has been reported to be high in hepatocytes. To determine whether this effect could explain the low hepatocyte degradation rate of LDL and Lp(a), we performed parallel degradation experiments in the three cell types in the presence of 5 nmol/L moniodotyrosine to inhibit the breakdown of [125I]iodotyrosine by deiodinases. At 50 nmol/L lipoprotein concentration, total degradation of Lp(a) increased 170%, 220%, and 290% in fibroblasts, HMDMs, and hepatocytes, respectively. The corresponding increases for LDL degradation were 164%, 150%, and 235%. Because the increases in the degradation rates were only marginal in hepatocytes relative to HMDMs and fibroblasts, this indicated that deiodinase activity could not account for the low degradation rate observed in hepatocytes.

Discussion

We initiated this study to clarify the role of the LDL receptor in the binding and degradation of Lp(a) by three important cell lines, ie, primary human hepatocytes, HMDMs, and human skin fibroblasts. Recent findings convincingly showed that the LDL receptor of fibroblasts binds Lp(a), although there is some disagreement over the strength of this interaction. That the LDL receptor functions to bind and mediate degradation of Lp(a) is supported by the observations that this lipoprotein is cleared more rapidly by transgenic mice overexpressing the LDL-receptor gene than by controls, and that subjects with familial hypercholesterolemia (FH) have higher than normal plasma Lp(a) levels, presumably due to impaired uptake caused by defective LDL receptors. However, several observations support the opposite view: (1) administration of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors increases LDL-receptor activity, thereby lowering plasma LDL levels without affecting the concentration of Lp(a); (2) hypercholesterolemic individuals of kindreds with defective apoB100 or defective LDL receptors do not have higher Lp(a) levels than unaffected siblings with the same apo(a) phenotype; (3) affected members of a family of LDL-receptor-defective rhesus monkeys do not have increased concentrations of Lp(a); and (4) the fractional catabolic rate of Lp(a) in subjects heterozygous for FH is the same as in subjects without FH, whereas the fractional catabolic rate of LDL is much lower in FH subjects than in subjects without FH, thereby lowering plasma LDL levels without affecting the concentration of Lp(a). The liver also...
appears to be the major organ responsible for the removal of Lp(a), as was shown in rats by the tissue distribution of [3H]cholesteryl linoleyl ether-labeled human Lp(a). It was therefore of interest to determine the degree of involvement of the LDL receptor of hepatocytes in the degradation of Lp(a). Our results indicate that LDL and Lp(a) are degraded poorly by primary hepatocytes because both LDL receptor-mediated and nonspecific degradation rates were higher in macrophages and much higher in fibroblasts. LDL receptor-mediated degradation of LDL and Lp(a) was 9- and 16-fold higher in macrophages and 65- and 141-fold higher in fibroblasts, respectively. Inefficient degradation of LDL by cultured human hepatocytes and Hep G2 cells was also noted by other investigators. Edge et al attributed the lower binding and degradation rate of LDL by hepatocytes to their larger size when compared with fibroblasts. We examined additional possible contributing factors but found that variation in cell density, age of cells, medium effects, upregulation of the LDL receptor, and deiodinase activity were not responsible for the low degradation rates of LDL and Lp(a). Lombardi et al described the low degradation rate of LDL in Hep G2 cells either to sluggish fusion between late endosomes and lysosomes or to inefficient lysosomal degradation.

The low hepatocyte degradation rate of LDL does not imply that the liver is not the major organ responsible for LDL clearance from the circulation. Quantitatively, hepatic LDL receptors far outnumber those of other tissues, and although extracellular LDL receptor-mediated degradation may be more efficient, this is compensated by the fact that concentrations of LDL in extravascular fluids that bathe these cells are low. In addition, there may be alternate pathways in existence for the removal of Lp(a) that involve not only the LDL receptor but also lipoprotein lipase. This pathway may be functionally postprandially in confined tissue compartments such as Disse’s space, where local concentrations of lipoprotein lipase would be high enough to affect uptake of Lp(a)–lipoprotein lipase complexes on binding to surface-bound heparan sulfate proteoglycans and the LDL receptor. However, the quantitative importance of this pathway in the clearance of Lp(a) from the circulation is not clear at present.

Previous in vivo studies with estrogen-treated rats indicated that Lp(a) interacts less efficiently than LDL with the estrogen-induced hepatic LDL receptor. Our results with human hepatocytes are in agreement with this conclusion: they show that LDL receptor-mediated catabolism of Lp(a) by cultured human hepatocytes is very poor, because the value obtained with 50 nmol/L LDL (see Table 4) was only 17% of the value obtained with 50 nmol/L LDL. Similar differences between LDL and Lp(a) were observed in the other two cell lines. One factor that may contribute to a lower degradation rate for Lp(a) than for LDL is that the actual process of internalization and degradation is less efficient for Lp(a). Previously, we found that the total intrinsic degradation rate for Lp(a) by HMMDMs, which includes both specific and nonspecific degradation, was lower than that for LDL. However, the evidence presented here (Table 4) suggests that LDL receptor-mediated degradation of Lp(a) is probably as efficient as that of LDL in all three cell types, because the proportion of Lp(a) to LDL that was LDL-receptor bound at 37°C was similar to the percentage degraded by the LDL receptor pathway. Interestingly, the nonspecific degradation rate of LDL did not differ significantly from that of Lp(a) in each cell line. The observed differences noted between LDL and Lp(a) are therefore LDL receptor mediated.

One aim of this study was to determine the affinity and binding capacity of the LDL receptor for Lp(a) and LDL. However, because LDL-specific binding of Lp(a) at 4°C in hepatocytes was such a small fraction of total binding (14.3%), we were not able to accurately determine a dissociation constant for the interaction of Lp(a) with the hepatocyte LDL receptor. We previously found that the affinity of both Lp(a) and LDL for the HMMD Lp(a) LDL receptor was low, because the respective dissociation constants were 0.80 and 0.23 μmol/L. Although this result indicated that the binding of Lp(a) to the LDL receptor of HMMDMs is almost fourfold weaker than the binding of LDL, there is considerable uncertainty in these values due to the difficulty in saturating the HMMD LDL receptor because of its low affinity for either lipoprotein. This contrasts with fibroblasts, for which LDL receptor saturation was readily achieved with either Lp(a) or LDL. We were able to accurately determine the binding constant for the interaction of Lp(a) with the LDL receptor of fibroblasts, and our results, shown in Table 1, indicate that the affinity of Lp(a) was equal to that of LDL. Therefore, the characteristic that distinguishes the binding of the two lipoproteins is not the strength of the ligand-receptor interaction, as was assumed by many investigators previously, but rather the mass of lipoprotein bound at saturation. In other words, the LDL-receptor occupancy of Lp(a) is lower than that of LDL when both are tested at the same molar concentration. As shown in Table 1 from the analysis of the 4°C binding curves, the amount of Lp(a) bound is 59% lower (significant at P<.01 using paired t test). Binding of Lp(a) at 37°C is even lower, being only 29% of the value obtained with LDL (see Table 4).

Our data can be used to reconcile the conflicting findings observed previously regarding the interaction of Lp(a) with the LDL receptor. We are in agreement with Hofmann et al who showed that Lp(a) binds with high affinity to isolated bovine LDL receptors. We also agree with their other important observation that the catabolism of Lp(a) in transgenic mice overexpressing LDL receptors is markedly accelerated, even though our data show that the LDL receptor has a lower binding capacity for Lp(a). This apparent discrepancy can be explained by the fact that the level of expressed LDL receptors is so high in these animals that a normal plasma concentration of LDL is ineffective in competing with Lp(a) for the LDL receptor. Our data also allow us to speculate that the reason HMG-CoA–reductase inhibitors do not lower Lp(a) levels despite the fact that they increase the number of hepatic LDL receptors is that this change is much lower than what was achieved in transgenic mice. For example, we were able to increase LDL receptor-mediated uptake of either LDL or Lp(a) with lovastatin only twofold in hepatocytes; Reinhäger et al observed a 2.8-fold increase in hepatic LDL receptors in patients given pravastatin, whereas the hepatic uptake of Lp(a) increased more than 10-
fold in transgenic mice. Because competition of LDL with Lp(a) for the LDL receptor is greater in humans than it is in mice because of higher LDL levels (possibly 40- to 50-fold higher than those of Lp(a) in a normal individual), the modest increase in LDL receptors achieved with HMG-CoA reductase inhibitors may not overcome competitive inhibition by LDL.

An explanation that might account for Lp(a) having an affinity equal to LDL but a lower Bmax could be that relative to LDL, the binding of Lp(a) to the LDL receptor is sterically hindered. Such a mechanism has been proposed by Chappel et al to explain the reduced binding of large LDL compared with small LDL particles by postulating that the binding of LDL to the LDL receptor of fibroblasts conforms to a lattice model. Because most LDL receptors are concentrated in coated pits, these authors suggested that, on a lattice of LDL receptors, large LDL would physically block binding to more adjacent receptors than would small LDL. This would reduce the number of available receptors for binding. Lp(a) is not only larger in mass than LDL but because of the asymmetric attachment of apo(a) to the lipoprotein particle, it probably has an even greater radius of gyration. Thus, sterically hindered binding of Lp(a) to the LDL-receptor lattice is an attractive hypothesis that might account for the lower binding of Lp(a). However, the results of the competition experiments are very difficult to reconcile with this mechanism. Implicit in the binding analysis is the fact that, with a similar binding constant, Lp(a) should at least be as effective as, if not better than, LDL in competing for the binding of radiolabeled LDL to the LDL receptor. Because the opposite is true, this implies that the proposed hypothesis is deficient and that the interaction of radiolabeled Lp(a) with LDL-specific binding sites on fibroblasts is more complex.

In conclusion, our results indicate that Lp(a) can definitely interact with the LDL receptor as demonstrated in fibroblasts, where Lp(a) has the same affinity as LDL but also a much lower receptor occupancy. However, LDL receptor-mediated degradation of Lp(a) varies greatly in different cell lines, with hepatocytes having the lowest and most nonspecific degradation rate, fibroblasts the highest and least nonspecific degradation rate, and HMDMs being intermediate. If we may extrapolate our in vitro results obtained with hepatocytes to the situation occurring in vivo, then the catabolism of Lp(a) must be mainly nonspecific because the already small LDL receptor-mediated component would be further suppressed by competition from LDL, which in a normal individual exceeds the molar concentration of plasma Lp(a) by a factor of 40 to 50.

Acknowledgments

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