Interaction of Lp(a) and of Apo(a) With Liver Cells

Gert M. Kostner

Lipoprotein(a) (Lp[a]) is a lipoprotein of high atherogenicity with unknown function. Although it binds in vitro to the low-density lipoprotein (LDL) receptor, it is not clear whether this mechanism also operates in vivo. We studied the interaction of Lp(a) and of apoprotein(a) (apo[a]) with hepatoma cells (HepG2 and Hep3B) with the following results. (1) HepG2 cells exhibited saturable high-affinity binding of LDLs, whereas the majority of Lp(a) binding was of low affinity and nonsaturable. Preincubation of HepG2 cells with LDL markedly reduced cholesterol biosynthesis, but Lp(a) had a much lower effect. (2) When HepG2 cells were preincubated for 48 to 72 hours with Lp(a) or apo(a), 125I-LDL binding was increased by a factor of >2. During this time, up to >1 µg of apo(a) per 1 milligram cell protein was found to be cell associated in an undegraded form. Monoclonal antibodies against the LDL receptor did not prevent the increase in LDL binding stimulated by apo(a). (3) Coincubation with LDL caused a significant increase of Lp(a) degradation by HepG2 cells that was probably caused by an increase of Lp(a) uptake in a "hitchhiking"-like process. (Arteriosclerosis and Thrombosis 1993;13:1101-1109)

KEY WORDS • HepG2 cells • LDL receptor • mevinolin • cholesterol biosynthesis • Lp(a) degradation

Because of its atherogenicity, there is currently great interest in lipoprotein(a) (Lp[a]) research (reviewed in References 1 through 4). Lp(a) consists of a low-density lipoprotein (LDL)-like particle containing apoprotein (apo) B-100 as the integral protein and apo(a) attached to it by a disulfide linkage. Apo(a) is a glycoprotein with approximately 28% of its molecular weight contributed by O- and N-linked sugars that are rich in sialic acid. Apo(a) exists in a great number of isoforms, which are determined by at least 24 alleles. These isoforms vary in their molecular weights from approximately 350 to 900 kD.

The physiological function of apo(a)/Lp(a) is completely unknown. Cloning of apo(a) cDNA revealed an unexpected striking homology of the apo(a) structure with plasminogen. In addition to the kringle-5 and the protease domains of plasminogen, from 17 to 34 kringle-4-like domains are also present in apo(a), depending on the size of the particular isoform. Studies by Utermann et al suggest that the size of apo(a) correlates negatively with the Lp(a) plasma concentration. Whether this correlation is relevant to the finding that individuals with Lp(a) levels >25 to 30 mg/dL are at a twofold to threefold higher risk for atherosclerotic diseases remains to be determined.

Earlier studies conducted in our laboratory revealed that, unlike LDL, Lp(a) is not a metabolic product of triglyceride-rich precursors. Plasma Lp(a) levels are largely determined by the rate of synthesis and not by the catabolic rate. Although small amounts of apo(a) mRNA have also been found in the brain and testes, the liver seems to be the predominant organ of Lp(a) synthesis.

At present, the in vivo mechanism of cellular interaction and uptake of Lp(a) and the organ responsible for its catabolism are unknown. Because apo B is a major constituent of the particle, it has been speculated that the LDL receptor may be involved in Lp(a) clearance. Reports from many laboratories provide evidence that Lp(a) binds to the LDL receptor in vitro. However, binding is significantly reduced in comparison to LDL. Few in vivo studies that address this question have been carried out. In a report by Hofmann et al, the overexpression of LDL receptors in the liver of transgenic mice was found to significantly increase the catabolism of human Lp(a). The strongest argument against the assumption that this mechanism plays a role in catabolism in vivo, however, is the fact that lipid-lowering drugs, such as cholestyramine or lovastatin, known to elevate the number of LDL receptors on the liver, do not reduce plasma Lp(a).

To further investigate these questions, we studied the interaction of Lp(a) with HepG2 cells and, to a lesser extent, the interaction of Lp(a) with Hep3B cells.

Methods

Cultivation of Liver Cells

Experimental cell work was carried out essentially as described previously. HepG2 and, for some control experiments, Hep3B cells, purchased from American Type Culture Collection, Rockville, Md, were seeded in 12-well Petri dishes and grown in Dulbecco's minimum essential medium (DMEM) supplemented with 10% fetal calf serum (FCS) and incubated in 95%
air/5% CO₂ at 37°C. After 2 to 4 days, the medium was replaced by DMEM containing 10% lipoprotein-deficient serum (LPDS) in addition to various lipoproteins or other substances known to affect LDL binding. After further incubation for 48 to 72 hours, cells were washed and then used for further experiments. Since HepG2 cells do not form monolayers, it is not possible to indicate their stage of confluency; at the time of the experiments, the cell number per plate was usually 2.5 to 3.1 million.

Binding and Degradation Studies

Lipoproteins were radiolabeled with ¹²⁵I according to McFarlane,²⁷ yielding preparations with specific activities of 300 to 640 cpm/ng protein. When lipoproteins were extracted twice with chloroform/methanol 2:1 at room temperature, >95% of the radioactivity resided in the protein portion of the lipoprotein. In the case of Lp(a), the percentage of radioactivity was distributed between apo B and apo(a) at a ratio of approximately 65:35. To determine this distribution, small amounts of ¹²⁵I-Lp(a) were treated with dithiothreitol and passed over a heparin-Sepharose column as described by Armstrong et al.¹⁹ The apo(a) and the Lp(a) after removal of apo(a) (Lp(a-)) peaks were collected separately, delipidated three times with chloroform/methanol (2:1, vol/vol), and counted in a gamma counter. In some cases, purified apo(a) was also radiolabeled according to McFarlane.²⁷

Specific binding and degradation of lipoproteins were measured according to Goldstein et al.²⁸ Cells were incubated for 3 hours at 4°C with increasing amounts of radiolabeled lipoproteins, and after washing (three times with 1% human serum albumin in phosphate-buffered saline and twice with albumin-free phosphate-buffered saline, 1.5 mL each), cells were solubilized in 0.3N NaOH and the radioactivity associated with the cells was quantified using a gamma counter. High-affinity binding was calculated from total binding minus unspecific bind-
ing, which was measured in the presence of a 50-fold excess of unlabeled ligand. In some cases, the binding, internalization, and degradation of lipoproteins and proteins were measured at 37°C as described.

The association of purified apo(a) with HepG2 cells and its subsequent degradation was also studied. Radiolabeled apo(a) with a specific activity of approximately 10 cpm/nmol was incubated for increasing time intervals with cultured HepG2 cells at 37°C; this low specific activity was chosen because it was sufficient for the type of experiments performed. The medium was removed and treated with trichloroacetic acid (TCA), and the radioactivity was counted (ie, association). Identical experiments were carried out using ovalbumin as a control.

To study the impact of LDL on Lp(a) degradation, the following experiments were conducted: HepG2 cells were grown as described above. Cells were then incubated for 2 hours at 4°C with radiolabeled Lp(a) or radiolabeled LDL in the absence or presence of increasing amounts of unlabeled LDL. The medium was removed, and cells were washed four times with ice-cold albumin-containing phosphate buffer, 1.5 mL each, followed by the addition of DMEM containing 10% LPDS. The cells were then incubated for 2 or 3 hours at 37°C, and the degradation of labeled lipoproteins (TCA-soluble material) was measured according to Goldstein et al. In all degradation experiments, dishes lacking cells were treated similarly and the values obtained were subtracted. This background amounted to <15% of the values observed with HepG2 cell-containing plates.

Measurement of Cholesterol Biosynthesis

For studying the influence of lipoproteins on cholesterol biosynthesis, HepG2 cells were cultivated as outlined above. The incorporation of 14C octanoate (2 mCi/mmol) over a period of 18 to 24 hours into the nonsaponifiable sterol fraction was measured according to Liscum and Faust.

For specifically blocking LDL-receptor-mediated LDL binding, the anti-LDL receptor–producing clone C7 was purchased from American Type Culture Collection and subcloned, and monoclonal antibodies were produced in mice. From the peritoneal fluid of these mice, pure antibodies were prepared by DEAE cellulose column chromatography. Cells were incubated with this antibody preparation (50 μg/mL) for 30 minutes at room temperature and then for 2 hours at 4°C, followed by LDL binding studies as outlined above.

Preparation of Lipoproteins

Plasma lipoproteins were purified from normolipemic plasma of healthy volunteers as described previously. The following density fractions (g/mL) were used: LDL, 1.025 to 1.055; high-density lipoprotein (HDL), 1.125 to 1.210; and Lp(a), 1.070 to 1.110. In most cases, Lp(a) was prepared from pooled plasma of donors with various isoforms. In some cases, donors with single isoforms were used. For the present work, it was essential to prepare absolutely LDL-free Lp(a).

This was achieved by affinity chromatography using specific immunoadsorbers or Lys-Sepharose as described earlier. Each particular preparation was checked for purity by double-antibody crossed immunoelectrophoresis. Apo(a) was prepared by passing Lp(a) that was first reductively cleaved with diithiothreitol over heparin-Sepharose. Desialylated apo(a) was prepared by incubating Lp(a) with neuraminidase (30 mU/mg lipoprotein for 3 hours at 37°C) as described in detail earlier. The sialic acid content of lipoproteins was determined by the thiobarbituric acid assay of Warren. All lipoprotein preparations were stored at 4°C and used within 2 weeks after purification. Plasminogen was a gift from Dr. Pathy, University of Budapest (Hungary).

Other Methods

Protein determinations were performed according to Lowry et al. Three test kits for cholesterol determination as well as culture media and FCS were from Böhringer, Mannheim, FRG. Disposable ware for the cell laboratory was purchased from Costar, Cambridge, Mass. All other chemicals were from E. Merck, Darmstadt, FRG.

Statistical Analysis

To evaluate the statistical significance of some of our results, data from several consecutive experiments (in most cases, two or three) that were carried out in triplicate were pooled, and mean values and standard deviations (of these six or nine values) were determined and plotted in the corresponding figures. Significances were calculated by Student's t test.

Results

Binding of Lp(a) to HepG2 Cells in Comparison to LDL

In the initial experiments, the binding of affinity-purified Lp(a), which was free of contaminating LDL, to

<table>
<thead>
<tr>
<th>Table 1. Competition of LDL Binding to HepG2 Cells</th>
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<tbody>
<tr>
<td>Competitor</td>
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<tr>
<td>------------------------------</td>
</tr>
<tr>
<td>LDL</td>
</tr>
<tr>
<td>Lp(a)</td>
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<tr>
<td>HDL</td>
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LDL, low-density lipoprotein; Lp(a), lipoprotein(a); HDL, high-density lipoprotein.

HepG2 cells were preincubated for 48 hours with lipoprotein-deficient serum. LDL binding at 10 μg/mL was measured in the presence of increasing amounts of LDL, Lp(a), or HDL at 4°C, as described in "Methods." LDL binding in the absence of competitor was taken as 100%. The values represent mean±SD of triplicate analyses.
substances as indicated. The experiments were carried out as described in Fig 1 except that on day 3, the medium was replaced by 10% lipoprotein-deficient serum (LPDS) alone or additionally supplemented with either 100 µg/mL of lipoprotein(a) (Lp(a)), high-density lipoprotein (HDL), or LDL (all cholesterol) or 10⁻³ mol/L mevinolin (MEV), 30 µg/mL of apoprotein(a) (apo-a), or 30 µg/mL of neuraminidase-treated apo(a) (n-apo-a). On day 5, total ¹²⁵I-LDL binding was measured as described in “Methods.” The curves are means of two (n-apo-a) or three experiments carried out in triplicate. The maximal binding of LDL to pretreated cells was significantly different from that of LPDS-incubated cells (P<.01) except for n-apo-a.

HepG2 cells at 4°C was measured and compared with that of LDL. Whereas LDL exhibited the expected saturable high-affinity binding (Fig 1, bottom), Lp(a) association was of low affinity and not saturable under the conditions at which LDL binding reached its plateau (Fig 1, top). Scatchard analysis of LDL binding to HepG2 cells preincubated with 10⁻⁴ mol/L mevinolin, which is known to increase the number of LDL receptors, revealed a marked increase in maximal binding capacity (Bmax). In contrast, for Lp(a) we observed little change.

We also performed competition experiments by incubating HepG2 cells with ²ⁱI-labeled LDL and adding increasing amounts of either unlabeled LDL, Lp(a), or apo E-free HDL (Table 1). By virtue of its apo B component, some interaction between Lp(a) and the LDL receptor was anticipated. As expected, Lp(a) was found to compete for LDL binding but only about one third as effectively as unlabeled LDL. Interestingly, apo E-free HDL was also capable of interfering with LDL binding. Thus, we assume that the interference of Lp(a) with LDL binding might be unspecific to some extent.

Taken together, these results suggested that Lp(a) binds to the LDL receptor of liver cells but to a much lesser extent than LDL.

Impact of Different Substances on LDL Binding to HepG2 Cells

To further investigate the response of the LDL receptor activity of HepG2 cells in the presence of Lp(a), receptor downregulation was probed. HepG2 cells were preincubated for 48 hours with LDL, Lp(a), HDL, or mevinolin, after which time ¹²⁵I-LDL binding was measured. HDL and mevinolin, which are known to upregulate LDL receptor number, were used as controls (Fig 2). Whereas preincubation with LDL caused the expected reduction in ¹²⁵I-LDL binding, Lp(a) had an opposite effect. Unexpectedly, when cells were preincubated with Lp(a), we observed a marked increase in LDL binding. In fact, measured values of LDL binding were even higher in these cells than in those preincubated with an equivalent amount of HDL cholesterol or with 10⁻³ mol/L of mevinolin.

The effect of apo(a) and of neuraminidase-treated apo(a) on LDL binding was also tested (Fig 2). Incubation with apo(a) stimulated LDL binding to HepG2 cells to an extent comparable to intact Lp(a). Conceivably, the carbohydrate moiety of apo(a) might be important to the observed effects on HepG2 cells. Apo(a) was therefore treated with neuraminidase to remove terminal sialic acids before incubation with the cells. The sialic acid content of apo(a), as measured by the method of Warren,³² was 155 µg/mg. In neuraminidase-treated material, the sialic acid content was below the detection limit of the method (<10 µg/mg). Neuraminidase-treated apo(a) also appeared to increase LDL binding; the effect, however, was statistically insignificant (Fig 2). We therefore believe that sialic acid plays an important role in the stimulation of LDL binding by apo(a).

Impact of Different Substances on Cholesterol Biosynthesis in HepG2 Cells

Normally, when the endogenous sterol pool is reduced, cells respond with an upregulation of LDL
TABLE 2. Binding Parameters of LDL to HepG2 and Hep3B Cells Preincubated With Various Substances

<table>
<thead>
<tr>
<th>Substance</th>
<th>HepG2 cells</th>
<th>Hep3B cells</th>
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<tbody>
<tr>
<td></td>
<td>$K_d$ (nmol/L)</td>
<td>$B_{max}$ (ng/mg CP)</td>
</tr>
<tr>
<td>LPDS</td>
<td>6.5 (0.3)</td>
<td>147 (11)</td>
</tr>
<tr>
<td>LDL (100 µg/mL)</td>
<td>4.2 (0.2)</td>
<td>66 (7)</td>
</tr>
<tr>
<td>Apo(a) (50 µg/mL)</td>
<td>8.4 (0.4)</td>
<td>528 (48)</td>
</tr>
<tr>
<td>Pig (50 µg/mL)</td>
<td>5.8 (0.3)</td>
<td>142 (14)</td>
</tr>
</tbody>
</table>

LDL, low-density lipoprotein; $K_d$, dissociation constant; $B_{max}$, maximal binding capacity; CP, cell protein; LPDS, lipoprotein-deficient serum; apo(a), apoprotein(a); Pig, plasminogen.

Cells were seeded, and on day 3 the medium was replaced by Dulbecco's minimal essential medium containing 10% LPDS supplemented with 100 µg/mL LDL cholesterol, 50 µg/mL apo(a), or 50 µg/mL plasminogen and further incubated for 48 hours. Cells were washed, and $^{125}$I-LDL binding was measured at 4°C at increasing concentrations of ligand in the presence or absence of a 50-fold excess of cold LDL as shown in Fig 1. Binding parameters were calculated from Scatchard transformation of binding curves. The values are mean ± SD of two experiments carried out in triplicate.

receptors and vice versa. We were interested, therefore, in measuring the influence of Lp(a) and of control substances on cholesterol biosynthesis in comparison to LDL or mevinolin. As known from earlier experiments, incubation of HepG2 cells with LDL or mevinolin reduced $^{14}$C-octanoate incorporation into the cellular sterol fraction. Incubation with Lp(a) was also found to reduce sterol biosynthesis; the effect, however, was by far less pronounced than with equivalent amounts of LDL. Apo(a) had virtually no effect on cholesterol biosynthesis (data not shown).

Since preincubation with apo(a) had such a profound influence on LDL binding, we asked whether this by itself might have an impact on cholesterol biosynthesis mediated by LDL. HepG2 cells were therefore incubated either with LPDS or with apo(a), and the inhibition of cholesterol biosynthesis mediated by 10 to 40 µg/mL of LDL cholesterol was studied. The downregulation of cholesterol biosynthesis was the same whether the cells were preincubated with apo(a) or not (Fig 3).

Control Experiments With Hep3B Cells and With Different Apo(a) Isoforms

Calculation by Scatchard analysis of the binding parameters of LDL to HepG2 cells preincubated with various substances revealed that, under all circumstances tested, the dissociation constant $(K_d)$ values were in the nanomolar range and apparently were not different from each other (Table 2). $B_{max}$ values, on the other hand, were reduced when cells were preincubated with LDL and markedly increased after preincubation with apo(a). Similar experiments were also carried out with another human hepatoma cell line (Hep3B). The results were comparable but somewhat less pronounced.

To determine whether various apo(a) isoforms may behave differently, we performed a control experiment using apo(a) isolated from homozygous donors that exhibited either the fast or the S-2 isoform as defined by Utermann et al.8 When HepG2 cells were preincubated with these two isoforms of apo(a), both isoforms stimulated LDL association to the same extent (data not shown).

Degradation of LDL by HepG2 Cells Pretreated With Different Substances

Although preincubation of HepG2 cells with Lp(a) and apo(a) caused a striking increase in LDL binding, the observed effects on cholesterol biosynthesis suggested that the increased LDL binding might not be the result of an increased number of LDL receptors per cell. To test this hypothesis, the degradation of LDL by HepG2 cells was studied. HepG2 cells were incubated with the substances shown in Fig 4, and the amount of TCA-soluble $^{125}$I radioactivity generated during a 4-hour incubation at 4°C was measured. As expected, mevinolin caused a significant increase (Fig 4, curve 1) and LDL a decrease (Fig 4, curve 4) of $^{125}$I-LDL degradation. Apo(a) had no effect (Fig 4, curve 3). For comparison, apo(a) alone was labeled with $^{125}$I and its degradation followed (Fig 4, curve 5). Apo(a) showed a definite but rather low degradation compared with LDL.

To substantiate our hypothesis that apo(a)-triggered binding of LDL is unrelated to LDL receptors, HepG2
cells preincubated with LPDS, HDL, Lp(a), or apo(a) were treated with monoclonal antibodies (C7) against the LDL receptor followed by measurement of \textsuperscript{125}I-LDL binding. Although antibody C7 almost completely inhibited \textsuperscript{125}I-LDL binding to LPDS-, LDL-, and HDL-pretreated cells, this antibody only partially inhibited LDL binding to apo(a)-pretreated cells (data not shown).

**Degradation of Apo(a) by HepG2 Cells**

To learn more about the fate of apo(a) during incubation, its degradation and association with HepG2 cells were studied and compared with that of ovalbumin. Radiolabeled proteins at concentrations of 30 and 60 \(\mu\)g/mL were incubated with the cells for 0 to 72 hours, and the material in the medium soluble in 10% TCA was analyzed. We observed a gradual increase of TCA-soluble material in the medium (Fig 5, top). After 72 hours, some 12% to 15% of the added apo(a) was degraded. Under these conditions, <1% of ovalbumin was degraded; therefore, ovalbumin data are not shown. There was also a steady increase of apo(a) association with HepG2 cells (Fig 5, bottom). After 72 hours of incubation with 30 and 60 \(\mu\)g/mL of apo(a), 565±39 ng and 952±72 ng of apo(a) per milligram cell protein, respectively, were associated with HepG2 cells in unde-
graded form. Also, in this case, the association of ovalbumin, which was used as a control, was minimal (5 to 42 ng/mg cell protein) and not related to the incubation time.

Impact of LDL on Lp(a) Degradation

From the results of all the studies mentioned above, we concluded that LDL must have a strong affinity for Lp(a) and apo(a). Thus, we studied the possibility that LDL, after binding to the apo B/E receptor, may indirectly trigger Lp(a) binding and degradation. HepG2 cells were therefore incubated with increasing amounts of radiolabeled Lp(a) at 4°C in the presence or absence of 1.5 μg/mL of unlabeled LDL protein. After removal of the medium and washing of the cells, Lp(a) degradation was studied at 37°C. As seen in Fig 6, Lp(a) degradation gradually increased with increasing Lp(a) concentrations and was significantly higher in the presence of 1.5 μg/mL of LDL. The fractional increase of LDL-triggered Lp(a) degradation was higher at low Lp(a) concentrations than at high concentrations.

To study this phenomenon in more detail, 5 and 10 μg of 125I-Lp(a) were incubated with cells at 4°C in the absence or presence of increasing amounts of unlabeled LDL (Fig 7). Unlabeled LDL greatly affected Lp(a) degradation. This effect, however, depended greatly on the particular Lp(a)-to-LDL ratio and was more pronounced at low LDL concentrations. Control experiments were also carried out similarly to those shown in Fig 7 using radiolabeled LDL instead of Lp(a). In these experiments, the addition of unlabeled LDL to the incubation in all cases reduced 125I-LDL degradation, as one would expect (data not shown).

Discussion

Structurally, Lp(a) is similar to LDL, with apo B as one major apoprotein component. Because of this structural similarity, one might expect that the catabolism of these two lipoproteins would proceed via common pathways, i.e., LDL receptor–mediated binding, internalization, and degradation by the liver. In fact, we found that the fractional catabolic rate of Lp(a) in humans was not much different from that of LDL.16 Many in vitro studies have also revealed that Lp(a) definitely binds to the LDL receptor but with a much lower affinity than that of LDL.

To gain a better understanding of the physiology of Lp(a), we investigated the downregulation of LDL receptor activity mediated by Lp(a) compared with LDL. Earlier experiments had used fibroblasts of normal persons and of patients with LDL receptor deficiency. In those studies, we were surprised to see that preincubation with Lp(a) in fact did not inhibit but rather increased the association of LDL with fibroblasts.43 Since this was the case with LDL receptor–positive as well as with LDL receptor–negative cells, the increase in binding was interpreted to be LDL receptor independent.

Our reasons for conducting this study of the interaction of Lp(a) with liver cells were twofold: (1) In vivo, the liver is the major organ of lipoprotein synthesis and catabolism, and (2) liver cells may behave differently from fibroblasts with respect to lipoprotein interaction. In that respect, it is noteworthy that 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase and LDL receptor regulation in human hepatoma cells were found to differ from those of fibroblasts, the former being relatively refractory to changes in external lipoprotein concentrations.36

The results of this study may be interpreted as follows. A minor fraction of Lp(a) binds to HepG2 cells directly via LDL receptors. These conclusions were derived from our observation that affinity-purified Lp(a) downregulates cholesterol biosynthesis in HepG2 cells to a small but reproducible extent. Lp(a) also competes for LDL binding to HepG2 cells. Yet, as we show in Table 1, not only Lp(a) but also apo E–free HDL interferes with 125I-LDL binding to liver cells. We have no direct explanation for this latter effect, but we assume that it may reflect unspecific displacement of LDL from HepG2 cells, irrespective of the nature of the LDL binding (receptor or unspecific binding). The fraction of Lp(a) bound to the LDL receptor downregulates cellular cholesterol biosynthesis by a mechanism similar to that of LDL. Whether this binding occurs with intact Lp(a) or whether it occurs only after dissociation of apo(a), as suggested by Knight et al,37 cannot be deduced from the experiments described in Fig 1. The majority of Lp(a), however, was shown to associate with HepG2 cells independent of the LDL receptor and to be degraded only slowly. This association is apparently mediated by the characteristic apoprotein, since apo(a) alone behaves similarly.

Incubation with Lp(a) or apo(a) causes a remarkable increase in LDL binding to HepG2 cells, with an affinity that is comparable to that of the LDL receptor. Treatment of apo(a) with neuraminidase, which removed virtually all sialic acids as monitored by the thiobarbituric assay of Warren,32 almost completely abolished the effect of apo(a) on LDL binding (Fig 2). The increase in LDL binding triggered by apo(a) is unrelated to LDL receptors, since monoclonal C7 antibodies did not interfere with this mechanism. The incubation of HepG2 cells with apo(a) apparently had no effect on LDL receptor–mediated LDL internalization and degradation, since the downregulation of cholesterol biosynthesis by LDL was the same whether or not cells were preincubated with apo(a) (Fig 3). To interpret the observations that Lp(a) and apo(a) stimulate the association of LDL with liver cells, observations from previously published work need to be considered. Lp(a) has a high affinity to surface proteins such as fibronectin,38 tetranectin,39 and proteoglycans.40 Whether some of these proteins may be involved in Lp(a) binding to HepG2 cells is not clear at the present time. In preliminary experiments, HepG2 cells were incubated with specific antibodies against fibronectin, followed by sequential incubations with apo(a) and 125I-LDL (G.M. Kostner, unpublished work). An excess of these antibodies, however, had virtually no influence on apo(a)-triggered LDL association.

Lp(a) is also known to bind to plasminogen receptors, thereby interfering with fibrinolysis.41,42 Yet here again, we have found that preincubation with apo(a) in the presence and absence of excess plasminogen did not cause a measurable reduction of the increased LDL association with HepG2 cells (authors’ unpublished results).

Also pertinent to this study is a recent report by Trieu et al15 showing that recombinant apo(a) binds apo
B-containing lipoproteins with a high affinity of approximately $10^{-8}$ mol/L. LDL binding to apo(a) was mediated by the kringle-4 domains and could be dissociated by proline and hydroxyproline.40 We thus considered the possibility that not only does LP(a) bind to cell surface matrices followed by LDL trapping, but also vice versa, ie, LDL interacting with specific receptors binds LP(a) and mediates LP(a) catabolism. To investigate this hypothesis, HepG2 cells were incubated at 4°C with radiolabeled LP(a) in the presence and absence of small amounts of cold LDL, followed by removal of the medium and incubation at 37°C. In this way, only the material originally bound to HepG2 cells was followed. These experiments demonstrated that LDL in fact increased LP(a) degradation markedly. The effect in our experiments was dependent on the LP(a)-to-LDL ratio: at higher ratios it was less pronounced. The reason for this might be a higher affinity of LDL than of LDL-LP(a) complexes to the LDL receptor.

If this mechanism is also operative in vivo, it might explain the raised LP(a) levels found in patients with LDL receptor defects and in familial defective hypercholesterolemia as well as many other in vivo findings.18,25,37 Our findings, however, do not explain the increase of LP(a) in some individuals treated with HMG-CoA reductase inhibitors.24

Taken together, we deduce from our results that little LP(a) may be catabolized in vivo directly by the LDL receptor. One portion of LP(a), the amount of which remains to be determined, probably interacts with connective tissue proteins (proteoglycans and others) and may not be able to enter the cells. Another fraction associates with LDL in circulation or is ready to bind to LDL receptors and is taken up and catabolized by a hitchhiking-like process. The net effect of this pathway may depend on individual LDL and LP(a) plasma concentrations, their local concentration at the surface of liver cells, and LDL receptor activity. All these factors finally mediate LP(a) homeostasis under normal conditions and in diseased states.

Acknowledgments

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