Interaction of Lipoprotein Lp(a) and Low Density Lipoprotein with Glycosaminoglycans from Human Aorta

Magdolina Bihari-Varga, Eva Gruber, Martina Rotheneder, Rudolf Zechner, and Gert M. Kostner

The lipoprotein complexing activity of glycosaminoglycans (GAG) prepared from human aortas with lipoprotein Lp(a) in comparison to low density lipoproteins (LDL) was determined turbidimetrically in the presence of Ca++. In control experiments, purified chondroitin-6 sulfate and proteoglycans (PG) were used. Lp(a) exhibited approximately a threefold higher reactivity. Analyzing the chemical composition of the complexes, we found that Lp(a) had greater than fourfold higher binding capacity for GAG. The binding capacity of Lp(a) to PG was 3.4-fold higher as compared to LDL. The binding capacity of both lipoproteins for chondroitin-6 sulfate was only 50% in comparison to GAG, but again Lp(a) was four times more reactive. Neuraminidase treatment of LDL or Lp(a) did not interfere with GAG or chondroitin-6 sulfate binding. If, on the other hand, Lp(a) was treated with dithiothreitol and the Lp(a)-specific protein (apo) a) was removed, the GAG binding was reduced by about 45%. Apo a by itself gave no insoluble complexes with GAG. LDL and Lp(a)-GAG and -LP(a)-PG complexes were incubated with mouse peritoneal macrophages (MPM), and the stimulation of cholesterol ester formation was studied. At identical lipoprotein cholesterol concentrations, Lp(a)-GAG complexes exhibited a 1.3-fold higher stimulation of cholesterol esterification as compared to LDL-GAG. This difference was even more striking if lipoproteins were compared at a molar basis. PG-lipoprotein complexes were much more active with respect to interactions with MPM. The highest amount of cholesterol ester formation upon incubation with MPM was found with PG-Lp(a) complexes. Since free GAGs, as well as PG, are present in circulating blood, we believe that this might contribute to the high atherogenicity of Lp(a). (Arteriosclerosis 8:851–857, November/December 1988)

Lp(a) is a plasma lipoprotein of high atherogenicity. Its plasma concentration is under genetic control, and evidence is accumulating that a plasma level above 25 to 30 mg/dl must be considered as an independent risk factor for atherosclerosis and myocardial infarction.

Size, morphology, and lipid composition of Lp(a) is similar to low density lipoprotein (LDL). The most striking difference between the two lipoproteins is the presence of an additional apolipoprotein (apo) in the Lp(a) particle. This protein is called apo a or Lp(a) specific antigen. Apo a may be dissociated from Lp(a) by treating with disulfide reducing agents, leaving "Lp(a-)", a lipoprotein that is chemically and immunochemically similar to LDL. Apo a exhibits a considerable size heterogeneity ranging from 300 to 700 kD. The carbohydrate content, notably that of sialic acids of apo a, is considerably higher than that of apo B.

Little is known concerning the high atherogenicity of Lp(a). Intact Lp(a) has been demonstrated in atherosclerotic lesions, pointing to the assumption that it is trapped by intracellular matrices. This, in fact, is supported by in vitro findings that Lp(a) interacts with glycosaminoglycans (GAG) in a manner similar to LDL.

To shed more light on the possible molecular mechanisms of atherogenicity, Lp(a) and LDL from the same donor were made to interact with GAG and proteoglycans (PG), and their capabilities to induce cholesterol ester accumulation in mouse peritoneal macrophages (MPM), were compared. Major emphasis was given to lipoprotein-GAG complexes, because free GAG, but little PG, is found in circulating plasma.

**Methods**

**Isolation and Characterization of Lipoproteins**

LDL and Lp(a) were prepared from pooled plasma of donors with Lp(a) levels of >40 mg/dl. In most cases, LDL and Lp(a) prepared from identical pools were compared. For a control, LDL was also prepared from individuals who were apparently Lp(a)-negative.
The purification of lipoproteins was performed as described earlier. Briefly, freshly drawn plasma was brought to a density of 1.070 g/ml by adding solid NaCl and was ultracentrifuged for 22 hours at 15°C at 140 000 g. The floating very low density lipoprotein (VLDL) + LDL fraction was diazoylated against NaCl of d = 1.1019 g/ml and was ultracentrifuged for the removal of VLDL (22 hours at 120 000 g). LDL were recentrifuged at d = 1.060 g/ml and were kept at 4°C in the presence of NaN₃ and ethylene-diaminetetraacetic acid (EDTA) for not longer than 14 days. Part of these LDL were acetylated as described. In control experiments, LDL were passed over an immunoadsorber specific for Lp(a) to remove contaminating Lp(a).

Lp(a) was prepared from the d = 1.070 bottom fraction after addition of solid NaBr up to a density of 1.120 g/ml and ultracentrifugation for 22 hours at 140 000 g. Further purification of Lp(a) was performed by column chromatography over Biogel A-5m.

To remove the sialic acid, LDL and Lp(a) were incubated with neuraminidase (NANA) from Clostridium perfringens (Sigma). Lipoprotein solutions containing 5 to 10 mg/ml LDL or Lp(a) were incubated for 6 hours with 0.05 U of NANA at 37°C. The progress of desialylation had been studied in previous experiments by measuring the residual sialic acid content with the thiobarbituric acid method of Warren. It was found to be virtually complete after 6 hours.

Lp(a) was prepared according to the method of Armstrong et al. Lp(a) fractions containing 10 to 15 mg protein were incubated for 3 hours with 10 mmol/l of dithiothreitol (DTT) at 37°C, and Lp(a) was separated by heparin sepharose column chromatography.

All lipoprotein preparations were analyzed by SDS polyacrylamide gel electrophoresis in 3.75% gels, and the possibility that proteolytic degradation occurred during preparation was excluded. The majority of the apo-Lp portion of LDL and of Lp(a)-) consisted of B-100; in addition to apo B-100, Lp(a) contained apo a. Only trace amounts of fragments were observable after Coomassie blue staining.

All experiments were performed within 3 days after lipoprotein purification.

Isolation and Analysis of Proteoglycans and Glycosaminoglycans

PG from human intima-media was prepared and characterized according to the methods of Camejo et al. After purification, the PG-containing material consisted of 35% protein and 65% GAG. For one control experiment, PG was radiolabeled with ¹²⁵I according to the method of McFarlane.

GAG was prepared according to a slight modification of our previous procedure. Freshly obtained human aortic intimal tissues were delipidated with diethyl ether followed by proteolytic digestion with papain for 24 hours at 65°C (2 mg papain, 6 mg EDTA, 2.7 mg Cys.HCl in 2 ml of 0.1M potassium phosphate buffer, pH 6.4). The samples were deproteinated by passing them over a DOWEX 50W-X2 column. The total GAG content was estimated by the measurement of the hexuronic acid content according to the method of Bitter and Muir.

Different types of GAG were characterized by a combination of electrophoresis and their susceptibility to digestion with specific enzymes. Electrophoresis was carried out on cellulose acetate strips (Sartorius GmbH, Göttingen) in barbital buffer, pH 8.6, ionic strength 0.04. Staining was performed with Alcian blue. The susceptibility of GAG to enzymes was tested with hyalurondase chondroitinase-AC or -ABC according to the method of Saito et al. Based on these analyses, our preparations consisted of 40% to 46% chondroitin-6 sulfate (CSF), 19% to 23% dermatan sulfate, 21% to 27% heparan sulfate, and 6% to 9% hyaluronic acid. The reference standard GAG was a generous gift from M.B. Mathews and J.A. Cifonelli (Chicago). In control experiments, pure chondroitin-6 sulfate (Sigma, Deisenhofen) was used.

For control experiments, small amounts of GAG were isolated from human plasma according to the method of Taniguchi et al.

Measurement of Lipoprotein-Proteoglycan and Lipoprotein-Glycosaminoglycan Interactions

Interaction of Lipoproteins with Proteoglycan

These experiments were performed in 5 mM Tris-HCl (pH 7.2), 4.4 mM CaCl₂, 1.5 mM MgCl₂ and 6 mM KCl. Solutions containing 1 to 5 mg/ml of lipoproteins were mixed with increasing amounts of PG and were incubated for 1 hour at 4°C, followed by centrifugation at 10 000 g. The pellet was washed twice with buffer and was analyzed.

In a control experiment, radiolabeled PG was added to freshly drawn human serum and was incubated for 2 hours at 37°C. Lp(a) and LDL were then separated by passing the mixture over immune specific adsorbers. The distribution of radioactivity was measured.

To determine the optimal complexing conditions, fixed amounts of lipoproteins were titrated with increasing amounts of GAG, and vice versa, as described in detail earlier. The turbidity of the formed GAG-lipoprotein complexes was measured at 680 nm. The insoluble complexes were precipitated in the presence of 1.4 mM CaCl₂, were centrifuged at 5000 g, were washed twice with 1.4 mM CaCl₂, and were solubilized in 0.3 M NaCl. This solution was analyzed for its content of cholesterol (CHOD-PAP method, Boehringer-Mannheim) and hexuronic acids.

Experiments with Mouse Peritoneal Macrophages

These experiments were carried out as outlined in detail in previous work. Mouse peritoneal macrophages (MPM) were harvested from the peritoneal cavity of unstimulated mice (about 3 x 10⁶ cells per mouse). Cells were suspended in Dulbecco’s modified Eagle medium (DMEM) containing 20% fetal calf serum (FCS), 100 U/ml penicillin, and 100 µg/ml streptomycin. Cells were dispensed into multiray dishes and were allowed to adhere for 2 hours at 37°C in a humidified 5% CO₂ 95% air atmosphere. Non-adherent cells were removed by washing with phosphate-buffered saline (PBS) and were further incubated for 24 hours in DMEM-FCS medium at 37°C. At the end of this incubation, each of the dishes contained about 50 mg cell protein.
Table 1. Chemical Composition of Lipoprotein Fractions Used for Complexing with Glycosaminoglycans

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>n</th>
<th>Protein</th>
<th>CE</th>
<th>FC</th>
<th>PL</th>
<th>TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL</td>
<td>14</td>
<td>21.5</td>
<td>42.8</td>
<td>11.0</td>
<td>21.3</td>
<td>3.8</td>
</tr>
<tr>
<td>LDL-NANA</td>
<td>4</td>
<td>21.7</td>
<td>42.6</td>
<td>10.8</td>
<td>21.2</td>
<td>3.9</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>13</td>
<td>29.5</td>
<td>37.1</td>
<td>10.1</td>
<td>19.7</td>
<td>4.0</td>
</tr>
<tr>
<td>Lp(a)-NANA</td>
<td>4</td>
<td>30.7</td>
<td>36.5</td>
<td>9.6</td>
<td>19.4</td>
<td>3.8</td>
</tr>
<tr>
<td>Lp(a–)†</td>
<td>5</td>
<td>23.6</td>
<td>41.5</td>
<td>10.3</td>
<td>20.5</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Values are % per weight and are means (±SD).

*Number of preparations analyzed; †Lp(a–) refers to Lp(a) treated with dithiotreitol so that it was free from apo a.

CE = cholesteryl ester, TG = triglyceride, LDL = low density lipoprotein, LDL-NANA = neuraminidase-treated LDL.

For studying the cholesteryl ester accumulation in the cells, the MPM were incubated for 24 hours with DMEM-FCS medium containing 20 mCi of 14C oleic acid plus various amounts of LDL-GAG or Lp(a)-GAG complexes. The medium was then removed, the monolayers were washed four times with PBS, and the radioactivity accumulating in the cholesteryl ester fraction was determined as described.

Control incubations were carried out with pure LDL, acetylated LDL (Ac-LDL), or Lp(a) as a control. All chemicals were reagents from E. Merck, Darmstadt, West Germany, unless stated otherwise.

Results

Table 1 displays the chemical composition of all lipoprotein fractions used in this study.

To determine binding, LDL and Lp(a) were titrated with increasing amounts of GAG and the resulting turbidity was measured photometrically at 680 nm (Figure 1). The turbidity observed with Lp(a) at a given GAG concentration was significantly higher than with LDL. Lp(a), as well as LDL, exhibited two shoulders in the titration curves before reaching saturation. The ratio of GAG concentrations at the three inflexion points was approximately 1:2:3.

Table 2 displays the amounts of GAG in the GAG-lipoprotein complexes expressed in a weight ratio. From these data, we conclude that Lp(a) has a more than fourfold higher binding capacity for GAG as compared to LDL. The difference was statistically highly significant (p<0.001).

In parallel experiments, we performed similar titrations with CSF and found that this compound was about 50% less reactive than our GAG preparations.

It is worth noting at this point that neither GAG nor PG exhibited any complex formation with high density lipoprotein or with different serum proteins, e.g., albumin or IgG, under our experimental conditions. In subsequent experiments, we addressed the question as to why Lp(a) might be much more reactive with GAG than LDL. A possible explanation is the difference in the sialic acid content between these two lipoproteins. Thus, LDL and Lp(a) were treated with NANA. By use of the thiobarbituric acid assay, it was ascertained that virtually all sialic acid had been removed by enzyme treatment. To verify that NANA treatment did not change the protein moiety of the lipoproteins, we further investigated apo LDL and apo Lp(a) by SDS polyacrylamide gel electrophoresis and compared them with the parent lipoproteins. LDL and NANA-LDL exhibited one major protein band (>90% of stainable material) migrating in the Mr region of 550 kD representing apo B-100. Lp(a) exhibited two bands, one representing apo B-100 and the other, apo-a. NANA treatment caused an anodical shift of the apo-a bands, giving further evidence that sialic acid was grossly reduced (pictures not shown). NANA treatment of LDL and of Lp(a) caused no fragmentation of the apolipoproteins.

NANA-treated lipoproteins were titrated with GAG and compared with their untreated counterparts. LDL and Lp(a) did not change their reactivity against GAG by
Table 2. Ratio of Glycosaminoglycans, Chondroitin-6 Sulfate, and of Proteoglycan/Lipoprotein Cholesterol in Insoluble Complexes

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>GAG/Lp-C</th>
<th>CSF/Lp-C</th>
<th>PG/Lp-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL</td>
<td>0.31 (0.10)</td>
<td>0.15 (0.03)</td>
<td>0.58 (0.03)</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>1.28 (0.31)</td>
<td>0.58 (0.32)</td>
<td>1.96 (0.1)</td>
</tr>
<tr>
<td>LDL-NANA</td>
<td>0.30 (0.07)</td>
<td>0.18 (0.15)</td>
<td></td>
</tr>
<tr>
<td>Lp(a)-NANA</td>
<td>1.26 (0.27)</td>
<td>0.55 (0.50)</td>
<td></td>
</tr>
<tr>
<td>LDL-DTT ²</td>
<td>0.31 (0.29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lp(a)-²</td>
<td>0.85 (0.22)</td>
<td>0.36 (0.32)</td>
<td></td>
</tr>
</tbody>
</table>

Values are w/w and are means (±SD).

Complexes were formed with different lipoproteins at saturation GAG/CSF/PG concentrations. (See Figure 1.)

GAG = glycosaminoglycans, Lp-C = lipoprotein cholesterol, CSF = chondroitin-6 sulfate, PG = proteoglycan, LDL = low density lipoprotein, NANA = neuraminidase, and DTT = dithiothreitol.

Table 2. Ratio of Glycosaminoglycans, Chondroitin-6 Sulfate, and of Proteoglycan/Lipoprotein Cholesterol in Insoluble Complexes

Interaction of Glycosaminoglycan and Proteoglycan-Lipoprotein Complexes with Macrophages

To measure the uptake of lipoprotein complexes, we determined the incorporation of $^{125}$I-olate into the cholesteryl ester fraction of HDL. The validity of this assay (i.e., a good correlation of cholesteryl ester (CE) formation with the binding and internalization of lipoproteins into macrophages) has been verified in previous reports. 22,23,24

LDL and Lp(a) were complexed with GAG and PG in the presence of Ca$^{++}$ and were incubated with MPM for 24 hours. The results shown in Figure 2 were derived from experiments where lipoprotein-GAG and lipoprotein-PG complexes were incubated with MPM at identical cholesterol concentrations.

In the first set of experiments, GAG complexes with LDL and Lp(a) were compared with Ac-LDL. Since lipoprotein-GAG complexes were less reactive than Ac-LDL, the experiments shown in Figure 2A relate to incubations with relatively high amounts of complexes (50 and 100 µg/ml cholesterol). MPM responded with Lp(a)-GAG complexes within approximately 1.3-fold higher cholesterol esterification as compared to LDL-GAG complexes. These differences were statistically significant. LDL or Lp(a) alone (without GAG) did not cause any appreciable cholesteryl ester formation in MPM (Tables 3 and 4) as reported earlier. 13 Tables 3 and 4 also reveal additional data from experiments with equimolar amounts of LDL and Lp(a) complexes and MPM.

In subsequent experiments, LDL and Lp(a) complexes with PG were compared with Ac-LDL with respect to their interaction with MPM (Figure 2B). It was found that Lp(a)-PG was significantly more reactive than LDL-PG, and even slightly more reactive than Ac-LDL if incubated at identical lipoprotein cholesterol concentrations. When the experiments were carried out at equimolar concentrations of lipoproteins, the differences were even more striking.

Discussion

Since the early publication of Virchow, 25 suggesting that mucous substances in the arterial wall play a dominant role in atherogenesis, the interaction of LDL with various kinds of sulfated polysaccharides has been studied extensively. 25,26,27 It is now generally agreed that the interaction of apo B-containing lipoproteins with sulfated GAG is one of the key features in the sequence of events leading to lipid deposition and plaque formation.

Since mostly free GAG, and not PG, circulate in the blood and are in close contact with plasma lipoproteins, we started our experiments with GAG. The Ca$^{++}$-mediated interaction of GAG from human aorta with LDL

NANA treatment (Table 2). This was true for the turbidity formation by the complexes, as well as for the GAG/ lipoprotein cholesterol ratios in the precipitate.

In further experiments, Lp(a) was incubated with DTT to remove the apo-a from the lipoprotein. Lp(a-) was purified by heparin sepharose column chromatography. The chemical composition of Lp(a-) listed in Table 1 revealed little difference from normal LDL. When tested for its GAG reactivity, Lp(a-) showed decreased binding toward GAG, but was still more reactive than LDL.

In control experiments, normal LDL was treated with DTT in the same way as Lp(a) and was titrated with GAG. It was found that DTT treatment did not alter LDL's interaction with GAG (Table 2).

Finally, the apo-a portion of Lp(a) regained from heparin sepharose column chromatography was tested by titration. Apo-a by itself yielded neither turbidity nor formation of a precipitate with GAG (data not shown).

Since our GAG preparation from human aorta represented a mixture of several sulfated polysaccharides, we repeated the titration of lipoproteins with pure commercial CSF. Under these conditions also, Lp(a) yielded a three-to-fourfold reactivity as compared to LDL (Table 2). Lp(a-) displayed an intermediate reactivity similar to the results obtained with GAG from human aorta. NANA treatment of LDL or Lp(a) had little influence upon the reactivity with CSF.

To prove the relevance of our findings for the situation in vivo, two control experiments were performed: 1) Radioactively labeled $^{125}$I-PG was added to two freshly drawn human serum batches containing 32 and 55 mg/dl of LDL. After incubation for 2 hours at 37°C, Lp(a) and LDL + VLDL were isolated by immunoabsorbers. In the first serum, 35% and 48% of the label, and in the second serum, 44% and 50% of the label eluted with Lp(a) and LDL, respectively. 2) GAG circulating in human blood was isolated in small amounts and was added to purified Lp(a) and to LDL. In both cases, >90% of the GAG were complexed to the lipoproteins.

Interaction of Glycosaminoglycan- and Proteoglycan-Lipoprotein Complexes with Macrophages

To measure the uptake of lipoprotein complexes, we determined the incorporation of $^{125}$I-olate into the cholesteryl ester fraction of HDL. The validity of this assay
and Lp(a) was studied. We carried out these studies on purpose with a mixture of sulfated polysaccharides from autopsic material, and not with pure single substances, to match the in vivo situation as closely as possible. Our results demonstrate that Lp(a) reacts with GAG to a significantly higher degree as compared to LDL. This was not due to the high content of sialic acids in Lp(a) because NANA treatment did not abolish the high binding capacity of Lp(a). These observations are in line with previously published results that showed that the sialic acid content of LDL does not interfere with its reaction with GAG.28 It might be worth noting that sialic acids are important for the interaction of LDL with PG.29

Also the Lp(a)-specific protein, apo-a, may not be solely responsible for the increased reactivity, since: 1) apo-a itself gave no visible complex formation with GAG, and 2) Lp(a-) exhibited a much higher reactivity with GAG than LDL, despite the fact that virtually all apo-a had been removed. So far we have no plausible explanation for these observations. The possibility that residual apo-a may have caused the increased reactivity of Lp(a-) as compared to LDL may be excluded since only trace amounts of apo a were found in our preparations (data not shown).

It is important to know more about the interaction of Lp(a) with GAG, which in fact is not even completely resolved in detail for LDL itself. From earlier work, it seems that phospholipids play an important role in GAG binding, since phospholipase A and phospholipase C treatment of LDL greatly diminish its reactivity.15,30,31 Similarly, the alteration of the surface lipids, lecithin and

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**Table 3. Cholesteryl Ester Formation in Mouse Peritoneal Macrophages during Incubation with Lipoproteins or Complexes of Lipoproteins with Glycosaminoglycans or Proteoglycans**

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Concentration (mg/ml cholesterol)</th>
<th>nmol of CE formed per mg cell protein in 24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL</td>
<td>50</td>
<td>0.8±0.2</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1.6±0.4</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>50</td>
<td>0.9±0.3</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1.8±0.5</td>
</tr>
<tr>
<td>GAG-LDL</td>
<td>10</td>
<td>2.1±0.7</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>11.4±1.3</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>15.3±1.2</td>
</tr>
<tr>
<td>GAG-Lp(a)</td>
<td>10</td>
<td>3.4±0.6</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>15.7±1.6</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>19.8±1.8</td>
</tr>
<tr>
<td>Ac-LDL</td>
<td>10</td>
<td>18.7±1.2</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>40.1±2.4</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>86.3±4.1</td>
</tr>
<tr>
<td>PG-LDL</td>
<td>10</td>
<td>8.6±0.5</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>12.6±1.0</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>21.3±2.1</td>
</tr>
<tr>
<td>PG-Lp(a)</td>
<td>10</td>
<td>22.8±2.6</td>
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<td></td>
<td>20</td>
<td>43.9±2.9</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>110.1±5.3</td>
</tr>
</tbody>
</table>

Values are means±SD of triplicate experiments. Incubations were carried out for 24 hours. Experimental details are given in the Methods section.

CE=cholesteryl ester, LDL=low density lipoprotein, GAG=glycosaminoglycans, Ac-LDL=acetylated LDL, and PG=proteoglycan.

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**Table 4. Cholesteryl Ester Formation in Mouse Peritoneal Macrophages during Incubation with Lipoprotein Complexes**

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Concentration ([mol/l]×10⁷)</th>
<th>nmol of CE formed per mg cell protein in 24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ac-LDL</td>
<td>0.1</td>
<td>15.8±1.1</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>36.3±1.8</td>
</tr>
<tr>
<td>GAG-LDL</td>
<td>0.1</td>
<td>2.0±0.2</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>4.7±0.3</td>
</tr>
<tr>
<td>GAG-Lp(a)</td>
<td>0.1</td>
<td>4.2±0.3</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>10.3±0.9</td>
</tr>
<tr>
<td>PG-LDL</td>
<td>0.1</td>
<td>7.4±0.5</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>11.7±0.8</td>
</tr>
<tr>
<td>PG-Lp(a)</td>
<td>0.1</td>
<td>31.7±2.4</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>64.5±3.8</td>
</tr>
</tbody>
</table>

Values are means±SD of triplicate experiments. See footnotes to Table 3 for abbreviations.

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**Figure 2.** Cholesteryl ester formation in mouse peritoneal macrophages stimulated with acetylated low density lipoprotein (Ac-LDL) in comparison to different lipoprotein complexes. A. Glycosaminoglycan (GAG)-lipoprotein complexes were added at identical total cholesterol concentrations, 50 and 100 mg/ml of medium. B. Proteoglycan (PG)-lipoprotein complexes were added at 10 and 20 mg/ml of medium, respectively. Ac-LDL at concentrations of 10 and 20 mg/ml were used as controls. The reactivity is expressed in nmol of cholesteryl ester (CE) per mg cell protein formed during a 24-hour incubation. For details, see the Methods section.
free cholesterol, by the action of lecithin-cholesterol acyltransferase strongly affects the reactivity of LDL with GAG.\textsuperscript{32}

The multiple inflection points observed in the titration curves imply the possibility that stable complexes of varying stoichiometry may be formed at lower GAG concentrations. The stepwise binding may involve different binding sites and various types of bonds (e.g., electrostatic forces or hydrogen bonds, as suggested by Srinivasan et al.\textsuperscript{25} and may play a role in the stepwise evolution of the atherosclerotic plaque. An alternative explanation might be that these inflection points reflect the heterogeneity of the native GAG mixture used and the presence of several different reactive polysaccharides. This might also explain our finding that purified CSF exhibited a markedly lower reactivity with LDL and with Lp(a) as compared to the mixture extracted from arteries (Table 2).

The data obtained in this study give a possible clue for the high atherogenicity of Lp(a). It is tempting to speculate that soluble GAG, which have been demonstrated in human plasma even under physiological conditions,\textsuperscript{7,10,11} interact with apo B-containing lipoproteins. Because of the higher reactivity of Lp(a) as compared to LDL, Lp(a) might be the favored substrate for such an interaction. These interactions with apo B-containing lipoproteins not only may take place with the B/E receptor, but also may lead to the catabolism of the complexes by the scavenger pathway known to operate in several cells (e.g., endothelial cells, smooth muscle cells, and macrophages). That the scavenger removal mechanism, in fact, occurs with these complexes has been demonstrated ex vivo in this study with MPM.

The feasibility of this theory is underlined by our two control experiments where we demonstrated that: 1) purified PG added to whole plasma complexes, to a major extent, to Lp(a) and to LDL, and 2) that GAG isolated from circulating human blood also binds to Lp(a) and LDL in a similar manner as GAG batches obtained from aorta.

Another explanation of the high atherogenicity of Lp(a) might be that Lp(a) that passed the endothelial layer and entered the arterial intima might become complexed with PG. Our data clearly demonstrate that Lp(a) exhibits a significantly higher reactivity with PG than LDL. These Lp(a)-PG complexes caused a high cholesteryl ester deposition in MPM, which was much greater than that found earlier with LDL-PG complexes. Lp(a)-PG were even more reactive than LDL-Ac.

Thus, Lp(a) may be retained in vivo in the subendothelial layer to a much higher degree than LDL. There, lipoprotein complexes may be taken up by smooth muscle cells, which are known to exhibit a limited capacity to catabolize cholesterol-rich lipoproteins. According to current theory, deposited lipoproteins are taken up by the scavenger pathway by monocytes/macrophages and are eventually transformed into foam cells. This process probably is greatly accelerated in individuals with high Lp(a) concentrations.

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References

3. Albers JJ, Cabana VG, Warnick GR, Hazzard WR. Lp(a) lipoprotein: Relationship to sinking pre-β lipoprotein, hyperlipoproteinemia and apolipoprotein B. Metabolism 1975; 24:1047–1054

Index Terms: Lp(a) • glycosaminoglycans • low density lipoprotein • proteoglycans • scavenger pathway

Correction

Since publication of our article in the January/February 1988 issue of Arteriosclerosis (Avogaro P, Bittolo Bon G, Cazzolato G. Presence of a Modified Low Density Lipoprotein in Humans. Arteriosclerosis 8:79-87, January/February 1988), we have partly modified our method. The first procedure actually contained an amount of EDTA which may be a matter for criticism. EDTA as a chelant of Ca++ interferes with the enzymatic determination of phospholipids. This is especially relevant because the decrease of phospholipids is a biochemical stigmata of our modified lipoproteins. In the new procedure, we have avoided the use of EDTA in the various buffers used for chromatography. Its use has continued for collection of plasma samples, for ultracentrifugation, and during the first hour of dialysis. The results obtained with the modified procedure substantially mimic our previous ones except for a minor, but significant, decrease of phospholipids in the modified low density lipoprotein. These data will be published shortly together with some other new findings.

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Interaction of lipoprotein Lp(a) and low density lipoprotein with glycosaminoglycans from human aorta.

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