Biochemistry and Clinical Relevance of Lipoprotein X

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ABSTRACT

Lipoprotein X (LP-X) is an abnormal lipoprotein that appears in the sera of patients with obstructive jaundice and is thus a marker for cholestasis. The presence of LP-X in serum does not allow discrimination between intra- and extra-hepatic cholestasis. In addition LP-X is present in the plasma of patients with familial plasma lecithin: cholesterol acyl transferase (LCAT) deficiency. It is a spherical particle that aggregates strongly. Phospholipids and unesterified cholesterol make up the bulk of LP-X, which is a low density lipoprotein. Protein, cholesterol esters, and triglycerides together make up 12 percent of the composition of LP-X. Lithocholic acid is the major bile acid in LP-X. Three species of LP-X have been isolated (LP-X₁, LP-X₂ and LP-X₃). Because of its aggregating properties, LP-X complexes with enzymes, such as alkaline phosphatase. Electrophoretic and immunochemical methods are available for assay of LP-X. The fact that bile lipoprotein can be converted to LP-X by addition of albumin, and LP-X can be converted to bile lipoprotein by the addition of bile salts may suggest that the integrity of the LPX molecule depends on a certain critical bile salts to albumin ratio. Phospholipase in plasma is implicated in the catabolism of LP-X. The role of LP-X in cholestasis is apparently related to the removal of free cholesterol from the circulation as a consequence of its aggregating properties.

Introduction

The presence of LP-X in the sera of patients with obstructive jaundice lends itself as a sensitive indicator of cholestasis.²,⁸,⁹,¹³ The specificity of LP-X for the demonstration of cholestasis becomes evident after the first year of life, since its usefulness in the newborn infant for detection of cholestasis is limited, perhaps, due to immature liver function.¹⁸ Demonstration of LP-X in sera does not, however, permit differentiation of intra and extra hepatic cholestasis.⁷ Demonstration of LP-X has also been made in the plasma of patients with familial plasma lecithin: cholesterol acyl transferase (LCAT) deficiency.¹⁵ There is an inverse relationship between LP-X and LCAT activity.¹¹

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Characterization of LP-X

It has been demonstrated by electron microscopy that LP-X is a spherical particle 30 to 70 nm in diameter. Utilizing ultra centrifugation, LP-X was found to be associated with the low-density lipoprotein fraction, which is isolated in a density gradient of 1.006 to 1.063 g per ml. Phospholipids constitute 66 percent of the LP-X molecule, with free cholesterol contributing 22 percent, cholesterol esters and triglycerides each amounting to 3 percent, and protein constituting 6 percent of the LP-X molecule. Albumin contributes to 40 percent of the protein content of LP-X, the remainder being made up of apolipoprotein C. Bile acids account for two to three percent by weight of LP-X, the major bile acid being lithocholic acid, which is known to be hepatotoxic. Lipoprotein-X has been fractionated into three species: LP-X₁, LP-X₂, and LP-X₃. Although there are slight differences in the content of very low density lipoprotein (VLDL), cholesterol, and protein between the three species of LP-X, they essentially have the same phospholipid to free cholesterol ratio found in the native LP-X. The major phospholipid in LP-X is lecithin, with sphingomyelin constituting the minor phospholipid fraction. Myristic acid is the major fatty acid found in the three species of LP-X. Carbon-13 nuclear magnetic resonance studies have demonstrated that the three species of LP-X have spectral characteristics different from normal human high density lipoprotein (HDL)-3 or low density lipoprotein (LDL). In table I are compared the density and composition of LP-X and other plasma lipoproteins.

Assay for LP-X

A variety of assay procedures are available. These include electrophoresis alone, electrophoresis and subsequent precipitation of LP-X with polyanionic compounds followed by densitometric scanning, or by using immunochromatographic techniques. Assay for LP-X in sera is based on the cathodal mobility of this component on Agar gel electrophoresis. Lipoprotein-X can be visualized by cathodal migration of sudan black added to the sample at the point of application, or it can be quantitated by precipitation by heparin and poly anions. Procedures that use polyanionic compounds, such as heparin and salts such as magnesium chloride to precipitate LP-X and the subsequent densitometric scanning of the precipitated band have a working range of 0.06 to 6.3 g per l. Levels of LP-X greater than 6.3 g per l can be measured by appropriate dilution with sera that do not contain LP-X.

Alternatively phosphotungstic acid can be used to precipitate the normal low density lipoprotein and very low density lipoprotein and LP-X is subsequently precipitated from the supernatant by the addition of magnesium ions at pH 9.0. Lipoprotein-X in the precipitate is quantitated by measuring phospholipids with a coupled enzyme assay. Lipoprotein-X can also be measured by immunochromatographic procedures. Because of cross-reactivity problems, it is best to precipitate lipoprotein B with specific antisera and measure LP-X in the supernatant by appropriate immunochemical techniques such as radial immunodiffusion and electro immunodiffusion. The former procedure is time consuming and is insensitive to levels less than 300 mg per l. In contrast, the electroimmunodiffusion procedure takes three hours to perform and can measure levels higher than 200 mg per l.

Aggregating Properties of LP-X

Membrane bound enzymes such as alkaline phosphatase, 5'-nucleotidase,
TABLE I

Comparison of Density and Composition of Lipoprotein X and Other Plasma Lipoproteins

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Density Range gm/ml at 4°C</th>
<th>Composition of Lipoprotein in Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Protein</td>
</tr>
<tr>
<td>Chylomicron</td>
<td>0.94</td>
<td>2</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.94 - 1.006</td>
<td>9</td>
</tr>
<tr>
<td>LDL</td>
<td>1.006 - 1.063</td>
<td>21</td>
</tr>
<tr>
<td>LP-X</td>
<td>1.006 - 1.063</td>
<td>5.8</td>
</tr>
<tr>
<td>LP-X&lt;sub&gt;1&lt;/sub&gt;</td>
<td>1.038</td>
<td>3.2</td>
</tr>
<tr>
<td>LP-X&lt;sub&gt;2&lt;/sub&gt;</td>
<td>1.049</td>
<td>6.4</td>
</tr>
<tr>
<td>LP-X&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1.058</td>
<td>6.7</td>
</tr>
<tr>
<td>HDL</td>
<td>1.063 - 1.21</td>
<td>50</td>
</tr>
</tbody>
</table>

\[\gamma\text{-glutamyl transpeptidase, and L-leucyl-p-naphthylamidase (LAP) have been shown to aggregate with LP-X.}\]

\[\text{Association of a fraction of serum alkaline phosphatase to LP-X results in its classification as a high molecular weight species when separated by gel permeation chromatography. It has been shown that high molecular weight alkaline phosphatase found in sera of LP-X positive patients having obstructive jaundice is the same enzyme associated with the liver cell membrane.}\]

\[\text{Clinical Significance of LP-X}\]

Lipoprotein-X is a sensitive marker for cholestasis. The presence of cholestasis, even in the absence of significant increases in alkaline phosphatase activity, can be corroborated by the demonstration of presence of LP-X in serum. The presence of alkaline phosphatase-LPX complex in serum is a reliable indicator of cholestasis. However, the presence of LP-X cannot discriminate between intra- and extra-hepatic cholestasis. The appearance of LP-X in sera of the newborn has limited usefulness as an indicator of cholestasis since liver function is still immature. As such, only in babies older than one year can the appearance of LP-X in serum be regarded as indicative of cholestasis. In addition, LP-X is present in sera of patients with deficiency of the enzyme lecithin-cholesterol acyl transferase (LCAT).

\[\text{Role of LP-X}\]

Why does LP-X arise in cholestasis? Does it arise from bile? If so, is it a modified form of bile lipoprotein? The recent demonstration that bile lipoprotein can be converted to LP-X by \textit{in vitro} addition of albumin and, conversely, that LP-X can be converted to bile lipoprotein by the addition of bile salts has thrown light on the formation of LP-X. Perhaps LP-X is formed when bile lipoprotein excreted from the liver, owing to obstruction, finds its way into the blood stream and there supplemented with serum albumin is transformed into LP-X. The explanation of high molecular weight alkaline phosphatase associated with LP-X in biliary obstruction can also be attributed to a reflux of bile lipoprotein into blood. In cholestasis, LP-X may serve to remove free cholesterol from circulation.

\[\text{Conclusion}\]

Lipoprotein-X is a sensitive indicator of biliary cholestasis. Apparently it arises from bile lipoprotein. Its association with additional albumin upon entry into the blood stream under cholestatic conditions confers upon it the properties
chemically determined for LP-X. Phospholipase of plasma might play a role in the catabolism of LP-X as has been demonstrated in in vitro studies.  

References