Abnormal lipoprotein-X

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Structure and Molecular Composition of Lipoprotein X

Lipoprotein X (LP-X) is an abnormal serum lipoprotein originating in several diseased conditions. It is a sub-fraction of low density lipoproteins (LDL), which does not react with antiseraum against normal serum [86, 105]. LP-X is a lamellar spherical particle with mean diameter 69 nm (range 40 to 100 nm) as revealed by electron microscopy, that aggregates strongly [33, 47, 90]. Compared to other lipoproteins LP-X particles are rich in phospholipids (60-67%) and cholesterol (23-30%) but poor in cholesterol esters (0.5-2%), triglycerides (2-3%), and protein (3-7%) [25, 37, 68, 90]. The following phospholipids are mainly presented in LP-X. lysophosphatidylcholine (4.1%), sphingomyelin (14.2%), phosphatidylcholine (77.5%), and phosphatidylethanolamine (2.5%) [92]. Bile acids are also often present in LPX, among which lithocholic acid is the major component [66]. The protein component of LP-X is dominated by albumin located in core and apolipoprotein C (I, II and III) located on the surface of the particle [34, 71]. Albumin contains 40% of total protein components of LP-X. It is located in the internal water compartment of LP-X covered by lipids and becomes visible only after complete delipidation of LP-X. [11, 96]. The role of this protein relevant to LP-X is not clear, however it has been shown that albumin is required for binding to LP-X cholesterol metabolizing enzymes [68]. It has been shown that addition of albumin to bile lipoprotein converts it to LP-X. This may offer possible explanation on the origin of LP-X [57].

Apolipoprotein C was discovered in 1964 by Gustafson and co-workers [32], who separated this apolipoprotein from partially dilapidated very low density lipoproteins (VLDL). It was characterized by a high capacity for lipid binding, immunological properties, and peptide patterns different from other plasma apolipoproteins, and by serine and threonine as N-terminal amino acids [32]. Later, apolipoprotein C was isolated from chyled VLDL [1] and from all lipoprotein classes from plasma of healthy subjects [2]. Apolipoprotein C has the same common to other apolipoproteins structural feature – domain containing amphipathic helix. Hydrophobic amino acids are located at one side of the helix, and hydrophilic amino
acids at the other side. The hydrophobic part of the helix is thought to interact with acyl chains of phospholipids, whereas hydrophilic part interacts with phospholipid hydrophilic groups. Immunochemical analysis of apolipoprotein C has shown that the protein moiety consists of at least three antigenic determinants [49]. There are identified three distinct classes of this apolipoprotein, C1, C2 and C3, which are encoded by different genes [52, 77] and differ in signal peptide amino acid sequence [49]. C1 and C2 are encoded by gene located on chromosome 19, and C3 is encoded by gene located on chromosome 11. [52, 77]. The major function of the apolipoprotein C as well as other apolipoproteins is maintaining of lipoprotein stability, binding and transportation of lipids in the blood stream [85].

Fig 1 demonstrates schematic structure of LP-X. In tables 1–3 the molecular composition and apolipoprotein content of LP-X in comparison to other lipoproteins are presented.

Hydroxylapatite chromatography revealed that LP-X has a higher molecular weight compared to LDL [108]. In addition, proton magnetic resonance studies showed motion of acyl chains and/or cholesterol rings much more restricted in LP-X compared to its normal counterpart (LDL) [10]. One important characteristic feature of LP-X is its mobility toward the cathode on agar-gel electrophoresis [91]. This phenomenon is not the result of a positive charge on the particle at pH 8.6, but of a pronounced electro-endosmosis in this medium, which strongly affects the migration of this rather large particle with its relatively small protein component [33, 95].

Using zonal ultracentrifugation, LP-X can be divided into three isoforms. LP-X1, LP-X2, and LP-X3, differing in density, phospholipid content and apolipoprotein composition [77]. In addition to apolipoprotein C, LP-X2, and LP-X3 also contain apolipoprotein A1 and apolipoprotein E, absent in LP-X1 [77].
Table 1. Molecular composition of lipoproteins

<table>
<thead>
<tr>
<th>Class</th>
<th>Diameter (nm)</th>
<th>Protein (%)</th>
<th>Cholesterol (%)</th>
<th>Phospholipid (%)</th>
<th>Triacylglycerol (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL</td>
<td>5-15</td>
<td>33</td>
<td>30</td>
<td>29</td>
<td>8</td>
</tr>
<tr>
<td>LDL</td>
<td>18-28</td>
<td>25</td>
<td>50</td>
<td>21</td>
<td>4</td>
</tr>
<tr>
<td>LP-X</td>
<td>40-100</td>
<td>6</td>
<td>27</td>
<td>65</td>
<td>2</td>
</tr>
<tr>
<td>VLDL</td>
<td>30-80</td>
<td>10</td>
<td>22</td>
<td>18</td>
<td>50</td>
</tr>
<tr>
<td>Chylomicrons</td>
<td>100-1000</td>
<td>&lt;2</td>
<td>8</td>
<td>7</td>
<td>84</td>
</tr>
</tbody>
</table>

Table 2. Classes of apolipoproteins (Apo) in different lipoproteins

<table>
<thead>
<tr>
<th>VLDL</th>
<th>LDL</th>
<th>LP-X</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major apolipoproteins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apo-B100</td>
<td>Apo-B100</td>
<td>Apo-C2</td>
<td>Apo-A2</td>
</tr>
<tr>
<td>Apo-C1</td>
<td>Apo-C1</td>
<td>Apo-C2</td>
<td>Apo-A2</td>
</tr>
<tr>
<td>Apo-C2</td>
<td>Apo-A1</td>
<td>Apo-C2</td>
<td>Apo-C3</td>
</tr>
<tr>
<td>Apo-C3</td>
<td>Apo-C3</td>
<td>Apo-C3</td>
<td>Apo-C3</td>
</tr>
</tbody>
</table>

Minor apolipoproteins

<table>
<thead>
<tr>
<th>Apo-D</th>
<th>Apo-E</th>
<th>Apo-C1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Apo-C2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Apo-C3</td>
</tr>
</tbody>
</table>

Table 3. Characteristics of some apolipoproteins

<table>
<thead>
<tr>
<th>Apolipoprotein</th>
<th>N-terminal</th>
<th>C-terminal</th>
<th>Antigenic determinants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo-A</td>
<td>Asp</td>
<td>Thr, Gln</td>
<td>two or more</td>
</tr>
<tr>
<td>Apo-B</td>
<td>Glu</td>
<td>Ser</td>
<td>one</td>
</tr>
<tr>
<td>Apo-C</td>
<td>Thr, Ser</td>
<td>Ala, Val, Glu</td>
<td>three or more</td>
</tr>
</tbody>
</table>

LP-X in liver and biliary tract diseases

For the first time LP-X was detected, isolated from the blood, and characterized upon studying patients with liver disease with obstructive jaundice [86, 105].
Subsequent studies demonstrated that the presence of LP-X is detected in 45% of cases of liver disease with cholestatic features, having raised levels of serum lipids, hypercholesterinemia, and a hyperlipoproteinemia, and was not detected in cases of liver disease without cholestasis [84, 91]. It was also shown that the incidence of LP-X in different causes of cholestatis varied, and while it was commonest in cases of extrahepatic obstruction of recent onset, occurring in 75% of cases, it was also found in primary biliary cirrhosis in 48% [84].

Further investigations performed by a number of research groups, including case- and large-scale studies of patients with different types of diseases of liver and bile tract, demonstrated presence of LP-X in the blood of patients with intrahepatic cholestasis (primary biliary cirrhosis, cholangitis, hepatic cirrhosis, hypercholesterolemia associated with pseudohyponatremia, graft-versus-host disease of the liver after allogeneic bone marrow transplantation, hepatic malignancy, etc.) and extrahepatic cholestasis (extrahepatic biliary obstruction by tumors and cholecystolithiasis, cholestasis of pregnancy, etc.) [9, 35-40, 48, 58, 62, 70, 72, 80, 81, 87, 88, 99, 100, 109, 114, 117, 120, 121, 126].

In patients suffering with different liver diseases the LP-X test was positive in 82-100% in whom histological evidence of cholestasis was observed, and negative in 95-98% in whom histological examination was negative [26, 27, 61, 69]. Notably that concentrations of LP-X in the patients with extrahepatic cholestasis were significantly higher than in those with intrahepatic cholestasis [9, 14, 39, 40, 82, 90, 94, 117].

LP-X was also detected in the blood of patients with acute viral and toxic hepatitis. Positive results were found in 96% of patients with hepatitis A and in 82% of hepatitis B. It was shown that in acute phase of viral hepatitis, LP-X is the most specific test in determining the presence of cholestasis [54, 82, 106, 122]. Increased blood levels of LP-X were reported in patients with drug-induced cholestatic hepatitis [104, 63], chronic cholestatic hepatitis [72] as well as viral hepatitis NANB [97].

High blood levels of LP-X were detected in children with liver diseases (progressive familial intrahepatic cholestasis, chronic graft-versus-host disease of the liver) and in infants with persistent cholestatic jaundice caused by biliary atresia and biliary agenesis of extra- and intrahepatic origin (prolonged jaundice, choledochal cysts, hypoplastic extrahepatic biliary tract, absence of extrahepatic biliary tracts, mechanical occlusion of bile ducts caused by a rhabdomyoblastoma, inborn enzymatic liver dysfunction, neonatal hepatitis, neonatal cholestasis, Alagille syndrome, etc.) [7, 15, 18, 20, 22, 30, 45, 46, 51, 64, 65, 73-76, 107, 111, 112, 118, 119, 124, 130].

Concentration of LP-X in the blood has been considered as an important and informative marker of the above mentioned liver and biliary tract diseases, and determination of LP-X blood level has been widely used for clinical diagnostics of cholestasis and for monitoring the efficiency of relevant therapeutic measures [3, 6, 9, 19, 20, 22, 26, 27, 42, 43, 51, 54, 58, 60, 61, 66, 69, 70, 74, 78, 89, 94, 103, 106, 122, 123, 125, 127]. The plasma concentration of LP-X was significantly corre-
lated to the plasma activity of alkaline phosphatase and serum bilirubin, but seemed to be superior to these two parameters in the differentiation between intrahepatic and extrahepatic cholestasis. Plasma levels of LP-X above 400 mg/100 ml are highly indicative of extrahepatic biliary obstruction [80].

In experimental models of cholestasis, LP-X may be detected in the plasma within the first 20 hours. The calculated fractional catabolic rate of LP-X was found to be 0.450 ± 0.069 for dogs and 1.553 ±0.096 for rats corresponding to a mean biological half life of 37.7 ± 6.4 h or 10.7 ±0.6 h, respectively [93].

The cause of the appearance of lipoprotein X is unknown, but the analysis of associated biochemical features suggested its relationship to physical biliary obstruction rather than a derangement of liver cell function [84].

Antiatherogenic properties of LP-X

The fact that hypercholesterolemia increases atherosclerosis incidence in the general population but not in patients with primary biliary cirrhosis, a cholestatic liver disease associated with marked increases in plasma LDL cholesterol [37, 41], raises the question on antiatherogenic properties of LP-X. Despite the highly increased cholesterol levels, prospective observation for a median of 7.4 years of 312 patients with primary biliary cirrhosis of various stages found no increased incidence of atherosclerotic death compared with age and sex-matched controls [17]. Based upon these results it was proposed that bioactivities of LP-X, may be responsible for this phenomenon by preventing origination of oxidized LDL products and thus reducing LDL atherogenicity [101]. This suggestion has been recently confirmed by in vitro study performed by Chang and co-workers [16]. This study revealed that after prolonged incubation with copper, LPX containing LDL isolated from the blood of patients with primary biliary cirrhosis failed to increase the oxidation index or electrophoretic mobility noted in control LDL. An admixture of LP-X containing LDL or LP-X with control LDL prevented oxidation of the latter in a dose-dependent manner. Furthermore, LP-X containing LDL isolated from the blood of patients with primary biliary cirrhosis was also noncompetitive against copper-oxidized LDL (oxLDL) for binding with a murine monoclonal anti-oxLDL antibody in a competitive ELISA. OxLDL exerts its proapoptotic and antiangiogenic effects in part by inhibiting fibroblast growth factor 2 (FGF2) expression. Preincubation of oxLDL with LP-X, containing LDL, but not control LDL, attenuated the inhibitory effects of oxLDL on FGF2 expression in cultured bovine aortic endothelial cells. Notably, the antioxidant and prosurvival properties of LP-X containing LDL isolated from the blood of patients with primary biliary cirrhosis diminished after the patients underwent orthotopic liver transplantation [16]. These results suggest that LP-X reduces LDL atherogenicity by preventing LDL oxidation to protect endothelial cells integrity in the presence of hypercholesterolemia [16, 99]. They also suggest that altering LDL composition may be as important as reducing LDL concentration in preventing or treating atherosclerosis.
**LP-X in familial lecithin-cholesterol acyltransferase deficiency**

Lecithin-cholesterol acyltransferase (LCAT) (also called phosphatidylcholine-sterol O-acyltransferase, EC 2.3.1.43) is an enzyme which converts free cholesterol into cholesteryl ester (a more hydrophobic form of cholesterol) which is then sequestered into the core of a lipoprotein particle, eventually making the newly synthesized lipoproteins spherical and forcing the reaction to become unidirectional, since the particles are removed from the surface. The enzyme is bound to both HDL and LDL in the blood plasma [128]. Familial LCAT deficiency is a monogenic autosomal recessive trait affecting cholesterol esterification, developed due to several allelic mutations of polymorphic gene on chromosome 16 (16q22.1) encoded LCAT [5, 12, 13, 28, 29, 44, 59, 110]. A deficiency of LCAT causes accumulation of unesterified cholesterol in certain body tissues. The disease is characterized by diffuse corneal opacities, target cell hemolytic anemia (normochromic normocytic anemia), and renal dysfunction (proteinuria with renal failure) [13, 44]. Familial LCAT deficiency is accompanied by low levels of HDL and LDL and the accumulation of LP-X in the plasma of patients [31, 44, 83, 113]. Plasma concentration of LP-X in familial LCAT deficiency ranges from 43 mg/100 ml to 251 mg/100 ml with a mean of 127 mg/100 ml. This is above the mean level of LP-X found in a group of patients with intrahepatic cholestasis (49mg/100 ml) and below the mean level found in patients with extrahepatic cholestasis (341 mg/100 ml) [82].

Pathogenic effects of LP-X in familial LCAT deficiency have been addressed in several human and animal studies, in vivo and in vitro. It has been demonstrated that high levels of LP-X cause glomerular capillary endothelial damage [98] and lead to progressive renal impairment and end-stage renal failure [31, 129]. The results presented by Lynn et al. suggest that LP-X participates in the pathogenesis of glomerulosclerosis and subsequent renal failure in familial LCAT deficiency by stimulating monocyte infiltration via a mechanism involving the expression MCP-1 by mesangial cells [53]. MCP-1 is an important chemoattractant for monocytes [115], and one key event in the pathogenesis of glomerulosclerosis is the infiltration of monocytes into affected glomeruli [24].

It was shown that the familial LCAT deficiency is characterized by the decreased catabolism of LP-X [67], however, the mechanisms for LP-X accumulation in this disease are unclear yet.

**LP-X in other diseased conditions**

Our own recent studies, for the first time have demonstrated the presence of the LP-X in the blood of patients with ischemic stroke and familial Mediterranean fever. The studies included 120 patients with ischemic stroke (each patient was examined at different time points after stroke onset) and 50 patients with familial Mediterranean fever (each was examined in remission stage and in acute stage of the disease). We found that in the blood of patients with ischemic stroke LP-X is present in both free form and cryoglobulin-bound form on days 1-7 after stroke onset [55, 56], and in the blood of patients with familial Mediterranean fever.
a free form of LP-X was detected (unpublished data). Figures 2, 3 demonstrate the results of our investigations.

Fig. 2. Typical electrophoretic patterns of LDL detection in the blood serum of patients with familial Mediterranean fever (A) and ischemic stroke (B)

Fig. 3. Typical electrophoretic patterns of LDL detection in cryoglobulins isolated from the blood of healthy subjects (a) and patients with ischemic stroke (b)

The above mentioned study of Lynn and colleagues [54] demonstrated that LP-X is stimulating monocytes infiltration via a mechanism involving MCP-1 ex-
pression. Both animal and human studies suggest that MCP-1-driven migration of inflammatory cells are implicated in the pathogenesis of ischemic stroke [21]. Furthermore, we found increased serum levels of MCP-1 in ischemic stroke patients on days 1-7 after stroke onset [4, 8]. Therefore, it is reasonable to propose a possible involvement of LP-X in the inflammatory response occurring after stroke onset [21]. The same applies to familial Mediterranean fever, which is a genetic autosomal recessive autoinflammatory disease developed due to several allelic mutations of polymorphic gene coding a protein, pyrin, on chromosome 16 (16p13.3). Pyrin is a part of regulatory pathway of inflammation and normally assists in keeping inflammation under control by deactivating the immune response. Mutations in pyrin encoding gene lead to malfunctioning protein and uncontrolled inflammation [102].

Thus, in case of ischemic stroke and familial Mediterranean fever, as well as in LCAT deficiency, LP-X probably acts as a trigger of inflammation through induction of MCP-1 expression. Further studies will clear if there are relationships between LP-X and other inflammatory mediators.

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