Abnormalities in lipoprotein metabolism in hemodialysis patients

MICHAELA KÖNIGER, THOMAS QUASCHNING, CHRISTOPH WANNE, PETER SCHOLLMEYER, and ANNETTE KRAMER-GUTH

Department of Medicine, Division of Nephrology, University of Freiburg, Freiburg, and Department of Medicine, Division of Nephrology, University of Würzburg, Würzburg, Germany

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Background. Patients on chronic hemodialysis treatment are at elevated atherogenic risk, and dyslipidemia appears to be one of the major risk factors. However, most of these patients exhibit elevated serum triglycerides, whereas serum cholesterol and low-density lipoprotein (LDL) cholesterol levels are in the normal range. This study was therefore designed to examine the influence of hypertriglyceridemia under the condition of hemodialysis and diabetes mellitus on LDL metabolism.

Methods. LDL was isolated from healthy controls, hypertriglyceridemic diabetic patients, and non-diabetic hemodialysis patients (N = 30, 10 in each group), which were separated into six subfractions by density gradient ultracentrifugation and were characterized concerning lipid/protein composition, degree of glycation, and oxidation. Uptake of 125I-labeled LDL was examined via LDL receptors of HepG2 cells and scavenger receptors of mouse peritoneal macrophages.

Results. In hemodialysis patients, serum triglycerides were significantly elevated, whereas cholesterol levels were within the normal range. Triglyceride enrichment occurred in the very low-density lipoprotein (VLDL) and LDL classes, and an accumulation of a highly atherogenic small dense LDL subfraction could be detected predominantly in patients with non-insulin-dependent diabetes mellitus. LDL of hemodialysis patients also contained elevated levels of lipid peroxidation products, which were even higher in diabetic patients. Alterations in composition, size, and configuration of LDL from diabetic and nondiabetic patients on hemodialysis impaired LDL receptor-mediated degradation and enhanced the uptake of these modified LDL particles via nonsaturable scavenger receptors.

Conclusion. Diminished LDL receptor-mediated uptake of modified, triglyceride-rich, small dense LDL most likely leads to accumulation of these lipoproteins in vivo, favoring the development of atherosclerotic lesions. Future clinical studies must demonstrate whether patients will benefit from reducing these atherogenic particles by lipid-lowering intervention.

Despite the tendency toward better survival in hemodialysis (HD) patients, cardiovascular disease has remained the most commonly reported cause of death. Multiple factors contribute to the atherosclerotic process, but dyslipidemia, which is frequently found in patients on HD, is one of the key factors in the initiation and acceleration of atherosclerosis. Lipoprotein metabolism is influenced by many determinants as the dialysis treatment itself, the use of heparin as anticoagulant, the membrane material, and the underlying uremia [1]. An atherogenic and typical lipoprotein profile can be detected consisting of hypertriglyceridemia with elevation of triglycerides in the very low-density lipoprotein (VLDL) class and LDL density class, low high-density lipoprotein (HDL) cholesterol and normal or only slightly elevated serum cholesterol levels [2]. In diabetic patients [non-insulin-dependent diabetes mellitus (NIDDM)] on HD treatment, diabetes mellitus additionally impedes lipoprotein metabolism by aggravation of hypertriglyceridemia and further modification of lipoproteins. This form of dyslipidemia, with LDL cholesterol levels in the normal range, may contribute to an elevated atherogenic risk, especially in the presence of diabetes mellitus. In this study, we therefore examined LDL metabolism under the condition of HD treatment and diabetes mellitus and their potential impact on the pathogenesis of atherosclerosis.

METHODS

Low-density lipoprotein was isolated from plasma of healthy controls (CO), hypertriglyceridemic diabetic (NIDDM-HD) HD patients, and hypertriglyceridemic nondiabetic HD patients (HD) (N = 30, 10 in each group) by sequential ultracentrifugation. LDL was characterized concerning lipid/protein composition, distribution of LDL subfractions by density gradient centrifugation [3], and glycation of apo B with the “Glycacor” enzyme immunoassay from Exocell (Philadelphia, PA, USA). Lipid peroxides in LDL and thiobarbituric acid reactive substances (TBARS) were determined [4]. Lag
Table 1. Lipid distribution among various lipoprotein density classes in hemodialysis patients

<table>
<thead>
<tr>
<th></th>
<th>Total Chol</th>
<th>LDL Chol</th>
<th>VLDL Chol</th>
<th>Total TG</th>
<th>LDL TG</th>
<th>VLDL TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
<td>4.20 ± 0.75</td>
<td>2.56 ± 0.54</td>
<td>0.23 ± 0.13</td>
<td>0.87 ± 0.38</td>
<td>0.14 ± 0.02</td>
<td>0.58 ± 0.38</td>
</tr>
<tr>
<td>HD</td>
<td>5.34 ± 0.98(^a)</td>
<td>3.21 ± 0.83</td>
<td>1.09 ± 0.47(^a)</td>
<td>3.39 ± 0.78(^a)</td>
<td>0.57 ± 0.16(^a)</td>
<td>2.48 ± 0.67(^a)</td>
</tr>
<tr>
<td>NIDDM-HD</td>
<td>6.37 ± 0.16(^a)</td>
<td>3.19 ± 0.73</td>
<td>2.20 ± 1.71(^a)</td>
<td>5.42 ± 3.24(^a)</td>
<td>0.68 ± 0.23(^a)</td>
<td>4.01 ± 3.57(^a)</td>
</tr>
</tbody>
</table>

Abbreviations are: CO, controls (N = 10); HD, hemodialysis patients (N = 10); NIDDM-HD, hemodialysis patients with type 2 diabetes mellitus (N = 10); Chol, cholesterol; TG, triglycerides. Data are given as mean ± sem.

\(^a\)P < 0.05 versus controls

RESULTS

In our HD patients, elevated serum triglycerides and triglyceride enrichment of VLDL and LDL were consistent findings. This was most pronounced in patients with diabetes mellitus. In contrast, total cholesterol levels were only slightly elevated (Table 1). Subfractionation of LDL revealed that hypertriglyceridemia was associated with an atherogenic LDL subfraction pattern. The increase in LDL triglycerides was due to an accumulation of a triglyceride-rich large, buoyant fraction (LDL-1, density range, 1019 to 1031 kg/liter) and a small dense fraction (LDL-6, density range, 1044 to 1060 kg/liter), both subfractions known for their low affinity to the LDL receptor and high atherogenicity. The largest enrichment in LDL-6 was detected in the hypertriglyceridemic diabetic HD patients (HD, 73.6 ± 4.6 mg/dl; NIDDM-HD, 84.4 ± 5.2 mg/dl vs. CO, 29.0 ± 4.5 mg/dl, P < 0.01).

To identify modifications of LDL that may additionally reduce uptake via LDL receptor, especially in diabetic patients, we measured the degree of glycation of apo B of LDL and found elevated concentrations in the diabetic patients (HD, 2.6 ± 0.4%; NIDDM-HD, 4.7 ± 1.6% vs. CO, 2.5 ± 0.4%, P < 0.05). Significant changes in lag time or diene formation could not be detected, but lipid peroxides in LDL were significantly elevated, especially in diabetic patients with higher concentrations of glycated apo B (HD, 0.058 ± 0.028; NIDDM-HD, 0.158 ± 0.126 vs. CO, 0.027 ± 0.016 μmol/100 μg LDL protein, P < 0.05). Binding experiments with \(^{125}\)I-labeled LDL in HepG2 cells (Fig. 1) showed that these modified LDL were taken up by the LDL receptor of HepG2 cells with lower affinity than control LDL but were enhanced taken up by scavenger receptors of macrophages. This was most pronounced for LDL of hypertriglyceridemic diabetic patients.

DISCUSSION

Hypertriglyceridemia, especially in combination with diabetes mellitus, contributes to the elevated cardiovascular risk of HD patients. In this study, LDL isolated from hypertriglyceridemic nondiabetic and diabetic HD patients, exhibited impaired receptor specific uptake via the LDL receptor of HepG2 cells. LDL degradation was even more reduced if hypertriglyceridemia was aggravated by coexisting diabetes mellitus.

In HD treatment and diabetes mellitus, a number of conditions are present, which may account for diminished cellular LDL metabolism: Independent of the etiology, hypertriglyceridemia leads to prominent compositional and configurational changes of LDL particles [7]. Triglyceride-rich LDLs are smaller and denser particles than control LDLs, and the alteration in the lipid-protein
S-250

ratio in dense LDL leads to configurational changes of apo B [3], which impair binding to the LDL receptor [8]. In this study, triglyceride enrichment in the LDL density class and an accumulation of a small dense LDL subfraction could be detected particularly in hypertriglyceridemic diabetic patients, thus contributing to the increased atherogenicity and the elevated cardiovascular risk in these patients [9].

In the presence of elevated blood glucose levels, apo B of LDL undergoes glycation and modification by advanced glycation end products (AGEs), which also reduce affinity to the LDL receptor [10]. Our results indicate that apo B from diabetic HD patients was glycated to a larger extent compared with nondiabetic patients.

In HD treatment, many factors promote oxidation of LDL like blood contact with dialysis membrane and acute phase reaction [11]. In addition, small dense LDL and glycated LDL are known to be prone to oxidation [10, 12]. In this study, LDLs from HD patients were more susceptible to oxidation than control LDLs. Especially in diabetic HD patients, elevated concentrations of lipid peroxides could be detected. Glycation and oxidation further reduce receptor-mediated LDL uptake by modification of the receptor-binding protein moiety (apo B) and contribute therefore to intravascular LDL accumulation. Prolonged circulation time further promotes modification of LDL.

These data suggest that modified LDL particles from HD patients were enhanced taken up by the nonsaturable scavenger receptors of macrophages and may therefore favor the development of atherosclerotic lesions in vivo. Whether patients will benefit from reducing these atherogenic LDL particles by lipid-lowering intervention has to be demonstrated by future clinical studies.

Reprint requests to Michaela Königer, M.D., Department of Medicine, Division of Nephrology, University of Freiburg, Hugstetterstr. 55, D-79106 Freiburg, Germany. E-mail: koeniger@mm41.ukl.uni-freiburg.de

REFERENCES