Down-regulation of hepatic LDL receptor-related protein (LRP) in chronic renal failure

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Background. Chronic renal failure (CRF) is associated with premature atherosclerosis, impaired high-density lipoprotein (HDL)-mediated reverse cholesterol transport, depressed clearance, and elevated plasma concentrations of very low-density lipoprotein (VLDL), chylomicrons, and their atherogenic remnants. LDL receptor-related protein (LRP) is a member of the LDL receptor gene family that is heavily expressed in the liver, and mediates removal of at least 30 different ligands, including VLDL remnants (IDL) and chylomicron remnants. This study was conducted to test the hypothesis that the well-known defect in clearance of IDL and chylomicron remnants in CRF may be indicative of diminished hepatic LRP abundance.

Methods. Hepatic tissue LRP mRNA abundance [reverse transcription-polymerase chain reaction (RT-PCR)] and protein abundance (Western blot analysis) were determined in rats 8 weeks after 5/6 nephrectomy (CRF group) or sham operation (control group).

Results. The CRF group exhibited hypertension, diminished creatinine clearance, increased plasma triglyceride concentration, and elevated total cholesterol-to-HDL cholesterol concentration ratio compared to the corresponding values found in the control group. This was associated with a significant down-regulation of hepatic LRP mRNA expression and undetectable LRP protein.

Conclusion. CRF results in down-regulation of hepatic LRP. This abnormality can, at least in part, account for the previously documented elevation of plasma concentration and depressed clearance of chylomicron remnants and IDL in CRF.

Chronic renal failure (CRF) is associated with increased risk of atherosclerotic cardiovascular disease [1, 2] and profound alterations of plasma lipid profile [3–5]. CRF-associated dyslipidemia is marked by impaired high-density lipoprotein (HDL) maturation, hypertriglyceridemia, elevated plasma concentration, and defective clearance of very low-density lipoprotein (VLDL), chylomicrons (CM), and their highly atherogenic remnants (IDL and CM remnants) [3–8]. Intermediate-density lipoprotein (IDL) and CM remnants are the by-products of lipolysis of VLDL and CM, respectively, by lipoprotein lipase in the circulation. CM remnants are removed from the circulation by the liver via hepatic LDL receptor-related protein (LRP) [9, 10]. IDL particles are either converted to LDL via further lipolysis by hepatic triglyceride lipase or are removed from the circulation by the liver via receptor-mediated endocytosis. LRP is a member of the LDL receptor gene family that is heavily expressed in the liver [11]. This high-molecular-weight (600 kD) receptor has substantial structural similarity to LDL receptor, and recognizes a wide array of diverse ligands, including ApoB 48- and ApoE-enriched lipoproteins (CM remnants, IDL, HDL), hepatic triglyceride lipase, coagulation factors, fibrinolytic enzymes, protease inhibitors, and protease-protease inhibitor complexes [12–15].

In view of the critical role of hepatic LRP in clearance of CM remnants, IDL, and proteinase-inhibitor complexes, we hypothesized that the reported increased in plasma concentration of CM remnants, IDL [6–8], and inactivated coagulation enzymes [16] in CRF may be indicative of hepatic LRP deficiency. Accordingly, hepatic tissue LRP gene expression and protein abundance were determined in rats 8 weeks after 5/6 nephrectomy or sham operation. The results showed significant down-regulation of hepatic LRP mRNA and protein expressions in the CRF animals, thus confirming the original hypothesis.

METHODS

Animal models

Male Sprague-Dawley rats weighing 225 to 250 g were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN, USA). They were housed in a climate-controlled, light-regulated facility with 12:12-hour day-night cycles. The animals were fed regular rat chow (Purina Mills,
Liver was removed immediately, snap-frozen in liquid urine collection. They were then killed by exsanguination the animals were placed in metabolic cages for a 24-hour observed for 8 weeks.

and aseptic techniques were observed. The animals were thesia (Na pentobarbital 50 mg/kg IP). Strict hemostasis surgical procedures were carried out under general anes-
sion, as described previously [17]. The animals assigned to the control group were subjected to sham operation. All 5/6 nephrectomy by surgical resection using a dorsal inci-

Brentwood, MO, USA) and water ad libitum, and were randomly assigned to the CRF and control groups. The animals assigned to the CRF group were subjected to sham operation. All surgical procedures were carried out under general anesthesia (Na pentobarbital 50 mg/kg IP). Strict hemostasis and aseptic techniques were observed. The animals were observed for 8 weeks.

At the conclusion of the 8-week observation period, the animals were placed in metabolic cages for a 24-hour urine collection. They were then killed by exsanguination via cardiac puncture between the hours of 9 and 11 a.m. Liver was removed immediately, snap-frozen in liquid nitrogen, and stored at −70℃ until processed. Serum triglyceride, VLDL, HDL, and creatinine concentrations and urinary creatinine were determined using standard laboratory methods. The protocol employed in this study was approved by the Institutional Committee for Care and Use of Animals at the University of California, Irvine.

RNA isolation and RT-PCR

RNA was isolated from frozen liver using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and purified by RNAeasy kit (QIAGEN, Valencia, CA, USA). One tenth micromgram total RNA from each sample was reverse transcribed to cDNAs by using MuLV reverse transcriptase (Applied Biosystems, Foster City, CA, USA) with a mixture of 1 mmol/L dNTP and 2.5 μmol/L random primers in a 10 μL volume for 10 minutes at 25℃ and 30 minutes at 48℃. The reaction was stopped by heating at 94℃ for 5 minutes.

Expression of LRP mRNA was assessed by reverse transcription-polymerase chain reaction (RT-PCR) using 18s ribosomal RNA as internal control. The primer sequences used are depicted in Table 1. The primers were designed with Primer3 program purchased from Invitrogen. For 18s rRNA amplification, we used alternate 18s primers (Ambion, Austin, TX, USA), which yields a 324-bp product. In each PCR reaction, 18s ribosomal RNA was coamplified with the target cDNA. The primers were tested for their compatibility with the alternate 18s primer. The cDNAs were amplified using standard PCR buffer, 0.2 mmol/L dNTP, 1 μmol/L LRP primer set, 0.5 μmol/L 18s primer/competimer mix, 2 mmol/L MgCl₂, and 1.25 U of Taq DNA polymerase (Applied Biosys-
tems) in 50 μL of total volume for 30 cycles. Each cycle consisted of 1-minute denaturation at 94℃ and 45 seconds annealing at 60℃, and 45 seconds extension at 72℃. PCR products were separated on a 1.2% agarose gel with ethidium bromide by electrophoresis. Signal intensity was determined by laser scanning densitometry. On each oc-
casion, the LRP mRNA abundance was normalized to the corresponding 18s ribosomal RNA.

Measurements of LRP protein

LRP receptor protein abundance in the liver tissue was measured by Western blot analysis using a monoclonal antihuman LRP antibody prepared in our laboratory using the IgG-11H4 hybridoma cell line purchased from ATCC (Manassas, VA, USA).

Data analysis

Student t test was used in statistical analysis of the data, which are given as mean ± SEM. P values less than 0.05 were considered significant.

RESULTS

General data

Creatinine clearance in the CRF group (0.56 ± 0.9 mL/min) was significantly lower than that found in the control group (2.6 ± 0.2 mL/min, P < 0.001). In contrast, systolic arterial pressure was significantly higher in the CRF group (165 ± 4 mm Hg) than in the sham-operated control rats (123 ± 2 mm Hg, P < 0.01). Body weight obtained at the conclusion of the study was significantly lower in the CRF group (366 ± 6 g) than in the control group (389 ± 16 g). Compared with the control group, the CRF animals showed a marked increase in plasma triglyc-cerides, VLDL cholesterol, and total cholesterol-to-HDL cholesterol ratio (Table 2).

Liver LRP data

Data are shown in Figures 1 and 2. Compared with the sham-operated control rats, the CRF group exhibited a

<table>
<thead>
<tr>
<th>Table 1. Primers used for measurements of LRP mRNA and 18s rRNA by RT-PCR</th>
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<tbody>
<tr>
<td>Gene</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>LRP Forward 5’-GGCTGTCACTGGCATCAGC-3′</td>
</tr>
<tr>
<td>Reverse 5’-TCGCTTTGCTGATGAC-3′</td>
</tr>
<tr>
<td>18s Forward 5’-AGGAATTCGAGGAGGAC-3′</td>
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<tr>
<td>rRNA Reverse 5’-GTGCAGCCGGGACATCTAAG-3′</td>
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<p>| Table 2. Creatinine clearance (Ccr), plasma concentrations of creatinine, triglycerides, total cholesterol, and VLDL cholesterol, and plasma total cholesterol-to-HDL cholesterol ratio in the sham-operated control rats (CTL) and rats with chronic renal failure (CRF) |
|---|---|---|---|</p>
<table>
<thead>
<tr>
<th>Groupa</th>
<th>CTL</th>
<th>CRF</th>
<th>P value</th>
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<tbody>
<tr>
<td>Creatinine m/L/mL</td>
<td>2.6 ± 0.2</td>
<td>0.56 ± 0.09</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Creatinine m/dL</td>
<td>0.4 ± 0.01</td>
<td>1.3 ± 0.2</td>
<td>&lt;0.01</td>
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<tr>
<td>Triglycerides mg/dL</td>
<td>47 ± 4.5</td>
<td>166 ± 17</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>59 ± 2.2</td>
<td>124 ± 11</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>VLDL cholesterol mg/dL</td>
<td>9.3 ± 0.9</td>
<td>33 ± 3.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total/HDL cholesterol, ratio</td>
<td>2.7 ± 0.3</td>
<td>3.7 ± 0.5</td>
<td>&lt;0.05</td>
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</table>

a N = 6 in each group.
significant reduction of LRP mRNA abundance normalized against 18s ribosomal RNA. Similarly, immunode-
tectable LRP protein abundance was significantly lower in the liver of the CRF rats than the corresponding value found in the sham-operated control group.

DISCUSSION

The main features of the CRF-induced dyslipidemia include hypertriglyceridemia, elevated plasma concentration, and impaired clearance of triglyceride-rich lipoproteins (VLDL and CM) and their remnants, coupled with a defective HDL maturation [3–8]. Hyper-
triglyceridemia in this model is not caused by increased hepatic triglyceride synthesis because acyl-CoA: diglycer-
erol acyltransferase (DGAT), which is the final step in triglyceride biosynthesis, is down-regulated in the CRF liver [18]. Instead, it is primarily caused by down-regulations of skeletal muscle and adipose tissue lipopro-
lipase lipase [17, 19–21], which is essential for the lipolysis of VLDL and CM, and of VLDL receptor [22, 23], which is involved in the peripheral uptake of VLDL. This is ac-
accompanied by hepatic triglyceride lipase deficiency [21, 24], which plays a critical role in the removal of the remaining triglycerides in the IDL particles and their eventual conversion to LDL. The role of lipoprotein lipase, hepatic triglyceride lipase, and VLDL receptor in metabolism of endogenous and exogenous plasma lipids is depicted in Figure 3. The effects of lipoprotein lipase, VLDL receptor, and hepatic triglyceride lipase deficiencies on metabolism of triglyceride-rich lipoproteins are compounded by impaired maturation of HDL-3 to HDL-2 in CRF [5]. The latter is due to down-regulation of lecithin: cholesterol acyltransferase (LCAT), the key en-
zyme involved in HDL-mediated cholesterol uptake in CRF [25]. HDL-2 serves as the ApoE (lipoprotein lipase and VLDL receptor ligand) and ApoC-II (lipoprotein lipase cofactor) donors to the nascent VLDL and chylom-
irons. Consequently, diminished HDL-2 level in CRF contributes to the defective lipoprotein lipase-mediated lipolysis of VLDL and CM and VLDL-receptor mediated uptake of VLDL by adipocyte and myocytes. The animal studies outlined above provided the molecular basis of clinical studies that have demonstrated depressed plasma LCAT activity, diminished plasma post heparin lipolytic activity, and elevated plasma prebeta HDL (lipid-poor HDL) in CRF patients [26–29].
The CRF animals employed in the present study exhibited a significant reduction of immunodetectable LRP in the liver tissue. This was accompanied by a parallel reduction of LRP mRNA abundance. Thus, CRF results in down-regulation of LRP at the level of gene transcription. The observed hepatic LRP deficiency, shown for the first time here, provides the molecular mechanism of the elevated plasma concentration of the atherogenic CM remnants in CRF [6–8].

In addition, hepatic LRP deficiency can, in part, contribute to elevation of plasma IDL in CRF. As noted earlier, CRF results in down-regulation of hepatic triglyceride lipase [21, 24], which, in turn, impedes conversion of IDL to LDL via the lipolytic pathway. This leads to a rise in the available supplies of IDL for clearance via the receptor-mediated endocytosis, commonly known as the shunt pathway. LRP plays an important part in hepatic clearance of IDL through the shunt pathway [11, 12]. Consequently, the combined down-regulations of the hepatic triglyceride lipase and LRP work in concert to impair IDL clearance and raise plasma concentration of this oxidation-prone, atherogenic lipoprotein remnant by limiting the lipolytic and shunt pathways of its metabolism. By promoting accumulation of highly atherogenic CM remnants and IDL in the plasma, hepatic LRP deficiency can contribute to the atherogenic diathesis in CRF. In addition, hepatic LRP deficiency has been shown to promote atherosclerosis in LRP knockout mice, independent of its action on lipoprotein metabolism [30]. This phenomenon has been attributed to the accumulation of other proatherogenic ligands of LRP. Thus, acquired hepatic LRP deficiency can be added to many previously identified risk factors for cardiovascular disease in CRF.

CONCLUSION

CRF results in acquired hepatic LRP deficiency, which can contribute to the atherogenic diathesis and elevated plasma lipoprotein remnants in chronic renal failure.

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REFERENCES


