HMG-CoA reductase inhibition reverses LCAT and LDL receptor deficiencies and improves HDL in rats with chronic renal failure

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Liang, K., C. H. Kim, and N. D. Vaziri. HMG-CoA reductase inhibition reverses LCAT and LDL receptor deficiencies and improves HDL in rats with chronic renal failure. Am J Physiol Renal Physiol 288: F539–F544, 2005. First published October 26, 2004; doi:10.1152/ajprenal.00074.2004.—Dyslipidemia is a prominent feature of chronic renal failure (CRF) and a major risk factor for atherosclerosis and the progression of renal disease. CRF-induced dyslipidemia is marked by hypertriglyceridemia and a shift in plasma cholesterol from HDL to the ApoB-containing lipoproteins. Several studies have demonstrated a favorable response to administration of 3-hydroxy-3-methylglutaryl (HMG-CoA) reductase inhibitors (statins) in CRF. This study was intended to explore the effect of statin therapy on key enzymes and receptors involved in cholesterol metabolism. Accordingly, CRF (% nephrectomized) and sham-operated rats were randomized to untreated and statin-treated (rosuvastatin 20 mg·kg⁻¹·day⁻¹) groups and observed for 6 wk. The untreated CRF rats exhibited increased total cholesterol-to-HDL cholesterol ratio, diminished plasma lecithin:cholesterol acyltransferase (LCAT) and hepatic LDL receptor, elevated hepatic acyl-CoA:cholesterol acyltransferase (ACAT), and no change in hepatic HMG-CoA reductase, cholesterol 7α-hydroxylase, or HDL receptor (SRB-1). Statin administration lowered HMG-CoA reductase activity, normalized plasma LCAT, total cholesterol-to-HDL cholesterol ratio, and hepatic LDL receptor but did not significantly change either plasma total cholesterol, hepatic cholesterol 7α-hydroxylase, total ACAT activity, or SRB-1 in the CRF animals. Statin administration to the normal control rats led to significant increases in plasma LCAT and hepatic LDL receptor, significant reductions of total cholesterol-to-HDL cholesterol ratio, hepatic HMG-CoA reductase activity, and cholesterol 7α-hydroxylase abundance with virtually no change in plasma cholesterol concentration. Thus administration of rosuvastatin reversed LCAT and LDL receptor deficiencies and promoted a shift in plasma cholesterol from ApoB-containing lipoproteins to HDL in CRF rats.

3-hydroxy-3-methylglutaryl; atherosclerosis; lipid disorders; renal disease; SRB-1; bile acid; cholesterol; triglyceride

CHRONIC RENAL FAILURE (CRF) results in profound abnormalities of lipid metabolism and marked alteration of plasma lipid profile. The associated dyslipidemia contributes to the athero- genic diathesis and progression of renal insufficiency in patients with chronic kidney disease (3, 7, 12, 28). The typical features of CRF-induced dyslipidemia include hypertriglyceri demia; elevated level and impaired clearance of VLDL, IDL, and LDL; inappropriately reduced HDL cholesterol; and impaired maturation of cholesterol-poor HDL-3 to cardioprotective cholesterol ester-rich HDL-2 (3, 7, 12, 28). The primary underlying mechanism of hypertriglyceridemia and elevation of plasma triglyceride-rich lipoproteins (VLDL, IDL, and chy- lomicrons) is downregulation of lipoprotein lipase, VLDL receptor (in adipose tissue and skeletal muscle), and hepatic triglyceride lipase (17, 20, 30, 31, 37). The main cause of depressed plasma HDL cholesterol and impaired HDL maturation is downregulation of lecithin:cholesterol acyltransferase (LCAT), which is essential for HDL-mediated removal of surplus cholesterol from the peripheral tissues (11, 24, 34). Impaired HDL-mediated reverse cholesterol transport in CRF plays an important role in the pathogenesis of atherosclerotic cardiovascular complications and progression of renal disease (12, 13, 18). In addition, the reduction in plasma concentration of HDL-2, which is the main vehicle for shuttling ApoC-II (lipoprotein lipase cofactor) and ApoE (lipoprotein lipase and VLDL receptor ligand) between VLDL remnants and nascent VLDL, contributes to defective clearance of triglyceride-rich lipoproteins (10).

Unlike CRF rats, which consistently exhibit moderate hypercholesterolemia, plasma total cholesterol concentration is usually normal or reduced and only occasionally elevated in CRF humans (3, 7). Moreover, due to the lack of cholesterol ester transfer protein (CETP) in rats (27) and its presence in humans, a greater relative portion of plasma cholesterol is transported by HDL in the rat compared with humans. Nonetheless, several studies have demonstrated significant improvements in plasma lipid profile and progression of renal disease with 3-hydroxy-3-methylglutaryl (HMG-CoA) reductase inhibitors (statins) in humans and animals with chronic renal insufficiency (4, 15, 25). The present study was intended to explore the effect of HMG-CoA reductase inhibition on key cholesterol-regulating enzymes and receptors in CRF rats.

MATERIALS AND METHODS

Animal models. Male Sprague-Dawley rats weighing 225–250 g were purchased from Harlan Sprague Dawley (Indianapolis, IN). They were housed in a climate-controlled, light-regulated facility with a 12:12-h light-dark cycle. The animals were fed regular rat chow (Purina Mills, Brentwood, MO) containing 49% carbohydrates, 24% protein, 5% fat, 7% minerals, 6% fiber, and various vitamins and water ad libitum and randomly assigned to the CRF and control groups. The animals assigned to the CRF group were subjected to % nephrectomy by surgical resection using a dorsal incision, as described previously (35). The animals assigned to the control group were subjected to sham operation. All surgical procedures were performed under general anesthesia (Nembutal, 50 mg·kg⁻¹·ip). Strict hemostasis and aseptic techniques were observed. After a 2-wk observation period, the CRF and sham-operated control rats were randomized into statin-treated and untreated groups. The statin-treated groups received rosuvastatin (20 mg·kg⁻¹·day⁻¹, AstraZeneca,....
Cheshire, UK) mixed in powdered rat chow. The treatment was continued for 6 wk. The untreated CRF and sham-operated control groups received drug-free powdered food. Six animals were included in each group.

At the conclusion of the treatment period, the animals were placed in metabolic cages for a 24-hr urine collection. They were then killed by exsanguination via cardiac puncture between the hours of 9 and 11 AM. The liver was removed, immediately frozen in liquid nitrogen, and stored at −70°C until processed. Serum cholesterol, triglyceride, LDL, VLDL, and creatinine concentrations and urinary protein and creatinine were determined as described previously (22). Plasma HDL cholesterol was measured in the supernatant of plasma after precipitation of LDL and VLDL using dextran sulfate (0.91 mg/ml), and HDL-3 cholesterol was measured after precipitation of HDL-2, LDL, and VLDL using dextran sulfate (1.74 mg/ml) as described by Warnick and Cheung (38). After centrifugation at 1,500 rpm for 30 min, supernatants were collected and analyzed for total HDL and HDL-3 cholesterol using a cholesterol reagent kit (Pointe Scientific). The HDL-2 cholesterol was calculated as the difference between total HDL and HDL-3 cholesterol concentrations. The protocol employed in this study was approved by the Institutional Committee for Care and Use of Animals at the University of California, Irvine.

Preparation of liver microsomes. Four hundred milligrams of frozen liver were homogenized in ice-cold buffer A containing 50 mM Tris·HCl, 250 mM sucrose, 1 mM EDTA, and 1 mM PMSF, pH 7.4. Microsomes were then prepared by differential centrifugation as described previously (23). The microsomal membranes were then resuspended in buffer A, divided into several aliquots, and stored at −70°C until used.

Measurement of LDL receptor protein. LDL receptor protein abundance in the liver tissue was measured by Western blot analysis using a mouse antibovine LDL receptor antibody (Cortex Biochem, Davis, CA) as described in our previous study (29).

Measurements of plasma LCAT concentration. Plasma LCAT concentration was determined by enzyme-linked immunosorbent assay (ELISA) using a rabbit anti-human LCAT antibody (generously provided by Dr. John S. Parks, Wake Forest University, Winston-Salem, NC) as described in our earlier studies (34).

Acy-CoA:cholesterol acyltransferase measurements. Acyl-CoA: cholesterol acyltransferase (ACAT)-2 protein abundance in the liver was determined by Western blot analysis using a polyclonal ACAT-2 antibody provided by Dr. John S. Parks, Wake Forest University, Winston-Salem, NC) as described in one of our earlier studies (21).

Analysis of plasma lipids and lipoproteins. Total cholesterol was determined by a cholesterol reagent kit (Pointe Scientific). HDL cholesterol was measured after precipitation of HDL-2 and HDL-3 using a cholesterol reagent kit (Pointe Scientific) as described in our previous study (33). Hepatic microsomal ACAT activity was quantified in a manner identical to that described in our earlier studies (33).

Data analysis. ANOVA and a multiple range test were used in the statistical evaluation of the data, which are shown as means ± SE. P values <0.05 were considered significant.

RESULTS

General data. Data are summarized in Table 1. The untreated CRF animals exhibited a significant reduction in creatinine clearance, mild hyperalbuninemia, severe hypertriglyceridemia, marked elevation of plasma VLDL, moderate elevation of plasma total cholesterol and LDL cholesterol concentrations, and a significant increase in plasma total cholesterol-to-HDL cholesterol ratio. Statin administration resulted in significant improvements in plasma VLDL, LDL, and triglyceride concentrations, marked elevation of plasma HDL cholesterol, normalization of plasma total cholesterol-to-HDL cholesterol ratio, with virtually no change in plasma total cholesterol concentration in the treated compared with the untreated CRF rats. In the sham-operated rats, statin administration resulted in no significant change in serum lipid levels except for a mild rise in HDL cholesterol and a modest decline in plasma total cholesterol-to-HDL cholesterol ratio. Statin administration resulted in a significant rise in both HDL-2 and HDL-3 cholesterol in the CRF group. However, the magnitude of the rise in HDL-2 cholesterol was greater than that of HDL-3 cholesterol concentration in the CRF animals. In contrast, the impact of statin therapy on cholesterol contents of HDL-2 and HDL-3 in the normal controls was less conspicuous than that seen in the CRF animals.

Body weight in the CRF groups was lower than that in the sham-operated controls. No significant difference was found in

Table 1. General data for the sham-operated control group, untreated rats with chronic renal failure, and 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor-treated control and chronic renal failure rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>CTL</th>
<th>CTL-HI</th>
<th>CRF</th>
<th>CRF-HI</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>59 ± 2.2</td>
<td>55 ± 4</td>
<td>124 ± 11*</td>
<td>124 ± 2.3*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>18.7 ± 2.7</td>
<td>19.1 ± 1.6</td>
<td>69 ± 10*</td>
<td>43 ± 8+*</td>
<td>0.02</td>
</tr>
<tr>
<td>VLDL cholesterol, mg/dl</td>
<td>9.3 ± 0.9</td>
<td>9.4 ± 0.8</td>
<td>33 ± 3.4*</td>
<td>19 ± 1.9†*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>23 ± 2.4</td>
<td>26 ± 2.3</td>
<td>36 ± 4.2*</td>
<td>63 ± 6.2+†</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL-2 cholesterol, mg/dl</td>
<td>10 ± 2.6</td>
<td>11 ± 0.9</td>
<td>19 ± 3.7*</td>
<td>36 ± 1.5†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-3 cholesterol, mg/dl</td>
<td>13 ± 0.8</td>
<td>15 ± 1.2</td>
<td>17 ± 3.3</td>
<td>27 ± 0.7+†</td>
<td>0.001</td>
</tr>
<tr>
<td>Total/HDL cholesterol ratio</td>
<td>2.7 ± 0.3</td>
<td>2.1 ± 0.2*</td>
<td>3.7 ± 0.5*</td>
<td>2.1 ± 0.2+†</td>
<td>0.004</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>46.6 ± 4.5</td>
<td>47.0 ± 4.1</td>
<td>166 ± 17*</td>
<td>94 ± 9.3+*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Albumin, g/dl</td>
<td>3.8 ± 0.1</td>
<td>3.7 ± 0.2</td>
<td>3.1 ± 0.06*</td>
<td>3.1 ± 0.2*</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Creatinine, ml/dl</td>
<td>0.38 ± 0.01</td>
<td>0.40 ± 0.03</td>
<td>1.3 ± 0.2*</td>
<td>0.9 ± 0.08+†</td>
<td>0.001</td>
</tr>
<tr>
<td>Cr, ml/24 h</td>
<td>2.6 ± 0.2</td>
<td>2.5 ± 0.2</td>
<td>0.56 ± 0.09*</td>
<td>0.98 ± 0.07†</td>
<td>0.001</td>
</tr>
<tr>
<td>Urine protein, mg/24 h</td>
<td>10.6 ± 1.3</td>
<td>10.7 ± 2.3</td>
<td>72 ± 7.1*</td>
<td>63 ± 6.7*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>365 ± 25</td>
<td>348 ± 28</td>
<td>339 ± 18*</td>
<td>283 ± 21+†</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6/group. CTL, sham-operated control; CRF, chronic renal failure; CTL-HI, HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA reductase-treated control; CRF-HI, HMG-CoA-treated CRF rate; Ccr, creatinine clearance. *P < 0.05 vs. CTL. †P < 0.05 vs. CRF.
body weight among the statin-treated and untreated control groups. However, body weight in the statin-treated CRF group was significantly lower than that in the untreated CRF group.

**Hepatic HMG-CoA reductase.** Data are depicted in Fig. 1. Hepatic HMG-CoA reductase protein abundance and microsomal HMG-CoA reductase activity in the untreated CRF rats were unchanged compared with the corresponding values found in the sham-operated control animals. Statin administration resulted in significant reduction of hepatic HMG-CoA reductase activity without significant change in the abundance of the immundetectable enzyme. Similarly, statin therapy led to a significant fall in HMG-CoA reductase activity with no significant change in its protein abundance in the treated controls.

**LDL and HDL receptors.** Data are given in Fig. 2. Hepatic tissue LDL receptor protein abundance in the untreated CRF animals was significantly lower than that found in the sham-operated control rats. Statin administration led to normalization of liver tissue LDL receptor in the treated CRF group. No significant difference was found in SRB-1 abundance among the three groups. Statin therapy raised hepatic LDL receptor but did not affect HDL receptor abundance in the liver of sham-operated controls.

**Plasma LCAT concentration.** Data are shown in Fig. 3. In confirmation of our previous studies (34), plasma LCAT concentration was significantly reduced in the untreated CRF rats compared with that in the control group. Plasma LCAT concentration was fully normalized in the statin-treated CRF group. A mild but significant rise was observed in plasma LCAT level in the statin-treated controls.

**ACAT.** Data are illustrated in Fig. 4. In confirmation of our recent studies (23), hepatic ACAT-2 protein abundance and microsomal ACAT enzymatic activity were significantly elevated in the untreated CRF group compared with the corresponding values in the control animals. Both ACAT-2 protein abundance and enzymatic activity remained significantly elevated in the statin-treated CRF group. In the treated subgroup of sham-operated control rats, statin administration resulted in a mild reduction in hepatic ACAT activity.

**Cholesterol 7α-hydroxylase.** Data are shown in Fig. 5. No significant difference was found in hepatic tissue cholesterol 7α-hydroxylase abundance between the untreated CRF rats and the sham-operated control animals. Although the mean value of the enzyme abundance was lower in the statin-treated CRF animals, the difference did not reach statistical significance. However, statin administration led to a significant fall in the enzyme abundance among the treated controls.

**DISCUSSION**

Cholesterol homeostasis in the peripheral tissues is primarily dependent on the balance between receptor-mediated cholesterol influx via ApoB-containing lipoproteins and HDL-mediated efflux of surplus cholesterol (10). Accordingly, the ratio of cholesterol in the ApoB-containing lipoproteins to that in HDL serves as a useful indicator of cholesterol homeostasis in the artery wall, glomerular mesangium, and a number of other tissues. The untreated CRF animals employed in the present study exhibited a significant increase in plasma total cholesterol-to-HDL cholesterol ratio, representing an atherogenic profile. This was associated with a severe reduction in plasma LCAT concentration, confirming the results of earlier studies.
in rats and humans with CRF (28, 34). LCAT deficiency can account for impaired HDL maturation, defective reverse cholesterol transport, and abnormal total cholesterol-to-HDL cholesterol ratio in patients and animals with CRF. Statin administration for 6 wk reversed CRF-induced LCAT deficiency. Correction of LCAT deficiency was associated with, and most likely responsible for, the marked rise in plasma HDL cholesterol concentration observed in the statin-treated CRF animals.

Long-term statin administration in the CRF rats resulted in a significant decline in plasma VLDL and LDL cholesterol concentrations. This phenomenon can be attributed to diminished cholesterol biosynthesis occasioned by the observed inhibition of hepatic HMG-CoA reductase activity and enhanced LDL clearance occasioned by the reversal of hepatic LDL receptor deficiency. The decline in plasma VLDL and LDL cholesterol concentrations in the statin-treated CRF rats was matched by a marked rise in plasma HDL cholesterol concentration. Consequently, plasma total cholesterol concentration remained virtually unchanged and total cholesterol-to-HDL cholesterol concentration ratio was normalized. It should be noted that, unlike humans, rats lack CETP (27). This protein mediates the exchange of cholesterol ester contained in HDL-2 for triglyceride contained in IDL within the circulation. The absence of CETP in the rat and its presence in humans are responsible for the relatively higher level of HDL cholesterol in the rat plasma than that in the human plasma. Accordingly, due to the CETP-mediated transport of cholesterol from HDL to ApoB-containing lipoproteins, the magnitude of the rise in HDL cholesterol and the corresponding fall in total cholesterol-to-HDL cholesterol ratio in response to inhibition of HMG-CoA reductase may be less striking in humans with CRF than in the rats with CRF.

Despite the inhibition of HMG-CoA reductase activity, the expression of HMG-CoA reductase and cholesterol 7α-hydroxylase (the rate-limiting enzymes in cholesterol biosynthesis and catabolism, respectively) remained unchanged in the CRF animals. This phenomenon is most likely due to enhanced hepatic cholesterol uptake from the circulation, occasioned by reversal of LDL receptor and LCAT deficiencies. Consequently, the resultant improvements in hepatic LDL uptake and HDL-mediated reverse cholesterol transport must have precluded the compensatory upregulation of HMG-CoA reductase.
and downregulation of cholesterol 7α-hydroxylase in response to the statin-induced reduction of cholesterol biosynthesis in the hepatocytes of these animals.

ACAT catalyzes intracellular esterification of cholesterol and as such is involved in intestinal absorption of cholesterol, hepatic assembly of ApoB-containing lipoproteins, foam cell formation, and atherogenesis (2, 5, 6, 8, 9, 16, 26). In confirmation of our earlier studies (23), the CRF rats employed here showed a marked upregulation of ACAT activity and liver-specific ACAT-2 protein, which remained elevated despite statin administration.

SRB-1 serves as a docking platform for unloading the HDL lipid cargo in the hepatocyte (1) and as such represents a critical link in reverse cholesterol transport. Hepatic SRB-1 protein abundance was unchanged in the liver of CRF animals and was not affected by statin administration.

In the sham-operated control rats, statin administration led to significant rises in plasma LCAT and the hepatic LDL receptor. This was coupled with a significant fall in cholesterol 7α-hydroxylase abundance and ACAT activity. The concurrent decline in cholesterol catabolism (downregulation of cholesterol 7α-hydroxylase) can largely account for the maintenance of normal plasma cholesterol concentration despite statin-induced reduction of cholesterol biosynthetic capacity among the statin-treated control rats. A slight rise in HDL cholesterol in the treated controls can be attributed to the mild rise in plasma LCAT level.

The statin-treated CRF rats exhibited a slightly higher creatinine clearance than that found in the untreated CRF rats. This observation is consistent with the results of a number of earlier studies (4, 15, 25) employing different HMG-CoA reductase inhibitors. Together, these observations suggest that statins may directly or indirectly (via an improvement of lipid profile) slow the progression of renal disease. In this regard, improvement in plasma HDL cholesterol is of particular interest since a relative reduction in HDL cholesterol has been recently identified as a strong independent risk factor for the progression of renal disease in humans (13). In fact, patients with hereditary LCAT deficiency, which leads to progressive impairment of HDL metabolism, frequently exhibit pronounced renal insufficiency and premature arteriosclerotic cardiovascular disease (18). Taken together, the available data (13, 18) substantiate the role of impaired HDL metabolism in the pathogenesis and progression of renal disease.

As expected, the CRF animals exhibited marked hypertriglyceridemia, which is a main feature of CRF-associated dyslipidemia (3, 7, 12, 28). This abnormality is primarily due to the downregulation of tissue lipoprotein lipase (30, 37), VLDL receptor (20, 31), and hepatic triglyceride lipase (17). In addition, the impaired maturation of HDL-3 to HDL-2, occasioned by LCAT deficiency, contributes to defective lipolysis and clearance of triglyceride-rich lipoproteins in the circulation. Thus a partial improvement in hypertriglyceridemia in the statin-treated CRF rats may be due, in part, to the improvement in HDL metabolism. Large-scale clinical trials of several different statins have shown favorable modifications of plasma LDL, HDL, and triglycerides in hyperlipidemic humans in the general population (14). Thus the effects observed in the CRF rats are directionally consistent with those found in hyperlipidemic humans.

The body weight of the statin-treated CRF rats was significantly below than that found in the untreated CRF animals. In contrast, the drug did not significantly affect body weight in the treated control rats. The reason for the observed difference in weight between the statin-treated CRF and control rats is not clear and warrants further studies of the effect of CRF on metabolism of the statin used here and other statins and the possible need for dosage adjustment. The lower body weight and food intake in the statin-treated CRF rats may have contributed, in part, to the observed changes in lipoprotein metabolism in these animals. It should be noted, however, that we have observed significant improvements in the lipoprotein profile, as well as hepatic expression, of the LDL receptor, HDL receptor, and plasma LCAT in rats with nephrotic syndrome (and normal creatinine clearance) with administration of the same statin preparation, despite there being no significant change in body weight (unpublished data).

In conclusion, long-term inhibition of HMG-CoA reductase reversed LCAT and LDL receptor deficiencies and improved the plasma lipid profile in rats with renal mass reduction.

GRANTS

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