Upregulation of hepatic LDL receptor-related protein in nephrotic syndrome: response to statin therapy

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Kim, Sara, Choong H. Kim, and Nosratola D. Vaziri. Upregulation of hepatic LDL receptor-related protein in nephrotic syndrome: response to statin therapy. Am J Physiol Endocrinol Metab 288: E813–E817, 2005. First published December 7, 2004; doi:10.1152/ajpendo.00266.2004.—Nephrotic syndrome (N-S) is associated with elevated plasma concentration and impaired clearance of VLDL, chylomicrons (CM), and their atherogenic remnants. These abnormalities are largely due to lipoprotein lipase, hepatic triglyceride lipase, and very-low-density lipoprotein (VLDL) receptor deficiencies and impaired HDL-mediated shuttling of apoE and apolipoprotein (apo)C between the nascent and remnant VLDL and CM. LRP is a multifaceted endocytic receptor that is heavily expressed in the liver. LRP recognizes at least 30 different ligands including VLDL and CM remnants. These observations prompted the present study to discern the effect of N-S on hepatic LRP gene and protein expressions. The study further sought to explore the effect of lipid-lowering therapy on LRP expression in N-S. Sprague-Dawley rats were randomized to the N-S (given ip injections of puromycin aminonucleoside; 130 mg/kg on day 1, 60 mg/kg on day 14) and placebo-injected control groups. On day 14, animals were subdivided into statin-treated (rosuvastatin; 20 mg·kg−1·day−1 mixed with powdered chow) and untreated groups and studied on day 28. The untreated N-S group exhibited severe proteinuria, hypoalbuminemia, hypercholesterolemia, and marked elevation of hepatic tissue LRP mRNA and protein abundance. Statin administration for 2 wk resulted in significant improvements of plasma lipid profile, proteinuria, and hypoalbuminemia as well as hepatic LRP mRNA and protein abundance. In contrast, statin administration had no significant effect on either plasma lipids or hepatic LRP levels in the normal control rats. In conclusion, N-S results in marked upregulation of hepatic LRP expression that is partly reversed with statin administration. These findings exclude depressed hepatic LRP expression as the primary cause of elevated plasma lipoprotein remnants in N-S.

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HYPERLIPIDEMIA IS ONE OF THE CARDINAL FEATURES of heavy glomerular proteinuria, otherwise known as nephritogenic syndrome (N-S) (7, 8, 43, 45). Nephrotic hyperlipidemia is characterized by hypercholesterolemia, hypertriglyceridemia, increased plasma concentration, and impaired clearance of low-density lipoprotein (LDL), high-density lipoprotein (HDL), very-low-density lipoprotein (VLDL), chylomicrons, and chylomicron remnants as well as depressed maturation of HDL (7, 8, 43, 45). In addition, N-S leads to severe abnormalities of coagulation, fibrinolytic, and protease inhibitory systems (3, 19, 28–31, 39–42). Together, these abnormalities contribute to the high risk of cardiovascular complications and progressive deterioration of renal function in patients with chronic N-S (21, 23).

LDL receptor-related protein (LRP) is a high-molecular-mass (500 kDa) membrane receptor that is abundantly expressed in the liver and has substantial structural similarity to LDL receptor (4). Like LDL receptor, LRP strongly binds calcium, which is a critical step in the receptor binding to apolipoprotein (apo)B and apoE. On the basis of these observations, Herz et al. (4) proposed that LRP is a lipoprotein receptor. Subsequent studies demonstrated the role of LRP as a receptor for chylomicron and VLDL remnants (apoE and apoB48 serving as the ligands) (1, 11, 12, 22, 44), hepatic lipase (9, 10), and lipoprotein lipase (2, 20, 46). In addition, LRP was shown to serve as a receptor for numerous other ligands including proteins involved in coagulation, fibrinolytic, and protease inhibitory systems (6, 28) (tissue-type plasminogen activator, plasminogen activator inhibitor-1, factors IXa, VIIIa and VIIa, tissue factor pathway inhibitor, antithrombin III, heparin cofactor II), regulation of proteolytic activity (α2-macroglobulin, α1-antitrypsin), and chaperone system (heat shock protein 96) among others (4). The ability of LRP to bind so many different ligands with high affinity is due to the existence of some 31 ligand binding-type repeats in the molecule, forming an array of surface contours and charge distributions. This structural attribute facilitates numerous interactions between the receptor and many different ligands, each recognizing a particular combination of the binding sites on the receptor molecule (4).

As noted above, N-S results in profound abnormalities of lipid metabolism, coagulation, fibrinolytic, and protease inhibitory systems (3, 7, 8, 19, 28–31, 39–43, 45). One of the features of nephrotic dyslipidemia is impaired clearance of chylomicron remnants and VLDL remnants, which are highly atherogenic (7, 8, 43, 45). Both chylomicron remnants and VLDL remnants are LRPI ligands (1, 11, 12, 22, 44). In addition, LRP serves as the receptor for several activated coagulation factors, fibrinolytic proteins, natural anticoagulants, and protease inhibitors (6, 27). Plasma con-
centrations of these LRP ligands are severely altered in N-S (3, 19, 28–31, 39–42). These observations provided the impetus to explore the effect of N-S on gene expression and protein abundance of LRP in the liver. To our knowledge, the effect of N-S on hepatic LRP expression has not been investigated previously and was studied here. In addition, the possible effect of lipid-lowering therapy on the hepatic LRP expression was evaluated. This was based on our recent study, which revealed amelioration of LDL receptor and HDL receptor deficiencies with statin administration in nephritic animals.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats were made nephrotic by sequential intraperitoneal injections of puromycin aminonucleoside (PAN; 130 mg/kg on day 1 and 60 mg/kg on day 14). Placebo-injected rats served as controls. The animals were observed for 2 wk after the last PAN or placebo injections. The PAN-treated animals failing to exhibit nephrotic proteinuria were excluded. The nephrotic animals were randomized to the hydroxymethylglutaryl (HMG)-CoA reductase inhibitor-treated and untreated subgroups. The statin-treated group received rosuvastatin (Astra-Zeneca, Cheshire, UK), 20 mg·kg⁻¹·day⁻¹, mixed with powdered standard chow for 2 wk. To compare the effects of statin administration between the nephrotic and normal animals, subgroups of statin-treated and untreated controls were similarly studied. General data from both untreated control groups were similar. The untreated nephritic and control groups were fed the powdered standard chow for 2 wk. To compare the effects of statin administration between the nephrotic and normal animals, subgroups of statin-treated and untreated controls were similarly studied. General data from both untreated control groups were similar. The untreated nephritic and control groups were fed the drug-free powdered food. The study animals (n = 6 rats in each group) were housed in a climate-controlled, light-regulated facility with 12:12-h light and dark cycles. At the end of the 4-wk observation period, the animals were placed in individual metabolic cages for a 24-h urine collection. Under general anesthesia (pentobarbital sodium; 50 mg/kg ip), the animals were euthanized by exanguination using cardiac puncture. The liver was immediately removed, snap frozen in liquid nitrogen, and stored at −70°C until processed. In addition, plasma and urine specimens were stored at −70°C. All experiments employed in the study were approved by the University of California, Irvine, Committee for the Use and Care of Experimental Animals.

RNA isolation and RT-PCR. RNA was isolated from frozen liver using TRIzol reagent (Invitrogen, Carlsbad, CA) and purified by RNeasy kit (Qiagen, Valencia, CA). One-fifth microgram total RNA from each sample was reverse transcribed to cDNAs by using murine leukemia virus reverse transcriptase (Applied Biosystems, Foster City, CA) with a mixture of 1 mM dNTP and 2.5 μM random primers in a 10-μl volume for 10 min at 25°C and 30 min at 48°C. The reaction was stopped by heating for 5 min at 94°C.

Expression of LRP mRNA was assessed by RT-PCR, using 18S ribosomal RNA as internal control. The primer sequences used are depicted in Table 1. For 18S ribosomal RNA amplification, we used alternate 18S (Ambion, Austin, TX), 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 mM dNTP, 1 μM LRP primer set, 1 μM 18S primer/competitor mix, 2 mM MgCl₂, and 1.25 units of Taq DNA polymerase (Applied Biosystems) in 25-μl total volume for 30 cycles. Each cycle consisted of 1-min denaturation at 94°C, 45-s annealing at 60°C, and 45-s extension at 72°C. PCR products were separated on a 1.5% agarose gel with ethidium bromide by electrophoresis. Signal intensity was determined by laser scanning densitometry. On each occasion, the LRP mRNA abundance was normalized to the corresponding 18S ribosomal RNA.

Measurements of LRP protein. LRP protein abundance in the liver tissue was measured by Western blot analysis using a monoclonal antibody prepared in our laboratory with the IgG-11H4 hybridoma cell line purchased from American Type Culture Collection (Manassas, VA).

Data analysis. Analysis of variance (ANOVA) and multiple-range test were used in statistical evaluation of the data. Data are given as means ± SE. P values <0.05 were considered significant.

Table 2. Plasma concentrations

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total cholesterol, mg/dl</th>
<th>LDL cholesterol, mg/dl</th>
<th>VLDL cholesterol, mg/dl</th>
<th>Total cholesterol/HD ratio</th>
<th>Triglycerides, mg/dl</th>
<th>Albumin, g/dl</th>
<th>Cretinine, mg/dl</th>
<th>CeR, ml/min</th>
<th>Urine protein, mg/24 h</th>
<th>Body weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTL</td>
<td>59±2.2</td>
<td>18±4.3</td>
<td>9.3±0.9</td>
<td>2.7±0.3</td>
<td>46±4.5</td>
<td>3.8±0.1</td>
<td>0.4±0.02</td>
<td>2.6±0.2</td>
<td>10±1.3</td>
<td>346±28</td>
</tr>
<tr>
<td>CTL-HI</td>
<td>55±4.0</td>
<td>19±3.7</td>
<td>9.4±0.8</td>
<td>2.1±0.2</td>
<td>47±4.5</td>
<td>3.7±0.2</td>
<td>0.4±0.03</td>
<td>2.5±0.2</td>
<td>11±1.3</td>
<td>332±28</td>
</tr>
<tr>
<td>N-S</td>
<td>264±17*</td>
<td>173±16*</td>
<td>33±3*</td>
<td>4.8±0.5*</td>
<td>167±15*</td>
<td>2.3±2.2</td>
<td>0.4±0.03</td>
<td>2.4±0.1</td>
<td>217±25*</td>
<td>329±7*</td>
</tr>
<tr>
<td>N-S + HI</td>
<td>99±5.7**</td>
<td>39±7.7**</td>
<td>19±3.3**</td>
<td>2.7±0.4**</td>
<td>94±6**</td>
<td>3.2±0.6**</td>
<td>0.5±0.02</td>
<td>2.4±0.4</td>
<td>77±7.8**</td>
<td>321±6*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Plasma concentrations of albumin, creatinine, total cholesterol, free cholesterol, low-density lipoprotein (LDL)-cholesterol, very-low-density lipoprotein (VLDL)-cholesterol, triglyceride, free fatty acids, total cholesterol-to-high-density lipoprotein (HDL) ratio, creatinine clearance (CeR), urinary protein excretion, and body weight in the control (CTL), untreated nephrotic (N-S), and hydroxymethylglutaryl (HMG)-CoA reductase inhibitor-treated control (CTL-HI) and nephrotic (N-S + HI) groups. NS, not significant. *P < 0.05 vs. control. †P < 0.05 vs. N-S.
RESULTS

General data. When compared with the control group, the nephrotic group exhibited heavy proteinuria, hypoalbuminemia, hypercholesterolemia, hypertriglyceridemia, elevated plasma LDL, VLDL and total cholesterol-to-HDL cholesterol ratio, unchanged creatinine clearance, and a mild but significant reduction in body weight (Table 2).

LRP data. Hepatic tissue LRP mRNA abundance was significantly increased in the nephrotic animals compared with the normal control group. Likewise, immunodetectable LRP protein abundance was increased in the liver of the nephrotic group relative to that found in the control group (Figs. 1 and 2).

Response to statin administration. Statin administration for 2 wk resulted in a significant amelioration of proteinuria and hyperlipidemia in the treated nephrotic animals without affecting creatinine clearance or body weight (Table 2). This was accompanied by a significant reduction of hepatic LRP mRNA abundance.
and protein abundance toward values found in the control group (Figs. 1 and 2). In contrast to the nephrotic animals, normal animals exhibited no significant change in either plasma lipid levels (Table 2) or hepatic LRP values in response to statin administration (Fig. 3).

DISCUSSION

In the past decade, considerable progress has been made in the understanding of the molecular mechanisms of nephrotic dyslipidemia. In this regard, N-S was shown to result in upregulation of the hepatic HMG-CoA reductase, insufficient expression of cholesterol 7α-hydroxylase (the rate-limiting step in cholesterol conversion to bile acid) (13, 36, 41), and acquired LDL receptor deficiency (35, 41). These abnormalities work in concert to raise plasma total cholesterol and LDL cholesterol concentrations. Moreover, N-S leads to severe hepatic HDL receptor [scavenger receptor B-1 (SRB-1)] deficiency (15) as well as urinary losses and diminished plasma concentration of lecithin-cholesterol acyltransferase (LCAT) (38, 41). Given the critical role of LCAT in HDL-mediated cholesterol uptake from the peripheral tissues, the identification of LCAT deficiency helped to unravel the mechanism responsible for impaired HDL maturation in N-S. Similarly, downregulation of SRB-1, which plays a critical role in hepatic clearance of HDL-born lipids, elucidated the reason for impaired HDL clearance in N-S (15). More recently, N-S was found to result in marked upregulation of hepatic acyl-CoA-cholesterol acyltransferase (ACAT) (33, 34). ACAT plays an important part in packaging and secretion of apoB-containing lipoproteins by the liver, and as such its upregulation in N-S contributes to the associated hyperlipidemia. In fact, inhibition of ACAT was recently shown to dramatically improve lipid profile in this model (33). In addition, N-S leads to downregulation of lipoprotein lipase (16, 24, 25), VLDDL receptor (17, 24, 25), and hepatic triglyceride lipase (18, 25) as well as upregulation of hepatic acyl-CoA-diacetylglcerol acyltransferase (DGAT; the final step in triglyceride biosynthesis) (32). Together, these abnormalities contribute to the pathogenesis and maintenance of hypertriglyceridermia and elevated plasma concentration and impaired clearance of VLDL, intermediate-density lipoprotein, and chylomicrons. Moreover, VLDDL clearance is further compromised by inefficient HDL-mediated apoE delivery to the nascent VLDDL (26). Comparison of nephrotic male rats exhibiting proteinuria and hypoalbuminemia with the male Nagase analbuminemic rats exhibiting severe isolated hypoalbuminemia (hereditary analbuminemia) without proteinuria has implicated the contribution of proteinuria as opposed to hypoalbuminemia in dysregulation of the majority of the lipid regulatory enzymes and receptors cited above (14).

As noted earlier, N-S is associated with elevated plasma concentration and impaired clearance of chylomicron and VLDL remnants, which are LRP ligands. It is therefore conceivable that accumulation of these lipoprotein remnants could be due to downregulation of LRP in the nephrotic liver. However, contrary to the expectation, the nephrotic animals employed in the present study exhibited a significant upregulation of LRP mRNA and protein expression compared with the normal control animals. This finding tends to exclude a quantitative deficiency of LRP as a culprit in the pathogenesis of impaired clearance of chylomicron and VLDL remnants in the nephrotic animals. It is of interest that N-S results in severe reductions of LDL receptor and VLDL receptor (17, 24, 25, 35, 41), two well-known members of the LDL receptor gene family. This is in clear contrast with the effect of N-S on LRP shown in the present study.

The mechanism by which N-S increases hepatic LRP expression is not clear. It is of note that lipid-lowering therapy with an HMG-CoA reductase inhibitor led to partial reduction of hepatic LRP mRNA and protein abundance in the nephrotic animals. The possible mechanisms by which statin administration reduced LRP abundance in the nephrotic liver include statin-induced reduction in proteinuria, amelioration of hyperlipidemia, or an unrelated effect of the drug. It should be noted that statin administration had virtually no effect on either plasma lipid levels or hepatic LRP abundance in the normal animals. This observation tends to argue against a direct effect of the drug on LRP expression. In view of the central role of proteinuria in the pathogenesis of all complications of N-S, statin-induced amelioration of proteinuria may have contributed to the partial reduction of hepatic LRP abundance.

In conclusion, N-S results in upregulation of hepatic LRP, which tends to exclude a quantitative alteration of this receptor as the primary cause of the defective clearance of chylomicron and VLDL remnants in this condition. Statin administration ameliorated proteinuria, improved hyperlipidemia, and partially reversed the upregulation of hepatic LRP in the nephrotic rats but had no effect in the normal animals.

REFERENCES


