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

Sara Kim, Choong H. Kim, and Nosratola D. Vaziri

Division of Nephrology and Hypertension and Department of Physiology

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Hyperlipidemia is one of the cardinal features of heavy glomerular proteinuria, otherwise known as nephrotic 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 LRP 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-

Address for reprint requests and other correspondence: N. D. Vaziri, Division of Nephrology and Hypertension, UCI Medical Center, 101 The City Drive, Bldg. 53, Rm. 125, Rt. 81, Orange, CA 92868 (E-mail: ndvaziri@uci.edu).

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centrations of these LRP ligands are severely altered in N-S (3, 19, 28–31, 39–42). These observations provided the

MATERIALS AND METHODS

Plasma concentrations

Table 2. Plasma concentrations

| Groups                     | CTL     | CTL-HI   | N-S      | N-S + HI | P
|---------------------------|---------|----------|----------|----------|-----
| Total cholesterol, mg/dl  | 59±2.2  | 55±4.0   | 264±17*  | 99±5.7†  | <0.001
| LDL cholesterol, mg/dl    | 18±4.3  | 19±3.7   | 173±16*  | 39±7.7†  | <0.001
| VLDL cholesterol, mg/dl   | 9.3±0.9 | 9.4±0.8  | 33±3*    | 19±1.3†  | <0.001
| Total cholesterol/HDL ratio| 2.7±0.3 | 2.1±0.2  | 4.8±0.5* | 2.7±0.4† | <0.001
| Triglycerides, mg/dl      | 46±4.5  | 47±4.5   | 167±15*  | 94±6†    | <0.001
| Albumin, g/dl             | 3.8±0.1 | 3.7±0.2  | 2.3±0.2  | 3.2±0.06† | <0.001
| Creatinine, mg/dl         | 0.4±0.02| 0.4±0.03 | 0.4±0.03 | 0.5±0.02 | NS
| Ccr, ml/min               | 2.6±0.2 | 2.5±0.2  | 2.4±0.1  | 2.4±0.4  | NS
| Urine protein, mg/24 h    | 10±1.3  | 11±1.3   | 217±25*  | 77±7.8*  | <0.001
| Body weight, g            | 346±8   | 332±8    | 329±7*   | 321±6*   | <0.05

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 (Ccr), 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

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Fig. 1. Representative RT-PCR and group data depicting hepatic LRP mRNA and ribosomal 18S RNA in the normal control (CTL), untreated nephrotic (N-S), and statin-treated nephrotic (N-S + HI) groups. *P < 0.01 vs. CTL. **P < 0.05 vs. N-S. #P < 0.05 vs. CTL.

Fig. 2. Representative Western blot and group data depicting hepatic LRP protein and β-actin in the normal control (CTL), untreated nephrotic (N-S), and statin-treated nephrotic (N-S + HI) groups. *P < 0.01 vs. CTL. **P < 0.05 vs. N-S. #P < 0.05 vs. CTL.

Fig. 3. Representative RT-PCR and group data depicting hepatic LRP mRNA and 18S ribosomal RNA abundance (top) and representative Western blot and group data depicting hepatic LRP and actin protein abundance in the untreated (CTL) and HMG-CoA reductase inhibitor-treated normal control (CTL-HI) rats.
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 regards, 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), VLDL receptor (17, 24, 25), and hepatic triglyceride lipase (18, 25) as well as upregulation of hepatic acyl-CoA-diaclyglycerol acyltransferase (DGAT); the final step in triglyceride biosynthesis (32). Together, these abnormalities contribute to the pathogenesis and maintenance of hypertriglyceridemia and elevated plasma concentration and impaired clearance of VLDL, intermediate-density lipoprotein, and chylomicrons. Moreover, VLDL clearance is further compromised by inefficient HDL-mediated apoE delivery to the nascent VLDL (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**