Nephrotic syndrome causes upregulation of HDL endocytic receptor and PDZK-1-dependent downregulation of HDL docking receptor

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Abstract

Background. Nephrotic syndrome (NS) is associated with dysregulation of lipid/lipoprotein metabolism and impaired high-density lipoprotein (HDL)-mediated reverse cholesterol transport and atherosclerosis. HDL serves as vehicle for uptake and transport of surplus lipids from the peripheral tissues for disposal in the liver via two receptors: (i) scavenger receptor class B type I (SR-BI) which serves as a docking receptor, enabling HDL to unload its lipid cargo and return to circulation to repeat the cycle, and (ii) beta chain ATP synthase which serves as the endocytic receptor mediating removal and catabolism of lipid-poor HDL. SR-BI abundance is regulated by PDZ-containing kidney protein 1 (PDZK1), a multifunctional protein, which prevents SRB-1 degradation at the post-translational level. This study explored the effect of NS on hepatic expression of these important molecules.

Methods. Gene expression, protein abundance and immunohistological appearance of the above proteins were measured in the liver of rats with puromycin-induced NS and control rats.

Results. The nephrotic animals exhibited severe proteinuria, hypoalbuminemia, hypercholesterolemia, hypertriglyceridemia, reduced HDL/total cholesterol ratio, normal glomerular filtration rate, significant upregulation of the endocytic HDL receptor messenger RNA (mRNA) and protein (P < 0.005) and significant reduction of SR-BI protein (P < 0.002) despite its normal mRNA abundance. The reduction in SR-BI protein abundance in NS animals was accompanied by parallel reductions in PDZK1 mRNA (P = 0.02) and protein abundance (P = 0.012).

Conclusions. NS results in elevation of hepatic HDL endocytic receptor and deficiency of HDL docking receptor. The latter is associated with and, in part, mediated by downregulation of PDZK1. Together, these abnormalities can increase catabolism and diminish recycling of HDL and contribute to the defective reverse cholesterol/lipid transport in NS.

Keywords: atherosclerosis; cardiovascular disease; dyslipidemia; proteinuria; reverse cholesterol transport

Introduction

Heavy glomerular proteinuria [nephrotic syndrome (NS)] is associated with profound dysregulation of lipid/lipoprotein metabolism and increased risk of atherosclerosis and thromboembolism [1–3]. By mediating reverse cholesterol transport, protecting vascular endothelium and exerting antioxidant, anti-inflammatory and antithrombotic actions, high-density lipoprotein (HDL) confers protection against atherosclerosis and cardiovascular disease [4, 5]. The importance of HDL in protection against atherosclerotic cardiovascular disease is evidenced by the inverse association of coronary atherosclerosis with plasma HDL cholesterol concentration in the general population [6]. In addition to its quantity, functional integrity of HDL is equally important for its antiatherogenic properties. This is clearly illustrated by the occurrence of atherosclerotic cardiovascular disease among patients with normal or even elevated but functionally abnormal HDL [7].

HDL serves as a vehicle for uptake and transport of surplus cholesterol from the peripheral tissues to the liver for disposal in the bile. This process depends on the cell-surface receptors for HDL on hepatocytes of which two have been thus far identified. These include the high-affinity, the HDL docking receptor, scavenger receptor class B type I (SR-BI) and endocytic receptor, beta chain of ATP synthase.

The classical function of SR-BI in the hepatocyte is to mediate selective uptake of cholesteryl esters from the hydrophobic core of HDL without transferring its apolipoprotein component, thus enabling the unloaded HDL to return to circulation and repeat the cycle [8]. SR-BI-knockout mice exhibit marked elevation of plasma cholesterol, abnormally large unesterified cholesterol-rich HDL particles, reduced biliary cholesterol secretion and accelerated atherosclerosis [9–13]. These observations clearly illustrate the central role of SR-BI in reverse cholesterol transport.

The stability of the SR-BI in the hepatocyte plasma membrane is dependent on its adapter protein, PDZ-containing kidney protein 1 (PDZK1). This is evidenced by the observation that PDZK1-knockout mice exhibit a
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>95% reduction in hepatic SR-BI protein [but not SR-BI messenger (mRNA)] resulting in hypercholesterolemia and large cholesterol-rich HDL particles [14, 15]. In hepatocytes, PDZK1 is predominantly associated with the basolateral plasma membrane where it interacts with the most distal region of the C-terminal cytoplasmic domain of SR-BI [16] via its N-terminal PDZ domain. Thus, as the major adapter protein for SR-BI, PDZK1 plays a critical role in HDL-mediated reverse cholesterol transport. The mechanism(s) by which PDZK1 controls the hepatic and intestinal levels of SR-BI remains unclear. PDZK1 could directly affect the synthesis, posttranslational processing, intracellular transport, subcellular localization, recycling and/or stability of SR-BI.

Several years ago, Martinez et al. [17] showed that the beta chain of ATP synthase (a principal protein complex of the mitochondrial inner membrane) is ectopically expressed in the hepatocyte cell membrane. They further showed that cell-surface-associated beta chain of ATP synthase serves as an apoA-I receptor, mediating endocytosis of the HDL particles including its protein and lipid contents. This process strictly depends on generation of ADP which is produced by the ATPase activity of the beta chain protein after its binding with apoA-I on the cell surface. Unlike SR-BI, which has high affinity for binding cholesterol-rich HDL-2 particles, beta chain ATP synthase mediates catabolism of apo A-1 and lipid-poor HDL particles.

Earlier studies conducted in our laboratories revealed marked reduction of SR-BI protein abundance despite normal SR-BI mRNA expression [18]. The present study was designed to test this hypothesis that the observed disparity in hepatic SR-BI protein and mRNA expression in NS may be due to downregulation of PDZK1. To further explore the effect of nephrotic proteinuria on HDL metabolism, we examined the effect of NS on hepatic expression of the HDL endocytic receptor, beta chain of ATP synthase.

Materials and methods

Animals
Male Sprague–Dawley rats were rendered nephrotic by sequential intraperitoneal injections of puromycin aminonucleoside (130 mg/kg on Day 1 and 60 mg/kg on Day 14). Placebo-injected rats served as controls. The rats were observed for 2 weeks following the second puromycin/placebo injection. They were housed in a temperature-controlled facility with 12-h light/dark cycles and were provided free access to regular rat chow and water. At the end of the 2-week observation period, animals were placed in metabolic cages for a 24-h urine collection. They were then anesthetized (pentobarbital 50 mg/kg IP) and euthanized by exsanguination using pentobarbital 50 mg/kg IP and euthanized by exsanguination using carbon dioxide. Liver was immediately removed, frozen in liquid nitrogen, and stored at −70°C until processed.

Quantitative reverse transcriptase–polymerase chain reaction
RNA was extracted from liver tissue using TRIzol (Invitrogen). First-strand cDNA synthesis was performed using the Bio-Rad iCycler and a Qiagen Quantitect SYBR green PCR kit (Valencia, CA). RNA from 100 μg of (five controls to five nephrotic) rat tissue was utilized for the SYBR green PCR assay. The reaction mixture consisted of 2 μl cDNA template, 1 μl of each primer, 10 μl of 2x SYBR green master mix, and water, giving a total reaction volume of 20 μl. The conditions of polymerase chain reaction (PCR) were: 95°C for 10 s, 60°C for 30 s, and 72°C for 30 s, for 40 cycles. PCR was performed in triplicate for each sample, and the cycle threshold (Ct) values were used to determine the relative expression of target genes. The specificity of the PCR was checked by melting curve analysis.

Real-time polymerase chain reaction
Quantitative polymerase chain reaction (qPCR) was performed using the Bio-Rad iCycler and a Qiagen Quantitect SYBR green PCR kit (Valencia, CA). RNA from 100 μg of (five controls to five nephrotic) rat tissue was utilized for the SYBR green PCR assay. The reaction mixture consisted of 2 μl of cDNA template, 1 μl of each primer, 10 μl of 2x SYBR green master mix, and water, giving a total reaction volume of 20 μl. The conditions of polymerase chain reaction (PCR) were: 95°C for 10 s, 60°C for 30 s, and 72°C for 30 s, for 40 cycles. PCR was performed in triplicate for each sample, and the cycle threshold (Ct) values were used to determine the relative expression of target genes. The specificity of the PCR was checked by melting curve analysis.

Immunohistochemical analysis
Briefly, cryopreserved liver tissues were cut at 8-μm thickness, mounted on slides, air dried and fixed in 4% paraformaldehyde and washed with phosphate-buffered saline (PBS). Endogenous peroxidase activity was removed using 3% hydrogen peroxide in water. The sections were incubated overnight at 4°C with rabbit anti-PDZK1 (Epitomics, Inc., Burlingame, CA), rabbit anti SR-B1 (Novus Biologicals Inc., Littleton, CO) or mouse anti-ATP synthase beta (BD Transduction Laboratories, Sparks, MD) antibody, diluted at 1:100 to 1:500 in a PBS buffer containing 1% bovine serum albumin. Antibody binding was amplified using an ABC kit (Vector Laboratories, Burlingame, CA) and the complex visualized using diaminobenzidine. Nuclei were lightly stained with Mayer’s hematoxylin.

Data analysis
Student’s t-test was used in statistical evaluation of the data which are shown as mean ± SEM. P-values <0.05 were considered significant.
Results

General data

Data are shown in Table 1. The nephrotic group exhibited severe proteinuria, hypoalbuminemia, normal plasma creatinine and creatinine clearance. Compared with the control group, the nephrotic animals had marked elevation of plasma free and total cholesterol, LDL and HDL cholesterol, triglyceride and free fatty acid concentrations and increased plasma total cholesterol-to-HDL cholesterol ratio.

SR-BI and PDZK1 data

In confirmation of our earlier study [18], SR-BI protein abundance was significantly reduced, whereas SR-BI mRNA abundance was unchanged in the liver of nephrotic group as compared to the corresponding values found in the control animals (Figure 1). The reduction of SR-B1 protein abundance observed by western blot analysis was confirmed by immunohistological analysis performed on the frozen liver tissue sections (Figure 2).

The reduction in SR-BI protein abundance in the nephrotic liver was accompanied by a parallel reduction in PDZK1 protein abundance (Figure 3). The reduction of PDZK1 protein abundance observed by western blot analysis was confirmed by immunohistological analysis.

Table 1. Plasma concentrations of cholesterols, albumin, creatinine, triglycerides, HDL cholesterol, free fatty acid, hepatic free fatty acid and urinary protein excretion in the NS and control (CTL) groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>95.5 ± 5.56</td>
<td>496.6 ± 28.3a</td>
</tr>
<tr>
<td>Free cholesterol, mg/dL</td>
<td>43.4 ± 5.56</td>
<td>217.6 ± 38.3a</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>46.3 ± 6.07</td>
<td>243.6 ± 7.99a</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>36.0 ± 3.74</td>
<td>169.8 ± 19.97a</td>
</tr>
<tr>
<td>Total HDL cholesterol ratio</td>
<td>2.72 ± 0.26</td>
<td>3.02 ± 0.22</td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>65.7 ± 8.73</td>
<td>416.1 ± 73.82a</td>
</tr>
<tr>
<td>Plasma albumin, g/dL</td>
<td>3.54 ± 0.09</td>
<td>1.89 ± 0.13a</td>
</tr>
<tr>
<td>Plasma creatinine, mg/dL</td>
<td>0.75 ± 0.04</td>
<td>0.74 ± 0.06</td>
</tr>
<tr>
<td>Creatinine clearance, mL/min</td>
<td>1.24 ± 0.16</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>Urine protein, mg/day</td>
<td>68.3 ± 12.66</td>
<td>646.5 ± 70.79a</td>
</tr>
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*P < 0.005 versus CTL.

![Fig. 1](https://academic.oup.com/ndt/article-abstract/26/10/3118/1905384)

Fig. 1. (A) Bar graphs depicting SR-BI/beta actin mRNA ratio in hepatic tissues of nephrotic and control rats. (B) Representative western blots and group data depicting protein abundance of hepatic tissue SR-BI/beta actin protein ratio in the NS and control (CTL) groups. n = 5 in each group. *P < 0.03.

![PDZK1](https://academic.oup.com/ndt/article-abstract/26/10/3118/1905384)

Fig. 2. Representative photomicrographs depicting PDZK1 immunostaining in the liver tissue of a NS compared to that of a control (CTL) rat, confirming the result obtained by western blot analysis.
performed on the frozen liver tissue sections (Figure 4). A direct correlation was found between SR-BI and PDZK1 protein abundance in the study groups.

**ATP synthase beta chain data**

In contrast to SR-BI and PDZK1, protein abundance of the beta chain of ATP synthase in the hepatic tissue was significantly increased in the nephrotic rats compared with the normal control group. This was accompanied by a parallel increase in the ATP synthase beta chain mRNA abundance in the liver of NS animals (Figure 5). The upregulation of beta chain ATP synthase mRNA and protein abundance was confirmed by immunohistological analysis performed on the frozen liver tissue sections (Figure 6).

**Discussion**

NS results in profound dysregulation of lipid metabolism which is marked by hypercholesterolemia, hypertriglyceridemia, elevated plasma LDL and lipoprotein (a) [LP(a)], impaired clearance of very low-density lipoprotein (VLDL) and chylomicrons and accumulation of their atherogenic remnants in the plasma [3, 20–23]. In addition, NS significantly increases the ratios of cholesterol-to-triglycerides, free cholesterol-to-cholesterol esters and phospholipids to proteins in all lipoprotein fractions [22]. Elevation of plasma cholesterol and LDL levels in NS is primarily caused by increased biosynthesis and reduced catabolism of Apo B and cholesterol [23–25]. These events are occasioned by upregulations of hepatic Apo B [23], 3-hydroxy-3-methylglutaryl-coenzyme A reductase [26, 27] and acyl-CoA cholesterolacyltransferase [ACAT-2] [28] coupled with reduced catabolism caused by acquired LDL receptor deficiency [29] and inappropriately low cholesterol to bile acids conversion capacity [30].

Hypertriglyceridemia in NS is largely due to impaired clearance of VLDL and chylomicrons and accumulation of their atherogenic remnants in the plasma [23–25]. These events are primarily caused by downregulation of adipose tissue and skeletal muscle lipoprotein lipase and VLDL receptor [31, 32], hepatic lipase [33] and cholesteryl ester acyltransferase [34]. In addition, NS results in upregulation of hepatic diacylglycerol acyltransferase [35], which is the rate-limiting enzyme in triglyceride biosynthesis pointing to increased endogenous triglyceride production capacity. Together, these alterations in lipid and lipoprotein metabolism result in a highly atherogenic plasma lipid profile in NS.

Via its central role in reverse cholesterol transport and its antioxidant, anti-inflammatory and antithrombotic actions, HDL confers protection against atherosclerosis and cardiovascular disease [4, 5]. NS adversely affects HDL metabolism in ways that limits reverse cholesterol transport. For instance, NS results in significant urinary losses of lecithin-cholesterol aclytransferase and consequent reduction of plasma LCAT concentration [36]. The associated LCAT deficiency, in turn, limits transformation of cholesterol ester-poor HDL to cholesterol ester-rich HDL-2 particle. The effect of LCAT deficiency in NS is compounded by upregulation of ACAT [28, 37], which limits free cholesterol efflux in peripheral tissues for uptake by HDL. The final step in reverse cholesterol transport is disposal of the
HDL’s lipid cargo in the liver. As noted in the introduction, SR-BI is the principal portal for unloading of the HDL lipid cargo in the liver. Previous studies by our group revealed marked reduction of SR-BI protein abundance despite normal SR-BI mRNA expression. Given the critical role of PDZK1 in stability of SR-BI, this study was undertaken to determine if the observed disparity in hepatic SR-BI protein and mRNA expression may be due to downregulation of PDZK1. In confirmation of our earlier study, we found significant reduction of SR-BI protein but not mRNA abundance in liver tissue of the nephrotic animals compared to the normal control group. This was associated with a significant reduction of PDZK protein and mRNA abundance in the liver of nephrotic animals. In view of the critical role of PDZK1 in stability of SR-BI in hepatocytes, the reduction of PDZK1 abundance accounts for the reduction of hepatic SR-BI abundance in the nephrotic liver tissue.

Nakamura et al. have shown that C-terminal region of PDZK1 is essential for upregulating SR-BI protein expression. They have further demonstrated that PDZK1 is phosphorylated at Ser-509 via a cyclic adenosine monophosphate-dependent protein kinase A and that phosphorylation of PDZK1 plays an important part in the regulation of hepatic SR-BI expression [38]. Due to the lack of commercially available antibody to phosphorylated PDZK1, we were not able to determine the effect of NS on PDZK1 phosphorylation. Further studies are required to explore this issue.

To further explore the effect of nephrotic proteinuria on HDL metabolism, we examined the effect of NS on hepatic expression of the HDL endocytic receptor, beta chain ATP synthase. The study revealed significant upregulation of this endocytic receptor in the nephrotic liver. As noted above, unlike SR-BI which serves as the docking receptor for cholesterol-rich HDL-2 particles, beta chain ATP synthase mediates removal and catabolism of apo A-1 and lipid-poor HDL particles. The negative impact of NS on HDL maturation occasioned by LCAT deficiency and upregulation of ACAT can render the circulating HDL particles increasingly susceptible for uptake and degradation by the endocytic receptor which can further compromise reverse cholesterol transport.

In conclusion, NS results in elevation of hepatic HDL endocytic receptor and deficiency of HDL docking receptor. The latter is associated with and, in part, mediated by downregulation of PDZK1. Together, these abnormalities can increase catabolism and diminish recycling of HDL and contribute to the defective reverse cholesterol/lipid transport in NS.

Conflict of interest statement. None declared.

References

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