Effect of nephrotic syndrome on homocysteine metabolism

Mohammad A. Aminzadeh, Pavan Gollapudi and Nosratola D. Vaziri

Division of Nephrology and Hypertension, University of California, Irvine, CA, USA

Correspondence and offprint requests to: Nosratola D. Vaziri; E-mail: ndvaziri@uci.edu

Abstract

Background. Proteinuria and hyperhomocysteinaemia are independently associated with increased risk of atherosclerosis and cardiovascular disease. The available data on plasma homocysteine (Hcy) level in patients with nephrotic syndrome (NS) are contradictory with increased, decreased and unchanged values reported by different investigators. The majority of Hcy in the plasma is bound to albumin and unchanged values reported by different investigators. The present study was designed to explore the effect of NS on plasma concentration and urinary excretion of Hcy and hepatic expression of methylenetetrahydrofolate reductase (MTHFR) and cystathionine-β-synthase (CBS), the key enzymes in remethylation and trans-sulphuration of Hcy, respectively.

Methods. Sprague–Dawley rats were rendered nephrotic by IP injection of puromycin aminonucleoside. Urine and plasma were used for measurement of Hcy, and the liver was processed for assessment of MTHFR and CBS protein expression.

Results. Compared with the controls, nephrotic rats showed heavy proteinuria, hypalbuminaemia, hypercholesterolaemia, normal plasma creatinine and creatinine clearance, reduced plasma Hcy, increased urinary Hcy, and downregulation of CBS but not MTHFR expression. Plasma Hcy correlated directly with plasma albumin and inversely with urinary protein excretion. The urinary Hcy excretion correlated directly with urine protein excretion.

Conclusions. NS results in significant reduction in plasma total Hcy concentration which is due to the reduction in albumin-bound Hcy as opposed to the free Hcy fraction. This is coupled with increased urinary excretion of albumin-bound Hcy. In addition, NS results in down-regulation of CBS which can curtail conversion of Hcy to cysteine and reduce production of H2S which is an important endogenous signalling molecule.

Keywords: atherosclerosis; cardiovascular disease; chronic kidney disease; inflammation; proteinuria

Introduction

Heavy glomerular proteinuria, commonly known as nephrotic syndrome (NS), is associated with increased risk of atherosclerosis and cardiovascular disease [1–3]. Similarly, elevation of plasma homocysteine (Hcy) is independently associated with adverse cardiovascular and non-cardiovascular outcomes [4–10]. For these reasons, several studies have sought to determine plasma Hcy concentration in patients with NS. However, the results of these studies have been contradictory. While some studies have reported elevated plasma Hcy levels in NS patients [11,12], others have shown normal [13] or reduced [14,15] values in this population. The reason for the difference in the results of the reported studies is uncertain. However, it may be due to differences in the magnitude and the underlying causes of proteinuria, severity of hypalbuminaemia, or concomitant renal insufficiency [7,16,17] which can potentially impact Hcy metabolism and its plasma concentration.

In the plasma, the majority of Hcy is bound to albumin, and only a small fraction is present in free form [18]. Moreover, under normal conditions, 99% of filtered free Hcy is reabsorbed in the proximal tubules [19]. Thus, under physiological conditions, disposal of Hcy primarily depends on its intracellular metabolism [20], and contribution of the kidney to this process is relatively small. However, heavy losses of albumin in the urine and the consequent fall of the albumin concentration in the plasma can profoundly affect plasma concentration and metabolism of Hcy. The present study was designed to test the hypothesis that severe albuminuria and the associated hypalbuminaemia may lead to a fall in plasma level and a rise in urine excretion of Hcy. The study further sought to determine the effect of nephrotic syndrome on the key enzymes involved in Hcy metabolism.

Materials and methods

Study groups

Male Sprague–Dawley rats weighing 180–200 g were housed in a temperature- and light-controlled space with 12-h light (500 lx) and dark (≤5 lx) cycles. The rats were allowed free access to food (Purina Rat Chow, Purina Mills Inc., Brentwood, MO, USA) and water. The animals were randomized into the nephrotic (NS) and control groups. The rats assigned to the NS group received sequential intraperitoneal injections of puromycin aminonucleoside (PAN) on Day 1 (130 mg/kg) and Day 14 (60 mg/kg). The control group received placebo injections of 5% dextrose in water instead. Thirty days after the initial PAN or placebo injections, the animals (n = 5 per group) were placed in individual metabolic cages for a 24-h urine...
collection. Food intake was monitored during the last week of the observation period and was found to be comparable in the two groups. At the conclusion of the observation period, under general anaesthesia, the animals were euthanized by exsanguination using cardiac puncture, and plasma and the liver were harvested. The tissue was immediately cleaned, then frozen in liquid nitrogen and stored at −70°C until processed. Plasma albumin, urinary protein excretion, plasma and urine concentrations of creatinine, plasma free and total cholesterol, LDL cholesterol, and triglyceride were measured using standard laboratory methods.

Western blot analysis

The frozen liver tissue was processed for determination of methylenetetrahydrofolate reductase (MTHFR) and cystathionine-β-synthase (CBS) abundance using antibodies against MTHFR (Santa Cruz Biotechnology Inc., CA, USA) and CBS (Santa Cruz Biotechnology Inc.). Briefly, the liver tissue was homogenized (25% wt/vol) in 10 mmol/L HEPES buffer, pH 7.4, containing 320 mmol/L sucrose, 1 mmol/L EDTA, 1 mmol/L DTT, 10 mg/mL leupeptin and 2 mg/mL aprotinin at 0–4°C with a tissue grinder fitted with a motor-driven ground glass pestle. Homogenates were centrifuged at 12 000 g for 5 min at 4°C to remove tissue debris. The supernatant was used for determination of MTHFR and CBS proteins by western blot analysis. The tissue extracts (75 μg protein) were size-fractionated on 4–12% Tris–glycine gel (Novex, Inc., San Diego, CA, USA) at 120 V for 3 h. After electrophoresis, the proteins were transferred onto Hybond ECL membrane (Amersham Life Science Inc., Arlington Heights, IL, USA) at 120 V for 3 h. The membrane was incubated for 1 h in blocking buffer (1× TBS, 0.05% Tween-20 and 3% non-fat milk) and then overnight in the same buffer containing the given antibodies. The membrane was washed three times for 5 min in 1× TBS with 0.05% Tween-20 prior to 2-h incubation in a buffer (1× TBS, 0.05% Tween-20 and 3% non-fat milk) containing horseradish peroxidase-linked anti-rabbit IgG and anti-goat IgG (Amersham Life Science Inc.) at 1:1000 dilution. The membrane was washed four times and developed by autoradiography using the ECL chemiluminescent agents (Amersham Life Science Inc.). The data were normalized against beta-actin which was used as a housekeeping protein.

Homocysteine assays

Plasma Hcy was measured by chemiluminescence technique using Immulite 2500 Homocysteine Diagnostic Test Kit (Diagnostic Products Corporation, Los Angeles, CA, USA) following manufacturer’s specification. Urine Hcy level was measured by liquid chromatography–mass spectrometry as described by Magera et al. [21].

Data analysis

Student’s t-test and regression analysis were used in statistical analysis of the data which are expressed as mean ± SE. P-values <0.05 were considered significant.

Results

General data

The data are shown in Table 1. The nephrotic group exhibited severe proteinuria, hypoalbuminaemia, and elevation of plasma concentrations of free and total cholesterol, LDL cholesterol and triglyceride. However, plasma creatinine concentration and creatinine clearance were unchanged in the nephrotic animals.

Plasma and urine homocysteine levels

The data are illustrated in Figure 1. Plasma Hcy concentration was significantly lower in the NS group than that found in the control rats. In contrast, urine Hcy excretion was markedly higher in the nephrotic group compared with that found in the control group. Plasma Hcy concentration in the study animals was directly related to plasma albumin level ($r = 0.770$, $P = 0.009$) and inversely related to urinary protein excretion ($r = -0.789$, $P = 0.02$). Urine Hcy excretion was directly related to the urine protein excretion ($r = 0.979$, $P < 0.001$).

Liver MTHFR and CBS data

The data are illustrated in Figures 2 and 3. CBS protein abundance was significantly reduced in the liver of the

Table 1. Plasma concentrations of cholesterols, albumin, creatinine and triglyceride, creatinine clearance (Ccr), and body weight in the nephrotic (NS) and control (CTL) groups

<table>
<thead>
<tr>
<th></th>
<th>CTL (n = 8)</th>
<th>NS (n = 8)</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Plasma total cholesterol, mg/dL</td>
<td>95.50 ± 8.10</td>
<td>496.68 ± 28.62</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Plasma free cholesterol, mg/dL</td>
<td>43.44 ± 5.56</td>
<td>217.58 ± 28.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Plasma LDL cholesterol, mg/dL</td>
<td>46.31 ± 6.07</td>
<td>243.62 ± 7.99</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Plasma triglyceride, mg/dL</td>
<td>65.72 ± 8.72</td>
<td>416.20 ± 73.81</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Plasma albumin, g/dL</td>
<td>3.35 ± 0.08</td>
<td>1.89 ± 0.13</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Plasma creatinine, mg/dL</td>
<td>0.75 ± 0.04</td>
<td>0.74 ± 0.08</td>
<td>Not significant</td>
</tr>
<tr>
<td>Ccr, mL/min</td>
<td>1.42 ± 0.21</td>
<td>1.36 ± 0.22</td>
<td>Not significant</td>
</tr>
<tr>
<td>Urine protein, mg/24 h</td>
<td>56.85 ± 6.75</td>
<td>691.87 ± 70.25</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>357.00 ± 7.36</td>
<td>265.00 ± 18.52</td>
<td>&lt;0.05</td>
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</tbody>
</table>

Values are presented as mean ± SE.

Fig. 1. Bar graphs depicting plasma concentration (micromole per litre) and urinary excretion (micromole per 24 h) of homocysteine in the nephrotic (NS) and normal control (CTL) groups ($n = 5$ in each group; *$P < 0.05$).
NS rats as compared with that found in the control group. No significant difference was found in hepatic tissue MTHFR protein expression between the nephrotic and control rats.

Discussion

Heavy proteinuria and hypoalbuminaemia in the nephrotic animals employed in this study were accompanied by significant reduction in plasma Hcy concentration and a marked increase of urinary Hcy concentration. This is not surprising since the bulk of Hcy in the plasma is bound to albumin [18,22,23]. Consequently, the reduction in serum albumin can account for the reduction in plasma Hcy in the nephrotic animals. Likewise, the elevation of urinary Hcy excretion can be explained by the presence of albumin-bound Hcy in the urine. Unlike the filtered free Hcy which is almost completely reabsorbed in proximal tubules, albumin-bound Hcy can escape tubular reabsorption and appear in the final urine as seen in our NS animals. It should be noted that the reduction in plasma Hcy concentration in NS should not be necessarily viewed as a favourable marker. This is because the reduction in plasma Hcy in NS is primarily due to diminished albumin-bound Hcy as opposed to the free Hcy fraction. In a large cross-sectional study of patients with type 2 diabetes and chronic kidney disease, Friedman et al. [16] found a weak association between serum Hcy and albumin concentrations but not with urine protein excretion. However, mean serum albumin concentration was nearly normal, urinary albumin excretion was relatively mild and renal function was significantly impaired in the study population.

Hcy is converted to methionine by re-methylation and to cysteine via trans-sulphuration. These reactions, which represent key pathways for disposal of Hcy, are catalysed by MTHFR and CBS, respectively. The NS animals employed in the present study exhibited significant down-regulation of CBS expression. This observation points to diminished Hcy metabolism through trans-sulphuration pathway and consequent impairment of Hcy to cysteine conversion. It is of note that CBS is one of the enzymes that catalyse biosynthesis of endogenous hydrogen sulphide (H₂S). Endogenous H₂S is a recently recognized gaseous mediator with diverse biological effects which include regulation of cardiovascular and neurological functions, modulation of inflammatory response and anti-atherogenic and antioxidant actions among others [24]. Thus, in addition to its impact on Hcy metabolism, down-regulation of CBS may contribute to adverse cardiovascular and other consequences of NS by limiting endogenous production of H₂S. Further studies are needed to explore this possibility.

In conclusion, heavy proteinuria results in significant reduction in plasma Hcy concentration which is due to diminished protein-bound fraction as opposed to its free fraction. This is associated with increased urinary Hcy excretion which is due to the loss of albumin-bound Hcy. In addition, NS results in down-regulation of CBS which can simultaneously reduce trans-sulphuration of Hcy and lower the endogenous production of H₂S.

Conflict of interest statement. None declared.

References

Normoalbuminaemia and IgA nephropathy

Min Chen¹,²,³, Fu-de Zhou¹,²,³, Ming-hui Zhao¹,²,³ and Hai-yan Wang¹,²,³

¹Renal Division, Department of Medicine, Peking University First Hospital, Beijing 100034, China, ²Institute of Nephrology, Peking University, Beijing 100034, China and ³Key Laboratory of Renal Disease, Ministry of Health of China, Beijing 100034, China

Correspondence and offprint requests to: Fu-de Zhou; E-mail: zhoufude1801@vip.sina.com

Abstract

Background. Massive proteinuria is often associated with hypoalbuminaemia in glomerulopathy. However, patients may have normal levels of serum albumin despite heavy proteinuria in many circumstances. This study analysed factors affecting serum levels of albumin in primary glomerulopathy patients with nephrotic-range proteinuria.

Methods. The renal histopathological data of 780 consecutive adult patients (age ≥ 18 years old) with primary glomerulopathy and nephrotic-range proteinuria, who received native renal biopsies in Peking University First Hospital from 1998 to 2007, were retrospectively analysed.

Results. Compared with patients with hypoalbuminaemia (serum albumin < 30 g/L), patients without hypoalbumi-