Mild hyperhomocysteinemia promotes renal hemodynamic dysfunction without histopathologic changes in adult rats

GEORGINA P. OSSANI, PATRICIA A. FISCHER, SILVIA G. CARAM, GRACIELA N. DOMINGUEZ, ALBERTO J. MONSERRAT, and LUCAS D. MASNATTA

Patología Experimental, Departamento de Patología, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina; Departamento de Fisiología, Farmacología y Bioquímica, Universidad Favaloro, Argentina; and Unidad de Farmacología Clínica, Sexta Cátedra de Medicina, Hospital de Clínicas José de San Martín, Universidad de Buenos Aires, Buenos Aires, Argentina

Mild hyperhomocysteinemia promotes renal hemodynamic dysfunction without histopathologic changes in adult rats.

Background. Hyperhomocysteinemia is able to promote glomerular damage and generate tubulointerstitial lesions. These findings were reported in rats with unilateral nephrectomy or in weanling rats with normal function, two experimental models that are exposed to other concomitant vascular risk factors. The aim of this work is to study whether mild hyperhomocysteinemia per se can induce renal histopathologic changes in adult rats with normal renal function at either 10 or 44 weeks of hyperhomocysteinemia.

Methods. Two months old male Wistar rats (N = 52) were randomly allocated to either a normal control (N = 26) or hyperhomocysteinemic (N = 26) group. Control and hyperhomocysteinemic groups had free access to either tap water or homocysteine thiolactone 50 mg/kg/day, during 10 or 44 weeks. Plasma homocysteine levels were determined by a high-performance liquid chromatography (HPLC) method. Glomerular filtration rate (GFR) and renal plasma flow (RPF) were calculated from inulin and sodium para-aminohippurate (PAH) clearance determinations. Structural renal changes were investigated in kidneys fixed by perfusion. Histopathologic and morphometric analysis were carried out by standard methods.

Results. Plasma total homocysteine levels were 53% (10 weeks) and 56% (44 weeks) higher in hyperhomocysteinemic group compared to the control group. GFR and RPF were significantly lower in hyperhomocysteinemic than in control group. The histopathologic and morphometric studies did not show any differences between the control and hyperhomocysteinemic rats at 10 or 44 weeks.

Conclusion. The present results show that mild hyperhomocysteinemia is able to induce renal functional and biochemical alterations in male adult rats that are not associated with renal histopathologic changes.

Key words: mild hyperhomocysteinemia, kidney.

Received for publication April 12, 2004 and in revised form May 10, 2004
Accepted for publication May 27, 2004

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In the normal kidney, nitric oxide plays a key role in the homeostatic regulation of vascular, glomerular, and tubular function maintaining normal renal perfusion, glomerular filtration rate (GFR), and renal vascular resistance (RVR) [1, 2].

Increased superoxide anion radical (•O2−) production inactivates the release of nitric oxide and reduces its bioavailability by peroxynitrite (OONO−) formation. Thus, a greater nitric oxide inactivation may reduce medullar blood flow, contributing to the development of renal failure [3].

Homocysteine is a thiol-containing amino acid. Homocysteine goes through the trans-sulphuration pathway which requires vitamin B6 as a cofactor to produce cystathionine, cysteine, and glutathione or is methylated to methionine taking a methyl group from choline-derived betaine (betaine methyl transferase) or 5-methyl-tetrahydrofolic acid (methionine sintetase, which needs vitamin B12 as a cofactor) (Fig. 1) [4, 5].

Different studies have shown that hyperhomocysteinemia is associated with increased risk of vascular disease involving coronary, cerebral, and peripheral arteries [6, 7]. Depending on the definition of hyperhomocysteinemia, 10% to 20% of the general population, more than 85% of patients with end-stage kidney disease (ESRD) [8, 9] and up to 32% of the individuals having premature peripheral arterial disease [10] have increased homocysteine levels. On the other hand, hyperhomocysteinemia is a frequent finding in heart, liver, and renal transplant recipients and is associated with renal dysfunction, even though the etiologic factors have not been clearly identified [11–13].

We recently studied the impact of increased level of homocysteine on renal function showing that hyperhomocysteinemia induces renal oxidative stress and renal hemodynamic dysfunction involving altered L-arg-nitric oxide pathway [14]. In addition, it has been found that hyperhomocysteinemia is also able to promote glomerular damage [15] or generate tubulointerstitial lesions [16].
However, these morphologic findings were reported in rats with unilateral nephrectomy or in weanling rats, two experimental models that are exposed to other concomitant vascular risk factors. Therefore, the aim of this work is to study whether mild hyperhomocysteinemia per se can induce renal histopathologic changes in adult Wistar rats with normal renal function at either 10 or 44 weeks of treatment.

METHODS

Animals

Two-month-old male Wistar rats ($N = 52$) (180 to 220 g) were used. The animals were maintained on a standard rat chow (Argentine Cooperative Association, Animal Nutritional Division) and tap water. They were housed under a 12/12-hour day/night cycle at a steady temperature of 25°C. After 1 week of acclimatization, they were randomly allocated to either a normal control ($N = 26$) or hyperhomocysteinemic ($N = 26$) group. Control and hyperhomocysteinemic groups had free access during 10 or 44 weeks to either tap water or homocysteine thiolactone (HTL) (50 mg/kg/day) respectively [17].

Plasma homocysteine levels

Femoral arterial blood samples (200 μL) were withdrawn at baseline, 10 or 44 weeks and immediately cooled in Eppendorf tubes containing 0.1% ethylenediaminetetraacetic acid (EDTA). Plasma was separated by centrifugation at 4°C and immediately frozen at −60°C until the day of analysis. Total plasma homocysteine levels were determined using the Bio-Rad high-performance liquid chromatography (HPLC) kit (Bio-Rad, Hercules, CA, USA) [18].

Renal hemodynamic function determination

Hemodynamic studies were performed in conscious unrestrained rats as previously described [19]. Briefly, at 10 and 44 weeks, 6 animals of each group (control and hyperhomocysteinemic) were anesthetized to cannulate both the femoral artery and the femoral vein. After recovery from surgery and in euvoletic conditions, a priming dose of inulin (16 mg/kg) and sodium para-aminohippurate (PAH) (8 mg/kg) were administered. Immediately after, a continuous intravenous infusion of 0.9% NaCl containing inulin (36 mg/mL) and PAH (11.6 mg/mL) was given at a rate of 0.0267 mL/min. After a 105-minute equilibration period, baseline arterial blood samples were taken.

Blood samples were used for hematocrit, inulin, and PAH determinations. Clearances of inulin (GFR) and PAH [renal plasma flow (RPF)] were calculated as previously described [20, 21]. RVR were calculated from mean arterial pressure (MAP) and renal blood flow (RBF) according to: $RVR = MAP/RBF$ (mm Hg × min × 100 g body weight/mL); $RBF = RPF/(1-hematocrit/100)$ (mL/min × 100 g body weight) [22].

Blood pressure and heart rate were monitored throughout the study via an arterial catheter connected to a Statham pressure transducer (Gould Instrument, Cleveland, OH, USA) and attached to a polygraph recorder (model 2400S) (Gould Instrument).
Table 1. Results of the morphologic studies

<table>
<thead>
<tr>
<th>Time weeks</th>
<th>Number Group</th>
<th>Glomerular area μm²</th>
<th>Mesangial expansion</th>
<th>Glomerulosclerosis</th>
<th>Interstitial fibrosis</th>
<th>Anti-a-smooth muscle actin</th>
<th>Vessels</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Control</td>
<td>7221 ± 321</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NEMA</td>
</tr>
<tr>
<td>10</td>
<td>Hyperhomocysteinemia</td>
<td>6489 ± 302</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NEMA</td>
</tr>
<tr>
<td>44</td>
<td>Control</td>
<td>7101 ± 299</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NEMA</td>
</tr>
<tr>
<td>44</td>
<td>Hyperhomocysteinemia</td>
<td>8462 ± 325</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NEMA</td>
</tr>
</tbody>
</table>

NEMA is not evident microscopic alterations.
Mean ± SE.

Histopathologic examination

Kidneys of 28 rats (Table 1) were fixed by perfusion [23] with 4% paraformaldehyde in 0.135 mol/L phosphate buffer, embedded in paraffin, and sections were stained with hematoxylin-eosin, periodic acid-Schiff (PAS), Masson’s trichrome, and Gallego (elastic fibers) following standard methods [24] for histopathologic and morphometric studies.

The presence of myofibroblasts was evaluated with immunomarcation against α-smooth muscle actin (α-SMA) (Sigma Chemical Co., St. Louis, MO, USA).

Glomerular measurements (Image Pro Plus program) were taken in each left kidney in 50 subcapsular, medium and yuxtamedullary glomeruli, in PAS-stained sections. Mesangial expansion and glomerular sclerosis were evaluated following the method of Raij et al [25], whereas interstitial fibrosis was estimated according to the method of Shih et al [26].

All these assessments were carried out under blind conditions.

Plasma homocysteine levels

Basal plasma homocysteine concentration did not differ between the two groups. After HTL administration, total plasma homocysteine levels were 53% (10 weeks) and 56% (44 weeks) higher in hyperhomocysteinemic group compared to the control group (8.11 ± 1.5 and 8.27 ± 1.6 μmol/L vs. 5.3 ± 0.8 μmol/L, respectively, P < 0.001).

Renal hemodynamic function determination

Under baseline conditions, MAP and RVR were significantly higher in the hyperhomocysteinemic group than in controls (Fig. 2A and D). Conversely, GFR (Fig. 2B) and RPF (Fig. 2C) were significantly lower in the hyperhomocysteinemic than in the control group.

Histopathologic evaluation

The histopathologic studies did not show any differences between the control and hyperhomocysteinemic rats at either 10 or 44 weeks (Figs. 3 and 4).

No renal vascular changes were observed either in the hyperhomocysteinemic or in the control rats.

DISCUSSION

The present study examined chronic effects of mild hyperhomocysteinemia on renal function and morphology in Wistar adults rats whose plasma homocysteine levels were increased by water supplementation of homocysteine thiolactone (50 mg/kg/day). Despite the fact that mild hyperhomocysteinemia induces renal functional and biochemical alterations and increases systemic blood
pressure, they are not associated with renal morphologic changes as evaluated by light microscopy at 10 or at 44 weeks of treatment.

Homocysteine is formed by the demethylation of methionine. Increased plasma homocysteine levels can occur as a genetic disease or as a consequence of a nutritional deficiency in those factors that promote homocysteine metabolism such as folic acid, vitamin B12, vitamin B6, and betaine (Fig. 1) [4, 5, 27–29]. Another major acquired cause of increased homocysteine values is chronic renal failure [30].

Homocysteine-induced oxidant injury of vascular endothelium has been proposed as a more contributing mechanism linking hyperhomocysteinemia to vascular disease. Numerous studies have suggested that the increased oxidative stress promoted by hyperhomocysteinemia is able to reduce nitric oxide bioavailability [31], increase vascular tone [32], and alter elastic properties of the vascular wall [33]. By this way, hyperhomocysteinemia is an important risk factor for cardiovascular disease development.

Recent investigations suggest that oxygen radicals may contribute to enhanced renal vascular tone, increased sensitivity to vasoconstrictors and impaired renal endothelium vasodilatation [34–36]. Hyperhomocysteinemia promotes the reaction between increased level of superoxide anion and nitric oxide, inducing peroxynitrite formation which, in turn, can nitrosylate...
membrane proteins and oxidized arachidonic acid, releasing F2-isoprostanes. These are potent renal vasoconstrictors, leading to a fall in glomerular capillary pressure and causing a marked reduction of GFR and RBF [37–39]. This related linear increase of F2-isoprostanes, at elevated plasma homocysteine levels [40] might be involved on homocysteine-induced renal deleterious function [14, 15] and could be an important pathogenic factor in glomerular damage associated with hypertension [41].

Structural alterations due to hyperhomocysteinemia have also been previously reported [16, 41]. Li et al [41] used uninephrectomized male Sprague-Dawley rats which, after 1 week recovery of the surgery, received methionine (1 g/kg per day for 6 weeks, orally). At the end of the experiment, homocysteine levels (≥12 μmol/L vs. 6 μmol/L) as well as proteinuria (≥60 mg/24 hours vs. ≥30 mg/24 hours) were higher in these treated rats. In addition, hyperhomocysteinemic rats showed increased glomerular extracellular matrix and expanded the glomerular mesangium with hypercellularity, capillary collapse, and fibrous deposition in the glomeruli. The glomerular injury score was higher than in control rats (3 vs. 1). In the same paper the authors showed that hyperhomocysteinemia is able to induce proteinuria and glomerular sclerosis in Dahl-sensitive hypertensive rats exposed to high salt (4% NaCl) diet [41].

Kumagai et al [16] studied the effects of folate deficiency, choline deficiency, and methionine loading on homocysteinemia, renal function, and renal histopathology on weanling male Fisher rats over a 12-week period. Folate deficiency, choline deficiency, and methionine loading (1.25% supplementation) synergistically induced hyperhomocysteinemia (up to 69.7 μmol/L) without any change in blood pressure. A negative correlation was found between creatinine clearance and homocysteine. The kidneys of hyperhomocysteinemic rats showed arterial and arteriolar wall thickening and focal tubulointerstitial fibrosis. The lesions of interstitial fibrosis appear wedge-shaped at the subcapsular cortex of the kidney; the expression of vascular endothelial growth factor was increased in the adjacent more intact area of the cortex.

Another important consideration could be the variability of the renal response to different types of injury depending on the animal age or renal growth. Thus, while a choline-deficient diet is able to induce acute renal failure with tubular or cortical necrosis in weanling rats [42], the same diet do not develops renal necrosis in adults unless it is submitted at the compensatory renal hypertrophy period after unilateral nephrectomy [46, 47]. In the same line, both in rats and human beings, the glomerular morphologic response to a diminished number of glomeruli induces more deleterious consequences when the loss of nephrons take place at an earlier period in life [48, 49]. With regard to this issue, Kumagai et al [16] highlighted that “our preliminary experiment with older rats failed to induce such severe renal injury, although the plasma homocysteine level of these rats reached the same level as that in the present study.”

CONCLUSION

Our results show that in adult rats with normal kidney function, mild hyperhomocysteinemia induces renal hemodynamic dysfunction and increased systemic blood pressure. However, we have found no differences be-
between cells expressing α-SMA (myofibroblasts) in the glomeruli or in the renal interstitium from controls or hyperhomocysteinemic rats. The present cells have been proved to be a good predictor of renal disease progression both in experimental animal [50] and human studies [51], supporting that no morphologic findings related to glomerular or interstitial sclerosis or fibrosis are induced by mild hyperhomocysteinemia if the animals are not exposed to other concomitant vascular risk factors. Taking into account that we have recently proved that antioxidant treatment may revert homocysteine-induced renal functional and biochemical deleterious effects, the present results may contribute to the treatment of incipient or moderate increments of homocysteine plasma levels before the development of structural alterations, preventing later vascular complications and reducing the cardiovascular risk.

ACKNOWLEDGMENTS

Alberto J. Monserrat is a member of the Research Career of the National Council of Research of Argentina (CONICET). This study is partially supported by grants from the University of Buenos Aires and CONICET.

Reprint requests to Georgina Ossani, J.E. Uriburu 950 5th Floor, CP 1114, Capital Federal, Buenos Aires, Argentina.
E-mail: georginaossani@yahoo.com.ar

REFERENCES

15. CHEN YF, LI PL, ZOU AP: Effect of hyperhomocysteinaemia on plasma or tissue adenosine levels and renal function. Circulation 106:1275–1281, 2002
38. TAKARASHI K, NAMBOUR TK, FUKUMAGA M, et al: Glomerular ac-


