Sustained Plasma Fibrinogen Elevation in Subtotal Nephrectomized Rats: Effect of Cilazapril, an Angiotensin-Converting Enzyme Inhibitor

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Abstract. The present study was undertaken to examine whether plasma fibrinogen persistently elevates in subtotal nephrectomized rats, an animal model with inflammatory renal changes. Eight weeks after the induction of 5/6 nephrectomy in male Wistar rats, plasma fibrinogen concentration was determined for the next 12 weeks in the animals received vehicle or an angiotensin-converting enzyme inhibitor, cilazapril (1 or 10 mg/kg per day) orally. In the vehicle-treated nephrectomized rats, plasma fibrinogen concentration significantly (P<0.001) increased (from 127.3 ± 4.6 [S.E.M.] to 182.3 ± 5.2 mg/dL) compared with that in the control rats (from 118.0 ± 2.0 to 153.5 ± 5.4 mg/dL). Cilazapril attenuated the increases in plasma fibrinogen concentration in a dose-dependent manner. Serum concentration of monocyte chemoattractant protein-1, a key macrophage chemoattractant and activator, increased in the vehicle-treated nephrectomized rats, which was also reduced by cilazapril. These results suggest that plasma fibrinogen elevates persistently in the nephrectomized rats. Local inflammation may be involved in the hepatic fibrinogen synthesis in this model.

Keywords: nephrectomized rat, cilazapril, fibrinogen, monocyte chemoattractant protein-1, inflammation

Introduction

A number of investigations revealed that elevated fibrinogen is one of the independent risk factors for cardiovascular disorders (1). The precise mechanism of an elevation of plasma fibrinogen is not fully understood in these patients, but inflammation is speculated to be involved (2, 3). In addition, epidemiological studies showed that the higher plasma fibrinogen is often associated with other cardiovascular risk factors such as advanced age, smoking, hypertension, diabetes mellitus, obesity, and hyperlipidemia (4–9). The combination of these risk factors enhances the chance for developing cardiovascular diseases. Therefore, agents with a fibrinogen-lowering effect would have merit for preventing these events.

To evaluate the effect of an agent on plasma fibrinogen, an animal model with a sustained elevation of this variable is useful. Kawabata (10) showed that the inhibition of nitric oxide synthesis by L-N(G)-nitroarginine methyl ester (L-NAME) elevates plasma fibrinogen in rats. However, we recently observed that hyperfibrinogenemia induced by L-NAME was transient and did not persist for more than 2 weeks in rats (11).

Previous studies showed that inflammatory cells including macrophage accumulate and proliferate within renal tissues in rats with reduced renal mass, a model of progressive renal failure. As the existence of inflammation contributes to the elevation of fibrinogen (12), it is anticipated that local inflammation in remnant renal tissues stimulates the synthesis of fibrinogen in the liver and increases plasma fibrinogen concentration in subtotal nephrectomized rat. We thus determined plasma fibrinogen concentration and serum concentration of monocyte chemoattractant protein-1 (MCP-1), a key macrophage chemoattractant and activator, in 5/6 nephrectomized rats. Blockade of angiotensin II (Ang II) action by an angiotensin-converting enzyme (ACE) inhibitor leads to a marked inhibition of macrophage proliferation in renal tissues in rats with 5/6 nephrec-
tony (13), which indicates that an ACE inhibitor could be effective for reducing plasma fibrinogen in this model. To examine this hypothesis, we also examined the effect of an ACE inhibitor, cilazapril, on this variable.

**Materials and Methods**

**Animals**

Male 6-week-old Wistar rats (Japan SLC Ltd., Shizuoka) were subjected to 5/6 nephrectomy. Under pentobarbital anesthesia (50 mg/kg, i.p.), renal ablation was achieved by ligation of the renal artery branches supplying two-thirds of the left kidney followed by a right unilateral nephrectomy 7 days later. Eight weeks after operation, the 5/6 nephrectomized rats were divided into three groups; group I (n = 8) received vehicle (0.5% methyl cellulose solution) alone, group II (n = 8) and group III (n = 10) received 1 and 10 mg/day of cilazapril, respectively. Animals with sham operation including kidney exteriorization and decapsulation were given vehicle and were used as controls (n = 6).

The compounds dissolved in 0.5% methyl cellulose solution were given by gastric gavage once daily for 12 weeks. Cilazapril was kindly donated by Eisai Co., Ltd. (Tokyo).

All rats were housed two per cage and were fed standard chow (CE-2; Japan Clea Co., Ltd., Tokyo) and allowed free access to water. Blood pressure was measured before and at every 4 weeks during the study period. Urine protein excretion was determined before and during the treatment period biweekly. Blood sample for plasma fibrinogen was obtained from the tail vein biweekly. To prevent blood coagulation, blood was collected in a tube containing 1/10 volume of 3.8% sodium citrate for plasma fibrinogen assay. At the end of the treatment period, blood was collected from the abdominal aorta under pentobarbital anesthesia (50 mg/kg, i.p.). The experiment was conducted in accordance with the Jichi Medical School Guide for Laboratory Animals.

**Blood pressure measurement and urine collection**

Blood pressure was measured in prewarmed, awake rats by a standard tail-cuff method (KN-210-1; Natsume Co., Ltd., Tokyo). To determine protein excretion and creatinine (Cr) clearance, 4-h urine sample was collected in metabolic cages following water loading (3% of body weight) by gastric gavage. Urine protein excretion was normalized in terms of urine Cr concentration. Endogenous Cr clearance was determined at the end of the treatment period.

**Plasma fibrinogen and chemistries in serum and urine**

Plasma fibrinogen concentration was determined by the thrombin time method. Serum total protein, albumin, and Cr in serum and urine were measured by an autoanalyzer (Hitachi 7170; Hitachi Co., Ltd., Tokyo). Urine protein concentration was measured by a pyrogallol-red molybdate complex method.

**Serum MCP-1 and transforming growth factor-β1 (TGF-β1)**

MCP-1 and TGF-β1 were determined using serum samples obtained at the end of the study. MCP-1 was measured by a commercially available ELISA kit (Cosmobio, Tokyo) according to the instruction manual. TGF-β1 was also measured by a human TGF-β1 ELISA kit (R&D, Minneapolis, MN, USA).

**Statistical analyses**

Results are expressed as the means ± S.E.M. The statistical significance of differences between group means was tested by one- and two-way analysis of variance. Fisher’s Protected Least Significant Difference test was used as a post hoc test. Statistical difference was considered significant for $P<0.05$.

**Results**

**Blood pressure and body weight**

Systolic blood pressure (SBP) in the vehicle-treated nephrectomized rats did not increase and was comparable with that of the control animals during the study period. Treatment with the lower dose of cilazapril (1 mg/kg, daily) tended to decrease and the higher dose (10 mg/kg, daily) significantly decreased SBP ($P<0.01$ by 2-way ANOVA). At the end of the treatment, SBP was 147±3 mmHg in the control, 146±4 mmHg in the vehicle-treated, 139±3 mmHg in the low-dose cilazapril-treated, and 127±4 mmHg in the high-dose cilazapril-treated groups ($P<0.01$ vs vehicle).

Body weight gain was comparable among the groups ($P>0.1$ by 2-way ANOVA). At the end of the treatment period, body weight was 390±10 g in the control, 372±5 g in the vehicle-treated, 376±6 g in the low-dose cilazapril-treated, and 378±8 g in the high-dose cilazapril-treated groups, without any significant differences between groups.

**Renal function**

At the initiation of the treatment with cilazapril, urinary protein excretion already elevated in the rats with 5/6 nephrectomy. Cilazapril attenuated the further development of proteinuria in a dose-dependent manner (Fig. 1). The higher dose of cilazapril markedly reduced
proteinuria, which was not significantly different from that of the control rats during the treatment period.

At the end of the study, Cr clearance in the nephrectomized rats was significantly lower than that of the control rats. Cilazapril did not significantly alter the decreased Cr clearance in rats with nephrectomy (Table 1).

**Plasma fibrinogen and serum chemistries**

Figure 2 shows the changes in plasma fibrinogen concentrations in the nephrectomized and control groups. Before the treatment, it was not significantly different among the groups. Plasma fibrinogen concentration gradually increased with age in the control and nephrectomized rats ($P<0.0001$ by 2-way ANOVA). In the vehicle-treated nephrectomized rats, its increase was significantly higher than that of the control rats (+55.0 vs +35.5 mg/dL, $P<0.01$). Cilazapril attenuated the increase in plasma fibrinogen concentration in a dose-dependent manner ($P>0.1$ in the lower dose, $P<0.01$ in the higher dose). During the study, plasma fibrinogen concentration increased by 48.5 and 37.2 mg/dL in rats treated with the lower and higher dose of cilazapril, respectively. The higher dose of cilazapril significantly attenuated the increase in plasma fibrinogen in the nephrectomized rats.

At the end of the study, serum albumin concentration was significantly lower in the nephrectomized groups than in the control group ($P<0.0001$). Cilazapril significantly increased serum albumin concentration in a dose-dependent manner (Table 1).

**Serum MCP-1 and TGF-β1**

At the end of the study, serum concentrations of MCP-1 and TGF-β1 in the vehicle-treated nephrectomized rats were significantly higher than those of the control rats (Fig. 3). Cilazapril significantly decreased serum MCP-1 concentration. Serum TGF-β1 was also reduced by cilazapril, but the difference did not reach statistical significance.

**Table 1.** Creatinine clearance and serum albumin in control rats and in the 5/6 nephrectomized rats with and without cilazapril for 12 weeks

<table>
<thead>
<tr>
<th>5/6 Nephrectomized rats</th>
<th>control</th>
<th>vehicle</th>
<th>cilazapril 1 mg/kg</th>
<th>cilazapril 10 mg/kg</th>
<th>$P$ value by ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Creatinine clearance (ml/min per kg)</td>
<td>6.86 ± 0.48**</td>
<td>4.37 ± 0.27</td>
<td>4.29 ± 0.28</td>
<td>5.09 ± 0.34</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum total protein (g/dL)</td>
<td>7.27 ± 0.05</td>
<td>6.86 ± 0.08</td>
<td>6.86 ± 0.15</td>
<td>6.92 ± 0.08</td>
<td>N.S.</td>
</tr>
<tr>
<td>Serum albumin (g/dL)</td>
<td>4.83 ± 0.05**</td>
<td>3.90 ± 0.12</td>
<td>4.18 ± 0.12*</td>
<td>4.41 ± 0.07**</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Mean ± S.E.M. *$P<0.05$, **$P<0.01$ vs the vehicle-treated group.
The present study showed that plasma fibrinogen concentration is persistently elevated in the 5/6 nephrectomized rats. Cilazapril blunted the elevation in this variable in a dose-dependent manner. The agent also exerted the renoprotective effect against the development of proteinuria in these animals.

In this study, plasma fibrinogen concentration was varied with the degrees of proteinuria and hypoalbuminemia. Fibrinogen is a glycoprotein synthesized primarily in the liver. In patients with nephrotic syndrome and animal model with massive proteinuria, fibrinogen and albumin synthesis proportionately increased in liver (14 – 16). There is general agreement that the decreased osmotic pressure due to hypoalbuminemia in nephrotic syndrome stimulates hepatic protein synthesis including the synthesis of fibrinogen. The nephrectomized rats in this study showed no edema or abnormal weight gain and had only mild hypoalbuminemia (~19% in serum albumin concentration), suggesting that these animals were not nephrotic. Therefore, we think that hypoalbuminemia is not the sole stimulant to the elevation of plasma fibrinogen concentration in 5/6 nephrectomized rats. In response to inflammation, proinflammatory cytokines, such as interleukin-6, are released that stimulate the liver to increase fibrinogen synthesis, and this fibrinogen is secreted into the circulation (12). In the nephrectomized rats, monocytes/macrophages infiltrate into the renal interstitial space (13). In this study, serum concentrations of MCP-1 and TGF-β1 significantly increased, which indicate that inflammatory process occurred in the remnant renal tissue. It is thus possible that local renal inflammation in the nephrectomized rats is also involved in the elevation of plasma fibrinogen. Such a local inflammation in atherosclerotic lesions is also reported to be involved in the increase in plasma fibrinogen in patients with coronary heart disease (2, 3).

Ang II plays some roles in the renal macrophage infiltration in several models of renal injury (17 – 19). Blockade of the renin-angiotensin system by ACE inhibitors or Ang II type 1 (AT-1) receptor antagonists reduces renal expression of MCP-1 and consequently prevents macrophage recruitment in the kidney (20 – 22). In the present study, cilazapril reduced serum MCP-1 concentration in the nephrectomized rats. Based on these findings, we think that in addition to hypoalbuminemia, fibrinogen synthesis in this model was enhanced by a stimulus through local inflammation in the remnant renal tissue. However, because cilazapril reduced not only serum MCP-1 but proteinuria in the nephrectomized rats, we could not clearly separate the contribution to the increased plasma fibrinogen of local inflammation from that of hypoalbuminemia in this model.

Ang II causes glomerular hypertension by preferential vasoconstrictive action on the efferent arteriole (23). ACE inhibitor and AT-1 receptor antagonist lower glomerular pressure and subsequently reduce glomerular sclerosis and proteinuria in this model (24 – 26). An increased level of bradykinin caused by the ACE inhibitors may be involved in the renoprotective effects. However, renoprotective effects of ACE inhibitors, including against tubulointestinal damages, were not influenced by the treatment with a bradykinin B₂-receptor antagonist (Hoe140: icatibant) in rats with renal ablation (27, 28). In addition, chronic administration of

![Fig. 3. Serum MCP-1 (A) and TGF-β1 (B) in 5/6 nephrectomized and control rats at the end of the treatment period. *P<0.05, **P<0.01 vs the vehicle-treated group. Mean ± S.E.M.](image-url)
ACE inhibitor and AT-1 receptor antagonist exerted similar renal protective effects in rats with reduced renal mass (29). Thus, the role of bradykinin in the renoprotective effect of ACE inhibitor appears to be small in the remnant kidney model. Proteinuria itself is reported to cause renal tubular damages, cellular infiltration into interstitial space, and tubulointerstitial fibrosis in several renal diseases (30, 31). Filtered protein can stimulate releases of platelet-derived growth factor, fibronectin, and MCP-1 from proximal tubule cells (32). This process may cause tubular overexpression of TGF-β1 and interstitial inflammation (33). It is likely that cilazapril decreases proteinuria in the nephrectomized rats primarily through a reduction in glomerular hypertension and hyperfiltration, which in turn improves tubulointerstitial remodeling. It is thus possible that cilazapril decreases hepatic fibrinogen synthesis through the alleviation of the local inflammatory process and the improvement of hypoalbuminemia. TGF-β1 produced by infiltrative monocytes (34) shows potent fibrogenic properties and plays an important role in glomerulosclerosis and renal tubulointerstitial fibrosis. As cilazapril did not significantly decrease serum TGF-β1 concentration in the nephrectomized rats, it is unclear whether the agent decreased plasma fibrinogen through the reduction of local TGF-β1 production.

The impacts of the elevated plasma fibrinogen and its correction by cilazapril on the cardiovascular system were unclear in rats. However, an elevated fibrinogen level in humans represents a risk factor for cardiovascular morbidity and mortality. Multiple mechanisms may contribute to this effect (1). These include increased blood viscosity and enhanced platelet adhesiveness and aggregation favoring the deposition of thrombi. Therefore, agents with a fibrinogen-lowering effect may have merit for the prevention of cardiovascular events. Fogari et al. measured plasma fibrinogen concentration in hypertensive patients treated with lisinopril (an ACE inhibitor), amlodipine (a calcium channel blocker), atenolol (a beta-blocker) or hydrochlorothiazide (a diuretic) for 8 weeks (35). They found that only lisinopril significantly reduced plasma fibrinogen concentration. In addition, perindopril, an ACE inhibitor, but not losartan, an AT-1 receptor antagonist, reduced plasma fibrinogen in overweight hypertensive patients (36). In that study, insulin sensitivity was also improved by perindopril, but not by losartan. The perindopril-induced decrease in fibrinogen was significantly correlated with the improvement of insulin sensitivity. The authors concluded that fibrinogen decrease by perindopril may be induced by its action on insulin sensitivity. It is thus possible that the alteration of insulin sensitivity by ACE inhibition may be also involved in the reduction of plasma fibrinogen. Similarly, ACE inhibitors, but not a diuretic or beta-blockers, reduced plasma fibrinogen in patients with hypertension (37) and chronic heart failure (38). Based on the present findings, we think that ACE inhibitors could decrease the elevated plasma fibrinogen. Further clinical studies are needed to evaluate the potential favorable effect of ACE inhibitors on plasma fibrinogen.

In summary, the present study showed that plasma fibrinogen concentration is persistently elevated in the subtotal nephrectomized rats. Cilazapril attenuated the increase in plasma fibrinogen and serum MCP-1. These results suggest that inflammation is one of the stimuli for fibrinogen synthesis in the nephrectomized rats. Cilazapril blunted the elevation of plasma fibrinogen, probably through the correction of the hypoalbuminemic state and the amelioration of inflammation in the remnant renal tissues in these animals.

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