Prevention of Renal Damage by Angiotensin II Blockade, Accompanied by Increased Renal Hepatocyte Growth Factor in Experimental Hypertensive Rats

Keiko Matsumoto, Ryuichi Morishita, Atsushi Moriguchi, Naruya Tomita, Yoshikage Yo, Tadahiko Nishii, Kunio Matsumoto, Toshikazu Nakamura, Jitsuo Higaki, Toshio Ogihara

Abstract—Hepatocyte growth factor (HGF) is a unique growth factor that has many protective functions against renal damage. Our previous study demonstrated that HGF stimulated the growth of endothelial and epithelial cells without the replication of mesangial cells. Moreover, angiotensin (Ang) II significantly decreased local HGF production in mesangial cells. Therefore, we examined the effects of Ang II blockade on renal HGF expression and renal damage in experimental hypertensive rats. An angiotensin-converting enzyme inhibitor (cilazapril; 10 mg kg\(^{-1}\) d\(^{-1}\)), an Ang II type 1 receptor antagonist (E-4177; 30 mg kg\(^{-1}\) d\(^{-1}\)), hydralazine (8 mg kg\(^{-1}\) d\(^{-1}\)), and vehicle were administered to 16-week-old stroke-prone spontaneously hypertensive rats (SHR-SP) for 3 weeks. Renal damage was evaluated with a computer analysis system, and renal HGF mRNA was measured by Northern blot analysis. Blood pressure of SHR-SP was significantly decreased by all drug treatments compared with vehicle. Moreover, cilazapril, E-4177, and hydralazine significantly decreased the thickening and necrosis of blood vessels compared with vehicle. Similarly, degeneration and necrosis of glomeruli were also markedly improved by cilazapril and E-4177 (P<0.01). We next examined the effects of Ang II blockade on renal HGF expression in SHR-SP. Renal HGF mRNA was markedly decreased in SHR-SP compared with Wistar-Kyoto rats, although Ang II blockade by cilazapril and E-4177 but not hydralazine significantly increased renal HGF mRNA in SHR-SP. Ang II blockade significantly increased renal HGF (a protective growth factor for tubular epithelial cells); thus, we examined tubular histological appearance. Degeneration and necrosis of tubules were significantly improved by cilazapril and E-4177 treatment (P<0.01). In addition, cell infiltration into the glomeruli and hemorrhage were also significantly reduced in SHR-SP treated with cilazapril or E-4177. The present data demonstrated the prevention of renal damage by Ang II blockade in SHR-SP, which was accompanied by a significant increase in renal HGF production. Given the strong mitogenic activity and antiapoptotic actions of HGF on endothelial and epithelial cells, we believe that increased local HGF production by the blockade of Ang II may improve renal function in hypertension. (Hypertension. 1999;34:279-284.)

Key Words: mesangium ■ kidney tubules ■ hypertension, renal ■ angiotensin-converting enzyme inhibitors ■ angiotensin II

Hypertension induces progressive damage to many systemic organs, including the kidneys. Antihypertensive therapy provides renal protection by reducing glomerular blood pressure.\(^1\) However, recent reports suggest that not all antihypertensive agents do produce an equal renal effect.\(^2,3\) The renin-angiotensin system plays a major role in the development of renal damage.\(^4,5\) Angiotensin-converting enzyme (ACE) inhibitors have been shown to produce a beneficial effect on the kidney.\(^5,7\) However, in addition to renin-angiotensin inhibition, ACE inhibitors have other effects, such as stimulation of the kallikrein-kinin system. Recently, specific nonpeptide angiotensin (Ang) II receptor antagonists have been developed. Ang receptor subtype I antagonists have been reported to have a protective effect on the kidney.\(^8-10\) In addition to the hemodynamic effects, Ang II is known to produce direct effects, such as stimulatory actions on mesangial cell growth and the production of extracellular matrix.\(^11-13\) Together with the presence of a local renin-angiotensin system in renal glomeruli,\(^14,15\) Ang II may directly modulate renal structure, which may lead to renal damage.

We previously reported that Ang II downregulated local hepatocyte growth factor (HGF) production in mesangial cells in a culture model.\(^16\) HGF was initially identified as the most potent growth factor for hepatocytes\(^17,18\) and is known to be a mesenchyme-derived pleiotropic factor that regulates...
cell growth, cell motility, and morphogenesis of various types of cells. HGF is also considered to be a humoral mediator of epithelial-mesenchymal interactions responsible for morphogenic tissue interactions during embryonic development and organogenesis. Recent studies suggest that HGF has many effects on the cells of other target organs, such as the kidney. For example, the administration of recombinant HGF promoted the regeneration of epithelial cells that were injured by antitumor drugs. These observations are important because acute renal failure is often reversible, and recovery depends on mitogenesis, motogenesis, and morphogenesis (tubular formation) of renal epithelial cells. From this viewpoint, the rapid regeneration of renal epithelial cells might be important for the treatment of acute renal failure. HGF enhances renal regeneration and suppresses the onset of acute renal failure caused by renal toxins, renal ischemia, or unilateral nephrectomy. In addition, our previous studies demonstrated that HGF is a potent antiapoptotic factor in endothelial and epithelial cells. Because it is apparent that cell-cell interactions among these cells are important in the control of renal function, HGF thus plays an important role in the maintenance of cell-cell interactions in the kidney.

Previously, we reported that local HGF production in the kidney was decreased in spontaneously hypertensive rats (SHR), which serve as a good experimental model of severe hypertension and end-organ damage, versus normotensive Wistar-Kyoto (WKY) rats. In addition, activation of the renal reticular activating system has been reported in this hypertensive animal. Thus, in this study, we examined the effects of Ang II blockade on renal damage in hypertension and the effects of Ang II blockade on local renal HGF expression and tubular structure.

Methods

Experimental Design

Fifteen-week-old male WKY and stroke-prone SHR (SHR-SP; Charles River Breeding Laboratories, Shiznoka, Japan) were divided into 4 groups (each n=13) and treated for 3 weeks with the following: vehicle (distilled water), cilazapril (10 mg·kg⁻¹·d⁻¹), E-4177 (30 mg·kg⁻¹·d⁻¹), or hydralazine (8 mg·kg⁻¹·d⁻¹). The drugs were donated by Eisai Pharmaceutical Company (Japan). The animals were randomly allocated to each group, and drugs were administrated by gavage. After the rats were treated, they were killed by decapitation and their blood was collected. Systolic blood pressure was measured in conscious rats by the tail-cuff method with a sphygmomanometer (Soffron Co. Ltd). All rats were free to drink water and eat standard laboratory rat chow (11.3 mEq Na⁺ per 100 g, 32.6 mEq K⁺ per 100 g, and 24.6% protein by weight; Oriental Kobo Co). Throughout the experiment, rats were housed in metabolic cages under light- and temperature-controlled conditions.

Evaluation of Renal Injury

The kidney was fixed with formalin and embedded in paraffin; 4-μm sections were stained with periodic acid–Schiff. Glomerular lesions were evaluated by light microscopy by an examiner without knowledge of the treatment. One hundred glomeruli were examined in each kidney for the presence or absence of sclerosis. The percentage of glomeruli with segmental or global sclerosis was used as an index of glomerulosclerosis. Another index was also evaluated from the measurement of 100 sections in each kidney. Animals were coded so that the analysis was performed without knowledge of which treatment each individual animal had received.

Blood Pressure of Rats

<table>
<thead>
<tr>
<th></th>
<th>16 Weeks, Before</th>
<th>19 Weeks, After</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>130±2 mm Hg</td>
<td>138±3 mm Hg</td>
</tr>
<tr>
<td>SHR-SP, vehicle</td>
<td>193±3 mm Hg</td>
<td>197±5 mm Hg</td>
</tr>
<tr>
<td>SHR-SP, cilazapril</td>
<td>193±3 mm Hg</td>
<td>169±4 mm Hg*</td>
</tr>
<tr>
<td>SHR-SP, E-4177</td>
<td>192±2 mm Hg</td>
<td>164±4 mm Hg*</td>
</tr>
<tr>
<td>SHR-SP, hydralazine</td>
<td>193±3 mm Hg</td>
<td>172±4 mm Hg*</td>
</tr>
</tbody>
</table>

*P<0.01 vs vehicle.

Northern Blot Analysis

RNA was extracted by RNazol (Tel-Test Inc) from the kidneys of SHR-SP and WKY. For Northern blot analysis, 20 μg of total RNA was subjected to electrophoresis on 1.5% agarose-formaldehyde denaturing gel and transferred to a nitrocellulose membrane (Amer sham International). The filter was baked, prehybridized, and hybridized. Full-length cDNAs for HGF and GAPDH labeled by a random primer kit (Amersham) were used as probes for Northern blotting. The filter was then washed and exposed to x-ray film.

Statistical Analysis

All values are expressed as mean±SEM. ANOVA with subsequent Bonferroni or Dunnett test was used to determine the significance of differences in multiple comparisons. Values of P<0.05 were considered statistically significant.

Results

Systolic blood pressure decreased similarly during treatment with cilazapril, E-4177, and hydralazine, as shown in the Table (P<0.01). There was no significant difference in blood pressure among all drug-treated groups. As shown in Figure 1, the renal morphology of SHR-SP was markedly improved by cilazapril or E-4177 treatment. The renal tissues in rats in the cilazapril, E-4177, and hydralazine groups showed significantly lower glomerular scores for mesangiolysis and sclerotic lesions versus the vehicle group (Figure 2, P<0.01). The degree of improvement in glomerular scores was significantly greater with cilazapril or E-4177 treatment than with hydralazine treatment (P<0.01). Similarly, glomerular hypertensive damage, such as thickening of the blood vessels and necrosis, in the cilazapril, E-4177, and hydralazine groups was significantly improved compared with the vehicle control group (Figure 3, P<0.01).

As mentioned earlier, HGF enhances renal regeneration and suppresses the onset of acute renal failure caused by renal toxins, renal ischemia, or unilateral nephrectomy, probably through potent mitogenic and antiapoptotic actions on endothelial and epithelial cells. In addition, our previous studies demonstrated that Ang II downregulated renal HGF expression in mesangial cells. Therefore, we further examined the effect of Ang II on local renal HGF production in SHR-SP, because Ang II is known to have a significant contribution to the pathogenesis of renal damage in this model. Indeed, a marked reduction of renal HGF mRNA was observed in SHR-SP compared with WKY, although no apparent difference in GAPDH mRNA was observed between WKY and SHR-SP (Figure 4). Thus, rats were treated with cilazapril, E-4177, hydralazine, or vehicle for 21 days. Renal HGF mRNA significantly increased in SHR-SP treated with cilazapril or E-4177 versus vehicle (Figure 4, P<0.01),
whereas there was no significant change in rats treated with vehicle or hydralazine.

Accompanied by a significant increase in renal HGF mRNA, cilazapril and E-4177 treatment significantly improved necrosis and degeneration of the tubules versus vehicle and hydralazine treatment (Figure 5, *P*<0.01). A small but significant decrease in tubular scores for necrosis and degeneration was also observed with hydralazine treatment. In addition, all drug treatments improved hemorrhage (Figure 6a, *P*<0.01). We measured the number of infiltrated cells in the glomeruli because endothelial cells act as a biological barrier against cell infiltration and HGF is a potent survival factor against endothelial cell death. When accompanied by a significant increase in renal HGF mRNA, cell infiltration into the glomeruli was significantly reduced by cilazapril and E-4177 treatment compared with vehicle and hydralazine treatment (Figure 6b, *P*<0.01).

**Discussion**

HGF is considered a humoral mediator of epithelial-mesenchymal interactions responsible for morphogenic tissue interactions during embryonic development and organogenesis. Recent studies suggest that HGF enhances renal regeneration and suppresses the onset of acute renal failure caused by renal toxins, renal ischemia, or unilateral nephrectomy. In addition, we reported that HGF is a unique growth factor that has potent mitogenic activity and an antiapoptotic action in endothelial and epithelial cells. Interestingly, renal HGF concentration decreased significantly in experimental hypertensive rats, probably as a result of an increase in renal Ang II, because Ang II downregulated local HGF expression in human mesangial cells in vitro. However, no direct evidence exists of how Ang II regulates renal HGF expression in vivo. Moreover, the contribution of renal HGF to the pathogenesis of renal injury in hypertension has not yet been clarified. Thus, in this study, we addressed the effects of Ang II blockade on renal damage in hypertension and the effects of Ang II blockade on local renal HGF expression and tubular structure.

Cilazapril, E-4177, and hydralazine reduced blood pressure and attenuated the development of renal hypertensive changes, such as glomerulosclerosis, in SHR-SP. However, the change in glomerular score was significantly greater with cilazapril or E-4177 treatment than with hydralazine treatment. These results suggest that the renal protective effect may be mediated by Ang II blockade, in addition to blood pressure reduction and decreased renal injury. The findings also suggest that HGF may play a role in the prevention of renal damage in hypertension.
pressure control. We then focused on the interaction of Ang II with the renal HGF system. Our present study documented a marked reduction of renal HGF mRNA in SHR-SP versus WKY. In experimental hypertensive models, activation of the vascular renin-angiotensin system has been reported in the kidney.28,29 As previously mentioned, in vitro studies also revealed that Ang II is a strong negative regulator of local HGF expression in human mesangial cells and vascular smooth muscle cells.16,30 Suppression of renal HGF expression might accelerate renal injury, such as glomerulosclerosis and tubular degeneration, because HGF prevents apoptosis of endothelial and epithelial cells mediated by several conditions.16,26,31,32 This phenomenon gives rise to the hypothesis that disruption of the autocrine-paracrine local HGF system in the kidney, which maintains endothelial and epithelial cell homeostasis, by Ang II may result in renal injury because endothelial cells secrete antiproliferative substances and renal injury depends on mitogenesis, motogenesis, and morphogenesis (tubular formation) of renal epithelial cells.33–35

We further investigated the tubular morphology in hypertensive animals treated with antihypertensive drugs. The

Figure 2. Effects of antihypertensive drugs on degeneration (a) and necrosis (b) of glomeruli in SHR-SP. Vehicle indicates SHR-SP treated with vehicle; cilazapril, SHR-SP treated with cilazapril; hydralazine, SHR-SP treated with hydralazine; and E-4177, SHR-SP treated with E-4177. n=8 per group. *P<0.01 vs vehicle, #P<0.01 vs WKY.

Figure 3. Effects of antihypertensive drugs increased the thickness (a) and necrosis (b) of blood vessels of SHR-SP. Labels as in Figure 2. n=8 per group. *P<0.01 vs vehicle.

Figure 4. A, Typical examples of HGF (top blot) and GAPDH (G3PDH; bottom blot) mRNA assessed by Northern blotting in the kidney of WKY and SHR-SP treated with antihypertensive drugs. Labels as in Figure 2. B, Effects of antihypertensive drugs on renal HGF expression. Labels are the same as in Figure 2. n=8 per group. *P<0.01 vs vehicle.

Figure 5. Effects of antihypertensive drugs on the degeneration (a) and necrosis (b) of tubules in SHR-SP. Labels as in Figure 2. n=8 per group. *P<0.01 vs vehicle, #P<0.01 vs WKY.
The present study demonstrated that Ang II blockade by cilazapril or E-4177 treatment resulted in the inhibition of tubular injury in SHR-SP, which was accompanied by a significant increase in renal HGF mRNA. As expected, no increase in renal HGF mRNA was observed with hydralazine treatment. Rapid regeneration of renal epithelial cells might be important for the treatment of acute renal failure, and the administration of recombinant HGF promoted the regeneration of epithelial cells injured by antitumor drugs. Increased renal HGF expression may participate in the improvement of tubular damage observed in SHR-SP treated with blockers of Ang II. The specificity of Ang II blockade is supported by the observation that the degree of improvement of tubular damage was significantly greater in the cilazapril and E-4177 treatment groups than in the hydralazine treatment group. By blockade of Ang II, increased renal HGF expression may have a therapeutic value in the prevention of tubular injury by enhancing the regeneration of epithelial cells in hypertension. Negative regulation of local HGF expression by Ang II may have physiological roles in renal disease.

**Acknowledgments**

This work was partially supported by grants from the Uehara Memorial Foundation, the Hoan-sya Foundation, the Japan Cardiovascular Research Foundation, and a Japan Heart Foundation research grant. R.M. is the recipient of a Harry Goldblatt Award from the Council of High Blood Pressure, the American Heart Association. We wish to thank Chihiro Noguchi and Shiori Takase for their excellent technical assistance.

**References**


*Figure 6. Effects of antihypertensive drugs on hemorrhage (a) and cell infiltration (b) into the glomeruli of SHR-SP. Labels as in Figure 2. n = 8 per group. *P < 0.01 vs vehicle, **P < 0.01 vs WKY.*
284 Hypertension August 1999


Prevention of Renal Damage by Angiotensin II Blockade, Accompanied by Increased Renal Hepatocyte Growth Factor in Experimental Hypertensive Rats
Keiko Matsumoto, Ryuichi Morishita, Atsushi Moriguchi, Naruya Tomita, Yoshikage Yo, Tadahiko Nishii, Kunio Matsumoto, Toshikazu Nakamura, Jitsuo Higaki and Toshio Ogihara

Hypertension. 1999;34:279-284
doi: 10.1161/01.HYP.34.2.279

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/34/2/279

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/