Angiotensin Blockade and the Progression of Renal Damage in the Spontaneously Hypertensive Rat

Katsuhiko Kohara, Hiroshi Mikami, Naoki Okuda, Jitsuo Higaki, and Toshio Ogihara

The pathophysiological role of angiotensin II in the development of renal sclerosis was investigated in 5/6-nephrectomized, 12-week-old male spontaneously hypertensive rats. After 1 week of a control period, nephrectomized rats received one of the following treatments for 4 weeks: the selective nonpeptide angiotensin II type 1 receptor antagonist TCV-116 (1 mg/kg per day), the angiotensin converting enzyme inhibitor delapril (30 mg/kg per day), hydralazine (15 mg/kg per day), or vehicle. Urinary protein and albumin excretions and systolic blood pressure were determined every week. Rats with reduced renal mass treated with vehicle had a poor survival rate (30%). Although TCV-116, delapril, and hydralazine treatment significantly improved the survival rate for 4 weeks, hydralazine failed to improve proteinuria and albuminuria as well as the decline in renal function compared with delapril or TCV-116. Histological examination revealed that both TCV-116 and delapril protected glomeruli from sclerosis, whereas hydralazine did not improve histological findings (5%, 7%, and 30% of glomeruli were affected, respectively). These results indicate that angiotensin II plays a dominant role through its type 1 receptor in the pathogenesis of renal deterioration by hypertension.

Key Words • receptors, angiotensin • nephrectomy • hydralazine • albuminuria • angiotensin converting enzyme inhibitors • glomerulosclerosis, focal

The renin-angiotensin system plays a major role in the pathogenesis of renal sclerosis in several renal injury models, including renal mass ablation, diabetic nephropathy, and membranous nephropathy. Angiotensin converting enzyme (ACE) inhibitor has been shown to be effective in reducing proteinuria and protecting glomerulus from sclerosis in both clinical and experimental conditions.

Recently, the development of specific nonpeptide angiotensin II (Ang II) receptor antagonists has made it possible to differentiate the physiological properties of Ang II into type 1 (AT₁) and type 2 (AT₂) receptor mediated responses. The AT₁ receptor is reported to be involved in vasoconstriction and aldosterone release by Ang II, and AT₁ receptor blockade successfully decreased blood pressure in renin-dependent hypertension models.

In the present study, we compared the effect of long-term administration of an AT₁ receptor antagonist with that of an ACE inhibitor and hydralazine in the spontaneously hypertensive rat (SHR) with reduced renal mass. The effects of the treatment on proteinuria, renal function, and glomerular morphology were investigated to try to separate the physiological role of blocking the AT₁ receptor from other factors associated with ACE inhibitor treatment.

Methods
Male 12-week-old SHRs were obtained from Charles River Japan, Inc., Kanagawa, Japan. All rats were allowed free access to water and standard laboratory rat chow (containing 11.3 mEq/100 g Na⁺, 32.6 mEq/100 g K⁺, and 24.6% protein by weight; Oriental Kobo Co., Osaka, Japan). Throughout the experiment, rats were housed individually in metabolic cages, and their urine was collected for 24 hours each week for the determination of urinary protein, albumin, and creatinine excretions. Systolic blood pressure (BP) was measured by tail plethysmography every week.

Surgical Procedure and Experimental Protocol
After basal BP measurement and urine collection, animals underwent 5/6 renal ablation. With animals under sodium pentobarbital anesthesia, a left flank incision was made, and approximately two thirds of the left kidney was infarcted by ligating two or three branches of the left renal artery. One week after the first surgery, the right kidney was removed with rats under light ether anesthesia. Animals with 5/6 nephrectomy were housed another week as a control period. On the last day of the control period, a blood sample was obtained with rats under light ether anesthesia for the determination of serum creatinine. Animals with 5/6 nephrectomy were divided into four groups and treated with one of the following medications for 4 weeks: vehicle, the selective nonpeptide AT₁ receptor antagonist TCV-116 (TCV, 1 mg/kg per day), the ACE inhibitor delapril (30 mg/kg per day), and hydralazine (15 mg/kg per day, Sigma Chemical Co., St. Louis, Mo.). TCV is a prodrug with a
bioavailability of 25% for rats. CV-11974, an active form of TCV, is a very potent noncompetitive AT₁ receptor antagonist with an IC₅₀ of 2.86×10⁻⁸ M for inhibition of Ang II binding to rabbit aorta membrane fraction. The ID₅₀ of TCV orally given for inhibition of the pressor response induced by Ang II is 0.06 mg/kg for rat. The above drug doses were determined in a preliminary study to induce significant BP reductions. All drugs were dissolved in drinking water. On the fourth week after initiation of treatment, an arterial catheter was implanted in the abdominal aorta through the femoral artery with rats under light ether anesthesia. The following day, mean BP and heart rate were determined in conscious freely moving rats. Blood was obtained through the arterial catheter for the determination of serum creatinine, blood nitrogen urea, plasma renin activity, plasma aldosterone concentration, and plasma angiotensin I (Ang I) and Ang II. Rats were killed with an overdose of sodium pentobarbital, and the remnant kidney was transcardially perfused with saline followed by 10% buffered formalin.

**Biochemical Determination**

Urinary protein was measured by the sulfosalicylic acid method. Urinary albumin was measured by an enzyme-linked immunosorbent assay using rat albumin antibody (Organon Teknika, Durham, N.C.). Plasma renin activity and plasma aldosterone concentration were measured with commercially available radioimmunoassay kits (Dainabot, Japan). For angiotensin measurement, plasma was concentrated on an Amprep C8 minicolumn (Amersham, Aylesbury, UK). Plasma Ang I and Ang II were separated by high performance liquid chromatography and measured using specific radioimmunoassays as reported previously.

**Statistical Analysis**

All values are expressed as mean±SEM. Differences in systolic BP and urinary protein and albumin excretions among treatment groups were evaluated by two-way analysis of variance followed by Duncan's multiple-range test. The differences in plasma electrolytes and humoral factors were analyzed by one-way analysis of variance. The criterion for statistical significance was a value of p<0.05.

**Results**

Figure 1 illustrates the survival curve. Treatment with TCV, delapril, or hydralazine significantly improved survival rate during the experimental period. Figure 2 depicts the change in systolic BP. Although hydralazine administration caused a greater fall in pressure in the early course of treatment, at week 4 all treated groups showed similar and significant BP reductions compared with the vehicle control in 5/6-nephrectomized SHRs. The mean BP obtained by direct measurement via an indwelling catheter on the fourth week confirmed these findings (186±9, 154±5, 164±9, and 158±18 mm Hg for vehicle, TCV, delapril, and hydralazine, respectively). Direct measurement also showed that neither TCV nor delapril changed heart rate, whereas hydralazine significantly increased heart rate on the fourth week (367±13, 366±11, 375±6, and 402±8 beats per minute for vehicle, TCV, delapril, and hydralazine, respectively). Figure 3 illustrates the change in urinary protein and albumin excretions during the experiment. In SHRs treated with either vehicle or hydralazine, proteinuria and albuminuria increased progressively during the course of the experiment. Administration of either TCV or delapril prevented this progression. The increase in albuminuria was associated with the deterioration of renal function (Table 1). Table 1 also summarizes the humoral factors measured on the fourth week. Any...
treatment significantly increased plasma renin activity compared with vehicle. Both TCV and hydralazine significantly increased plasma Ang I and Ang II, whereas delapril significantly increased only Ang I, resulting in the decrease in the Ang II/Ang I ratio as an index of ACE activity. Figure 4 illustrates the typical histological findings of glomeruli. The glomerular injuries classified as segmental or global lesions are summarized in Table 2. TCV or delapril significantly improved both segmental and global glomerular lesions, whereas hydralazine improved only global changes.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Sodium (mmol/L)</th>
<th>Potassium (mmol/L)</th>
<th>Creatinine (mg/dL)</th>
<th>BUN (mg/dL)</th>
<th>CCr (mL/24 hours per 100 g body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>5</td>
<td>140±1</td>
<td>5.7±0.4</td>
<td>0.95±0.08</td>
<td>72±5</td>
<td>508±60 (15)</td>
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<tr>
<td>TCV-116</td>
<td>8</td>
<td>142±2</td>
<td>5.9±0.2</td>
<td>0.67±0.04*</td>
<td>54±5*</td>
<td>518±46 (10)</td>
</tr>
<tr>
<td>Delapril</td>
<td>9</td>
<td>140±1</td>
<td>5.1±0.3</td>
<td>0.74±0.04*</td>
<td>40±2*</td>
<td>491±40 (9)</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>5</td>
<td>141±2</td>
<td>4.6±0.2†</td>
<td>0.92±0.06</td>
<td>52±4*</td>
<td>477±86 (6)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>PRA (ng/mL per hour)</th>
<th>PAC (pg/mL)</th>
<th>Ang I (pg/mL)</th>
<th>Ang II (pg/mL)</th>
<th>Ratio of Ang II/Ang I (x10^(-1))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>5</td>
<td>1.3±0.2</td>
<td>389±86</td>
<td>169±33</td>
<td>11.6±2.1</td>
<td>8.0±1.3</td>
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<tr>
<td>TCV-116</td>
<td>8</td>
<td>9.8±1.7†</td>
<td>492±46</td>
<td>338±28*</td>
<td>26.6±3.6*</td>
<td>7.7±0.5</td>
</tr>
<tr>
<td>Delapril</td>
<td>9</td>
<td>5.1±0.5†</td>
<td>369±54</td>
<td>279±21†</td>
<td>9.3±1.4</td>
<td>3.4±0.4*</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>5</td>
<td>5.5±1.7†</td>
<td>581±92</td>
<td>281±41†</td>
<td>16.0±3.0†</td>
<td>5.9±0.4</td>
</tr>
</tbody>
</table>

BUN, blood urea nitrogen; CCr, creatinine clearance; PRA, plasma renin activity; PAC, plasma aldosterone concentration; Ang I, angiotensin I; Ang II, angiotensin II. Values are mean±SEM. Numbers in parentheses indicate number of rats during the control period.

Discussion

In the present study, we investigated the effect of the AT1 receptor antagonist TCV-116 on renal function in SHR with reduced renal mass with an emphasis on the comparison with an ACE inhibitor and hydralazine. Because the survival rates of all three treated groups were significantly improved compared with vehicle controls, BP reduction seems to be the most important factor for improving the mortality in this chronic renal failure model in SHR. However, the effect on the preservation of renal function was quite different among treatments. The ACE inhibitor and AT1 receptor antagonist significantly improved the deterioration of albuminuria, renal function, and glomerular sclerosis, whereas hydralazine improved only the global lesion of glomeruli compared with vehicle controls.

Although several mechanisms have been proposed as underlying the protective action of the ACE inhibitor, the elimination of the renal effect of Ang II is considered the principal mechanism. Ang II plays an important role in the genesis of proteinuria by changing the intrarenal hemodynamics, glomerular capillary permeability, and filtration surface area. Ang II also causes hypertrophy in glomerular cells. ACE inhibitor administration produces greater vasodilation in efferent than in afferent arterioles, and these hemodynamic changes reduce the intraglomerular pressure, resulting in renal protection.

In situ autoradiographic study using specific AT1 and AT2 receptor antagonists revealed that high concentrations of Ang II receptors were identified in the glomeruli and vascular bundles, and almost all of them were AT1 receptors. Our finding in the present study that TCV was as effective as delapril in preserving renal function indicates that the AT1 receptor is a principal Ang II receptor involved in the progression of glomerulosclerosis in SHR. The renal protection of AT1 receptor blockade was reported to be mediated by ameliorating glomerular transcapillary pressure in a study that used Munich-Wistar rats. In SHR, acute AT1 receptor blockade was reported to induce either little or a positive effect on urinary volume and electrolyte out-
FIGURE 4. Light photomicrographs show glomeruli from rats treated with hydralazine (panel A) and TCV-116 (panel B). Glomeruli from hydralazine-treated rats show segmental and global glomerulosclerosis (panel A); glomeruli from rat treated with TCV-116 is free from these changes (panel B). Periodic acid–Schiff stain, ×66.

TABLE 2. Incidence of Glomerulosclerosis

<table>
<thead>
<tr>
<th></th>
<th>Glomerular injury</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>Segmental</td>
<td>Global</td>
</tr>
<tr>
<td>Vehicle</td>
<td>5</td>
<td>36.2±6.3</td>
<td>19.3±6.0</td>
</tr>
<tr>
<td>TCV-116</td>
<td>8</td>
<td>5.3±1.5*</td>
<td>0*</td>
</tr>
<tr>
<td>Delapril</td>
<td>9</td>
<td>7.3±2.1*</td>
<td>0.5±0.3*</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>5</td>
<td>29.6±6.0</td>
<td>6.7±1.4*</td>
</tr>
</tbody>
</table>

Incidence is expressed as number of affected glomeruli per 100. *p<0.05 compared with vehicle control.

Renal mass ablation in SHR resulted in further augmentation of hypertension and a poor survival rate during 5 weeks of observation. In normotensive rats, it usually takes 3–4 months for the development of renal sclerosis after subtotal nephrectomy. Pre-existing hypertension or renal abnormality in a genetically hypertensive model may accelerate the process of glomerular sclerosis.

Tsuruda et al16 showed that, unlike the present study, hydralazine decreased proteinuria and glomerulosclerosis in 5/6-nephrectomized SHR. This difference could be due to the method of renal ablation; they surgically removed tissue to reduce renal mass. The finding in their study that untreated SHR did not increase BP indicates that the progression of renal damage in their model was slow compared with the present study. The failure of hydralazine administration to affect renal protection was also reported in another study using Munich-Wistar rats 5/6 nephrectomized by ligation.14

In summary, the present study showed that selective AT1 receptor blockade was as effective as ACE inhibi-
tion in ameliorating albuminuria, renal function, glomerulosclerosis, and survival rate in SHR with reduced renal mass. These findings indicate that Ang II plays a crucial role in the pathogenesis and progression of renal damage through its AT1 receptor.

Acknowledgments
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References
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