Effects of chronic inhibition of ACE and AT1 receptors on glomerular injury in Dahl salt-sensitive rats

FUMIO OTSUKA,1 TAKAYOSHI YAMAUCHI,2 HIDEO KATAOKA,1 YUKARI MIMURA,1 TOSHIO OGURA,2 AND HIROFUMI MAKINO1,2

Effects of chronic inhibition of ACE and AT1 receptors on glomerular injury in Dahl salt-sensitive rats. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1797–R1806, 1998.—To elucidate the contribution of the renin-angiotensin system (RAS) to glomerular injury in salt-sensitive hypertension, we investigated the chronic effects of the angiotensin I-converting enzyme inhibitor cilazapril and the angiotensin II type 1-receptor antagonist (AT1a) TCV-116 in Dahl-Iwai rats. Dahl salt-sensitive (S) rats receiving 8% salt diet for 6 wk were simultaneously treated with cilazapril (n = 6), TCV-116 (n = 6), or saline (n = 14). The 8% salt diet markedly increased systolic blood pressure (SBP), urinary protein, and N-acetyl-β-glucosaminidase (NAG) excretion compared with 0.3% salt-treated S (n = 6) or salt-resistant (n = 6) rats. Although neither cilazapril nor TCV-116 reduced the elevated SBP, TCV-116 significantly lowered urinary protein and NAG excretion. Histologically, 8% salt treatment in S rats induced progressive sclerotic and proliferative glomerular changes, which were ameliorated by both drugs. TCV-116 increased the glomerular diameter. Immunofluorescence demonstrated the increased level of type III collagen in the mesangium of 8% salt-treated S rats, which was completely reversed by TCV-116. Competitive RT-PCR of mRNA extracted from the glomeruli revealed that 8% salt treatment significantly increased the levels of proliferating cell nuclear antigen (PCNA) and platelet-derived growth factor B-chain and that TCV-116 significantly reduced the levels of PCNA and transforming growth factor-β1 (TGF-β1). Thus, although the chronic RAS-inhibition in salt-sensitive hypertension exerted a histologically renoprotective effect by both ways without lowering blood pressure, the RAS inhibition due to AT1a had more beneficial advantages of reducing proteinuria and attenuating the levels of glomerular TGF-β1 and extracellular matrix.

Hypertension is a major risk factor for renal injury; however, the mechanism underlying the development and progression of renal damage in hypertensive patients and experimental animals remains to be elucidated. ANG II, the main molecular effector of the renin-angiotensin system (RAS) and one of the most important regulators of systemic blood pressure, controls vasoconstriction, the facilitation of central sympathetic outflow and peripheral neurotransmission, and the release of vasopressin and aldosterone and has direct volume-retaining effects (12). ANG II also exhibits growth-promoting effects, particularly on the renal cells (31). Two major types of ANG II receptors (type 1 and type 2) have been identified and cloned to date (11, 16). The hemodynamic and nonhemodynamic effects of ANG II, however, are mediated primarily by the ANG II type 1 receptor (AT1) (5, 28). Because of the pivotal role of ANG II, pharmacological blockade of the RAS recently has become a mainstay in the treatment of renal and cardiovascular diseases. The effects of RAS-inhibiting agents, such as ANG I-converting enzyme inhibitors (ACEI) and AT1 antagonists (AT1a), on histological and molecular renal alterations have been studied in vivo in various experimental models of hypertension (21, 22). We have reported previously that enhanced expression of the platelet-derived growth factor (PDGF) B-chain mRNA in the glomeruli preceded the appearance of histological changes in spontaneously hypertensive rats (SHR) and that the glomerular PDGF-B-chain mRNA was suppressed by the administration of the ACEI cilazapril (33). In addition, treatment with cilazapril or the AT1a L-158,809, attenuated the expression of PDGF-B-chain and transforming-growth factor (TGF)-β1 mRNA in the glomeruli of deoxycorticosterone acetate (DOCA) salt-treated hypertensive rats without reducing blood pressure (25). Thus the renoprotective effects of RAS inhibitors are mediated in part by unknown mechanisms and are independent of the antihypertensive effects of the agents. To investigate the role of RAS on the hypertension and renal injury in salt-sensitive hypertension, we have evaluated the effects of chronic RAS inhibition on glomerular injury in Dahl salt-sensitive rats using an ACE inhibitor and an AT1-receptor antagonist.

METHODS

Materials. Dahl-Iwai salt-sensitive (DS) and salt-resistant (DR) rats (5 wk old) were purchased from Japan SLC (Shizuoka, J apan). All rats were housed in climate-controlled metabolic cages with a 12:12-h light-dark cycle. The animals received a low (0.3%)-NaCl or high (8%)-NaCl diet (MF, Oriental Yeast, Tokyo, J apan), with water provided ad libitum. Oligonucleotides for RT-PCR were synthesized with a model 380B DNA synthesizer (Applied Biosystems, Foster City, CA). The location of the oligonucleotides of primer pairs were as follows: PDGF-B-chain, 1079–1098 and 1601–1620 (27). Cilazapril, an ACEI, and TCV-116, an AT1a, were donated by Eisai Pharmaceutical (Tokyo, J apan). At 7 wk of age, the DS rats were divided randomly into two groups. One group received a diet containing 0.3% NaCl (DSL; n = 6), the other group received a diet containing 8% NaCl (DSH) for 6 wk. The DSH rats were...
in RPMI-1640 medium for 30 min at 37°C. Total RNA was extracted by the acid-guanidium-phenol-chloroform method. The final RNA pellets were washed with 70% ethanol and resuspended in 100 µl diethylpyrocarbonate-treated water. The RNA was quantified by measuring the absorbance at 260 nm and stored at −20°C until the assay.

Quantification of RNA expression by competitive RT-PCR. First, mutant cDNAs for competitive PCR were generated using the PCR MIMIC Construction Kit (Clontech, Palo Alto, CA). The mutant fragments for G3PDH and TGF-β1 messages were 556-bp fragments long, and the mutant fragments for the PDGF-B-chain and PCNA messages were 276-bp fragments long. Subsequently, these fragments were reamplified with four sets of specific primers to determine their ability to act as competitors for the native mRNAs. The obtained products were as follows: G3PDH, 596 bp; TGF-β1, 598 bp; PDGF-B-chain, 316 bp; and PCNA, 316 bp. These fragments were purified using a CHROMA SPIN Column (Clontech) and diluted to 100 amol/µl with 10 µg/µl ultrapure glycerol.

Extracted glomerular RNA was reverse transcribed using a GeneAmp RNA PCR kit (Perkin Elmer Cetus, Norwalk, CT). RT was performed with 10 ng of RNA per reaction using random hexamer (2.5 µM), reverse transcriptase (2.5 U/µl), and deoxynucleotide triphosphate (dNTP; 1 mM) at the following conditions: 42°C for 5 min, 99°C for 5 min, and 4°C for 5 min. The resulting cDNA was resuspended in 50 µl deionized, autoclaved water for competitive PCR analysis. The linear portion of the relationship between the native cDNAs and competitive mutant cDNAs then was determined for G3PDH, TGF-β1, PDGF-B-chain, and PCNA. For a preliminary analysis, a fixed amount (0.5 ng) of cDNA derived from control DSH RNA was coamplified with tenfold serial dilutions (1–10 n) of the competitive mutant cDNAs using the four primer sets and the preceding PCR kit. For fine-tuned competitive PCR, another reamplification was performed using twofold serial dilutions (2–2) of one of the dilution steps of the preliminary PCR as a starting point (G3PDH, 10 n; TGF-β1, 10 n; PDGF-B-chain, 10 n; PCNA, 10 n). The competitive PCR was performed using the preceding PCR kit and a thermal cycler (TP Cycler-100, Toyobo, Osaka, J apan) under the following conditions: G3PDH and PDGF-B-chain, 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min; TGF-β1, 40 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min; and PCNA, 37 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min. Aliquots of the PCR products of cDNA and competitive mutant cDNA were electrophoresed on 1.5% agarose gels, visualized by ethidium bromide staining, and photographed using an instant positive/negative film (337; Polaroid, Cambridge, MA). The negatives were analyzed by a scanning densitometer (Scanning Imager 300-SX, Molecular Dynamics, Sunnyvale, CA), and the relative integrated density of each band was calculated by taking the absorbance multiplied by the surface area. Finally, the ratios between the densitometric readings of the native cDNA- and mutant cDNA-PCR products were plotted using logarithmic scale on the y-axis against the logarithmic dilutions of the mutant cDNA on the x-axis. After establishment of the working ranges in which a linear relationship existed, cDNAs from all individual samples were subjected to competitive PCR analysis, using the TGF-β1, PDGF-B-chain, and PCNA primers. Control analyses were carried out using G3PDH primers.
Statistical analysis. All results were expressed as means ± SE and statistically analyzed by ANOVA. P values < 0.05 were accepted as statistically significant.

RESULTS

Time courses of SBP, HR, and body weight. In the control DSH group, the SBP increased markedly with age and was significantly higher than in the DRL and DSL animals throughout the ages of 7–13 wk and 8–13 wk, respectively (Fig. 1). The cilazapril + DSH and TCV + DSH animals generally did not exhibit significantly reduced SBP compared with the control DSH animals, except for 11-wk-old TCV + DSH animals. Moreover, no significant difference in SBP existed between the cilazapril + DSH and TCV + DSH groups. The HR was significantly lower in the DRL animals compared with the control DSH animals. Neither cilazapril nor TCV-116 treatment significantly altered the HR during the experimental period (data not shown). The animals’ body weights throughout the observation period were as follows: DRL > DSL > control DSH > TCV + DSH > cilazapril + DSH (data in 13-wk-old animals are shown in Table 2). During the experimental period, the mortality rate in each group was as follows: control DSH, 14.3%; DSL, DRL, cilazapril + DSH, and TCV + DSH, 0%.

Changes in urine volume, urinary protein, and NAG excretion. The urine volume was highly increased in the control DSH animals compared with the DSL and DRL animals (Fig. 2A). The increase in urine volume also occurred in cilazapril + DSH and TCV + DSH animals. Especially in the cilazapril + DSH animals, the urine volume tended to increase between the ages of 9 and 12 wk. Both urinary protein and NAG excretion was significantly increased in control DSH animals compared with DRL and DSL animals (Fig. 2B and C). TCV-116 treatment significantly reduced the increase in urinary protein and NAG excretion in the DSH animals. Cilazapril treatment did not significantly decrease these two parameters compared with control DSH animals.

Evaluation of renal function and RAS. The effects of a high-salt diet and treatment with cilazapril or TCV-116 on numerous indicators of renal function were evaluated at the end of the experimental period (i.e., in 13-wk-old animals). The levels of serum sodium, potassium, chloride, total protein, and urea nitrogen in the trunk blood did not differ significantly among the groups (Table 1). The serum creatinine levels, however, were significantly elevated in control DSH animals compared with the DSL and DRL animals. Treatment with cilazapril or TCV-116 prevented this elevation. Serum uric acid levels were lower in the DSL group than in the control DSH group. The PRA level under TCV-116 treatment was increased significantly above those of control DSH animals, which was rather elevated compared with DSL or DRL despite the high-salt treatment. Finally, urinary aldosterone levels were measured in 7- and 12-wk-old animals (Table 1). DRL animals that had received a low-salt diet for 5 wk demonstrated markedly increased aldosterone levels. A high-salt diet, in contrast, reduced urinary aldosterone concentrations to undetectable levels, independent of the treatment with cilazapril or TCV-116.

Organ weights and body weight ratios. We also analyzed the body weights and organ weights of the five groups at the end of the experimental period. The kidney weights of TCV + DSH animals, however, were significantly higher than those of the DSH animals. Heart weight was significantly lower in the DSH and DRL groups than in the control DSH group and was not affected by cilazapril or TCV-116. Kidney weight-to-body weight and heart weight-to-body weight ratios were significantly lower in DSL and DRL groups than in the control DSH group, and these ratios were not affected by either drug.

Histological findings. The glomerular structures in the five experimental groups were compared using light microscopy (Fig. 3). DSL and DRL animals showed...
normal glomeruli. Control DSH animals, in contrast, exhibited severely damaged glomeruli characterized by mesangial expansion, increases in the mesangial matrix accompanied by sclerotic changes, and cell proliferation. In animals treated with cilazapril or TCV-116, the extent of the glomerular injury was markedly decreased compared with the control DSH animals. These findings were confirmed by mesangial-injury scoring and analysis of glomerular cellularity. Both of these parameters were elevated in the control DSH animals compared with DSL and DRL animals (Table 3). Moreover, the glomerular cellularity was significantly lower in DRL than in DSL animals. Treatment with cilazapril or TCV-116 significantly improved both indicators of glomerular injury to the same extent. We also examined the glomerular sizes in the five groups. The glomerular size distribution indicated that the glomeruli in DSL animals tended to be smaller and those in TCV + DSH animals tended to be larger among the five experimental groups (Fig. 4). Finally, as an indicator of the extracellular matrix (ECM), glomerular collagen (type III, IV, VI) expression was assessed by immunofluorescence. Among the three types of collagens examined, type III collagen was strongly expressed in the
mesangial region of control DSH animals, but not of DSL and DRL animals (Fig. 5). Treatment with TCV-116 markedly suppressed the expression of type III collagen. The expression of type IV and VI collagens did not differ significantly among the five groups.

Quantification of glomerular expression of PDGF B-chain, TGF-β1, and PCNA mRNAs. To quantify the glomerular expression of PDGF B-chain, TGF-β1, and PCNA by competitive RT-PCR, we first determined the linear range of the ratios of coamplified mutant cDNAs and native cDNAs reverse transcribed from glomerular RNA as described in Methods (Fig. 6A). For the quantification of all native cDNA samples, we chose the following logarithmic dilutions of the mutant cDNAs: 2^-7 (7.8-10^-3 amol/µl) for G3PDH, 2^-5 (3.125-10^-4 amol/µl) for PDGF B-chain, 2^-3 (3.125-10^-5 amol/µl) for TGF-β1, and 2^-1 (1.56-10^-6 amol/µl) for PCNA. For the competitive PCR reactions, 2 µl of these dilutions were added to 2 µl of each native cDNA (0.25 ng/µl). The resulting PCR products were quantified by densitometric scanning as described in the Methods (Fig. 6B). The results demonstrated that control DSH animals exhibited significantly enhanced levels of glomerular PDGF B-chain and PCNA mRNAs than did DSL and DRL animals. TCV-116 treatment significantly reduced the levels of glomerular PCNA and TGF-β1 compared with the control DSH group. The PDGF B-chain level reduced by TCV-116 treatment but not significantly. Cilazapril treatment also reduced the levels of glomerular PDGF B-chain, TGF-β1, and PCNA compared with control DSH animals, but these differences were not statistically significant. G3PDH levels did not differ significantly among the groups.

**DISCUSSION**

Dahl rats are considered a useful model of human salt-sensitive hypertension, because high dietary sodium levels exaggerate the development of hypertension in strains that are genetically predisposed to hypertension (7). Although the pathogenesis of salt-sensitive hypertension in these animals remains to be elucidated, genetic factors (7), as well as nitric oxide (14), have been implicated in determining the individual’s sensitivity to salt ingestion. Several lines of evidence implicate the kidney as a primary determinant of arterial blood pressure in Dahl rats (8). The mechanism underlying hypertensive renal damage, including nephrosclerosis, however, is unknown, as are the factors that contribute to the progression of hypertension-related nephropathy and glomerulosclerosis. We therefore investigated the potential therapeutic effects of RAS inhibition by administration with either ACEI (cilazapril) or AT1a (TCV-116) to examine the role of RAS in glomerular damage observed in high-salt-treated DS rats. Some of our experimental results, the histological analyses and analysis of ECM expression patterns by immunofluorescence, demonstrated that salt-sensitive hypertension was complicated with significant glomerular injury. In contrast, the inhibition of the RAS by both

---

**Table 1. Effects of salt-sensitive hypertension and treatment with RAS inhibitors on the levels of serum electrolytes, total protein, urea nitrogen, creatinine, uric acid, PRA, and urinary aldosterone excretion**

<table>
<thead>
<tr>
<th></th>
<th>Control DSH (n = 12)</th>
<th>DSL (n = 6)</th>
<th>DRL (n = 6)</th>
<th>Cilaza + DSH (n = 6)</th>
<th>TCV + DSH (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium, meq/l</td>
<td>139.7 ± 1.96</td>
<td>139.0 ± 3.09</td>
<td>138.0 ± 5.76</td>
<td>140.3 ± 0.67</td>
<td>138.8 ± 2.44</td>
</tr>
<tr>
<td>Potassium, meq/l</td>
<td>7.87 ± 0.34</td>
<td>7.65 ± 0.29</td>
<td>7.50 ± 0.33</td>
<td>7.07 ± 0.12</td>
<td>7.33 ± 0.30</td>
</tr>
<tr>
<td>Chloride, meq/l</td>
<td>101.1 ± 1.61</td>
<td>104.2 ± 2.30</td>
<td>105.0 ± 4.57</td>
<td>98.8 ± 0.93</td>
<td>99.0 ± 2.05</td>
</tr>
<tr>
<td>Total protein, g/dl</td>
<td>5.11 ± 0.15</td>
<td>5.33 ± 0.23</td>
<td>4.85 ± 0.22</td>
<td>5.42 ± 0.11</td>
<td>5.37 ± 0.16</td>
</tr>
<tr>
<td>Urea nitrogen, mg/dl</td>
<td>21.8 ± 1.22</td>
<td>18.8 ± 1.20</td>
<td>19.2 ± 1.22</td>
<td>19.7 ± 0.80</td>
<td>21.0 ± 1.37</td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>0.733 ± 0.053</td>
<td>0.533 ± 0.033*</td>
<td>0.567 ± 0.049*</td>
<td>0.633 ± 0.033</td>
<td>0.633 ± 0.033</td>
</tr>
<tr>
<td>Uric acid, mg/dl</td>
<td>1.96 ± 0.28</td>
<td>1.32 ± 0.075*</td>
<td>1.37 ± 0.095</td>
<td>1.62 ± 0.098</td>
<td>1.77 ± 0.19</td>
</tr>
<tr>
<td>PRA, ng·ml⁻¹·h⁻¹</td>
<td>3.60 ± 1.32</td>
<td>2.49 ± 0.625</td>
<td>3.23 ± 0.453</td>
<td>5.72 ± 1.36</td>
<td>8.35 ± 2.34‡</td>
</tr>
<tr>
<td>Urinary aldosterone, ng/day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-wk old</td>
<td>ND</td>
<td>5.22 ± 3.04</td>
<td>39.87 ± 11.4†</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7-wk old</td>
<td>13.84 ± 1.27</td>
<td>15.37 ± 1.03</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. For a description of the experimental groups, see Table 1. *P < 0.05 vs. control DSH; †P < 0.05 vs. control DSH, DSL, Cilaza + DSH, and TCV + DSH; ‡P < 0.05 vs. control DSH, DSL, and TCV.

---

**Table 2. Effects of salt-sensitive hypertension and treatment with RAS inhibitors on body, kidney, and heart weights and body weight ratios**

<table>
<thead>
<tr>
<th></th>
<th>Control DSH (n = 12)</th>
<th>DSL (n = 6)</th>
<th>DRL (n = 6)</th>
<th>Cilaza + DSH (n = 6)</th>
<th>TCV + DSH (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>347.6 ± 6.85</td>
<td>374.7 ± 7.32</td>
<td>405.0 ± 17.8*</td>
<td>319.3 ± 7.49*</td>
<td>343.8 ± 10.05</td>
</tr>
<tr>
<td>Kidney weight, g</td>
<td>1.832 ± 0.032</td>
<td>1.514 ± 0.024*</td>
<td>1.426 ± 0.037*</td>
<td>1.676 ± 0.057*</td>
<td>1.836 ± 0.047†</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>1.440 ± 0.026</td>
<td>1.185 ± 0.017*</td>
<td>1.232 ± 0.051*</td>
<td>1.380 ± 0.040</td>
<td>1.493 ± 0.050</td>
</tr>
<tr>
<td>Kidney wt/body wt, %</td>
<td>0.537 ± 0.011</td>
<td>0.407 ± 0.004*</td>
<td>0.361 ± 0.004*</td>
<td>0.530 ± 0.020</td>
<td>0.539 ± 0.015</td>
</tr>
<tr>
<td>Heart wt/body wt, %</td>
<td>0.415 ± 0.010</td>
<td>0.317 ± 0.006*</td>
<td>0.305 ± 0.005*</td>
<td>0.432 ± 0.007</td>
<td>0.436 ± 0.018</td>
</tr>
</tbody>
</table>

Values are means ± SE. For a description of the experimental groups, see Table 1. *P < 0.05 vs. control DSH; †P < 0.05 vs. Cilaza + DSH.
cilazapril and TCV-116 significantly attenuated these histological changes using a light microscope. The renoprotective effects of these agents were independent of their antihypertensive actions. These findings suggest that glomerular injury in salt-sensitive hypertension is attributable to the activation of RAS.

In vitro analyses found that, among the components of the RAS, ANG II directly stimulates the growth of renal mesangial cells via AT1 receptors (5). In in vivo models of renal disease caused by excessive ANG II expression, mesangial matrix expansion rather than cell proliferation usually occurs, indicating that the increased protein synthesis is a dominant response of mesangial cells to ANG II in vivo (26). Moreover, in vivo transfection of the genes for renin and angiotensinogen into the glomeruli has been shown to induce mesangial matrix expansion (2). These observations suggest that the increased protein synthesis by mesangial cells in response to ANG II may play a key role in the development of glomerulosclerosis. This fibrogenic effect of ANG II likely is mediated by TGF-β (20). Transfection of TGF-β1 into the kidney induces glomerulosclerosis characterized by the accumulation of ECM proteins, supporting the hypothesis that an increase in TGF-β1 expression also is responsible for glomerulosclerosis in vivo (17). Renal TGF-β1 gene expression reportedly is enhanced in several renal disease models, including glomerulonephritis (4) and obstructive nephropathy (19), as well as in some models of hypertensive nephropathy, including DOCA salt hypertensive rats (21) and spontaneously hypertensive stroke-prone (SHRSP) rats in the malignant phase (22). However, the role of TGF-β1 in salt-sensitive hypertension has not yet been determined. In this study, we detected the enhanced levels of TGF-β1 and type III collagen in the glomeruli of rats with salt-sensitive hypertension. Conversely, RAS inhibition by TCV-116 significantly reduced the glomerular TGF-β1 level accompanied by the suppression

Table 3. Effect of salt-sensitive hypertension and treatment with RAS inhibitors on mesangial injury score and glomerular cellularity

<table>
<thead>
<tr>
<th></th>
<th>Control DSH (n = 12)</th>
<th>DSL (n = 6)</th>
<th>DRL (n = 6)</th>
<th>Cilaza + DSH (n = 6)</th>
<th>TCV + DSH (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesangial injury score</td>
<td>185.7 ± 8.45</td>
<td>58.5 ± 5.09†</td>
<td>39.3 ± 1.76†</td>
<td>121.1 ± 24.1*</td>
<td>120.9 ± 9.56*</td>
</tr>
<tr>
<td>Cellularity (nuclear cells/glomerulus)</td>
<td>77.8 ± 1.44</td>
<td>41.2 ± 1.37†</td>
<td>33.0 ± 0.75‡</td>
<td>51.7 ± 1.20†</td>
<td>50.4 ± 1.25†</td>
</tr>
</tbody>
</table>

Values are means ± SE. For a description of the experimental groups, see Table 1. *P < 0.01; †P < 0.0001 vs. control DSH; ‡P < 0.0001 vs. DSL.
sion of type III collagen without antihypertensive effect. The TCV-116 treatment also significantly suppressed the mRNA level of glomerular PCNA. PCNA is a nuclear protein that is expressed from late G1 through the M phase of the cell cycle (27). PCNA expression previously has been shown by immunostaining to be elevated in ANG II-infused Sprague-Dawley rats (18). These findings indicate that RAS may contribute to glomerular cell proliferation in salt-sensitive hypertension in vivo. This hypothesis is consistent with the change of glomerular cellularity due to RAS blockade using TCV-116. We further investigated the glomerular mRNA levels of the PDGF B-chain, a growth factor that accelerates mesangial cell proliferation (1). The PDGF B-chain mRNA levels were increased significantly in animals with salt-sensitive hypertension. In contrast to the change of PCNA level, however, RAS inhibition did not significantly alter the glomerular PDGF B-chain level. These findings suggest that cell proliferation in high-salt-treated DS rats may be associated with factors other than the PDGF B-chain, at least in this chronic hypertensive phase.

Fig. 4. Effects of salt-sensitive hypertension and treatment with renin-angiotensin system inhibitors on glomerular diameter. For a description of experimental groups, see Fig. 1. Frequency distribution of glomerular size demonstrated the tendency that DRL animals had smaller and TCV + DSH animals had larger glomeruli among the experimental groups.

Fig. 5. Analysis of glomerular collagen expression by immunofluorescence. For a description of experimental groups, see Fig. 1. Type III collagen (top row) was strongly expressed in the mesangium of control DSH animals (A) but not in the glomeruli of DSL (B) and DRL animals (C). In comparison with the cilazapril (D) treatment, TCV-116 (E) markedly suppressed the expression of type III collagen in DSH. Expression of type IV (middle) and VI (bottom) collagens did not differ significantly among the 5 groups.
Fig. 6. Quantification of glomerular mRNA expression by competitive RT-PCR. For a description of experimental groups, see Fig. 1. A: linear range in the ratios of coamplified PCR products was determined using native cDNA that was obtained by reverse transcription of glomerular RNA from a control DSH animal and competitive mutant cDNAs for G3PDH, platelet-derived growth factor (PDGF) B-chain, transforming growth factor (TGF)-β1, and proliferating cell nuclear antigen (PCNA) at serial logarithmic dilutions of $2^{-1}$–$2^{-9}$. B: for quantification of native cDNA samples, competitive PCR was performed by mixing a constant amount (2 µl) of native cDNA (0.25 ng/µl) with the following dilutions of mutant cDNAs; $2^{-7}$ (7.8 × $10^{-4}$ amol/µl) for G3PDH, $2^{-5}$ (3.125 × $10^{-4}$ amol/µl) for PDGF B-chain, $2^{-5}$ (3.125 × $10^{-5}$ amol/µl) for TGF-β1, and $2^{-6}$ (1.56 × $10^{-4}$ amol/µl) for PCNA. Densitometric quantification of the PCR products demonstrated that control DSH animals exhibited significantly higher levels of glomerular PDGF B-chain and PCNA than did DSL and DRL animals. TCV-116 treatment significantly reduced the levels of glomerular PCNA and TGF-β1 compared with the control DSH animals. G3PDH levels did not differ significantly among the groups. Bars represent means ± SE. Representative blots are shown above each graph, lane 1–5, control DSH, DSL, DRL, Cilaza + DSH, and TCV + DSH. *P < 0.05 vs. control DSH; **P < 0.01 vs. control DSH. MM, molecular wt marker.
High-salt treatment usually suppresses the renin secretion from the juxtaglomerular apparatus; however, it is noted that the suppression of PRA caused by high-salt intake is blunted in DS rats compared with DR rats in the literature (10). Von Lutterotti et al. reported that PRA gradually increases after 4 wk of high salt loading in DS rats, presumably secondary to the glomerulosclerosis and renal arterial injury observed in salt-loaded DS rats with hypertension (29). In our experiment, the period of blood sampling for PRA was 6 wk after the initiation of high-salt treatment. If the sampling for renin were right after the high-salt treatment, the suppression of PRA might be apparent. The chronic high-salt-treated DS rats in our study showed markedly elevated blood pressure and histopathologically severe arterial and renal damages. When the data are taken into consideration, it is presumable that the RAS is one of the major hypertensinogenic factors related to the kidney of DS rats in the chronic phase. Furthermore, PRA was significantly elevated in the TCV-116 group more than the cilazapril group. This finding was also agreeable with the results of the previous literature, which had demonstrated the other hypertension model, with DOCA salt hypertension (21) or SHRSP rats (22), indicating that the AT₁ receptor plays a role in the negative feedback regulation of renal renin secretion and that AT₁a is a more potent inhibitor of this regulatory mechanism than ACEI, although neither drug reduced the SBP. The urinary aldosterone excretion of DR rats was increased after 5 wk on a low-salt diet, whereas it was suppressed completely in DS rats receiving a high-salt diet; however, the differences between the RAS-inhibitory ways by ACEI and AT₁a were not found as difference of urinary aldosterone level. The suppression of aldosterone secretion in high-salt-treated DS rats was probably associated with not only the renin-ANG II-aldosterone cascade but also adrenocortical suppression due to the extremely hypertensive state during the chronic experimental period. Additionally, the blockade of adrenocortical AT₁ receptors, which stimulate aldosterone secretion, is also involved in the suppression of aldosterone under the TCV-116 treatment (30).

Although the degree of the histological improvement of the glomerular injury between ACEI and AT₁a is not significantly different, the glomeruli in the TCV + DSH or cilazapril + DSH group have been damaged compared with those in the low-salt-treated groups. During our experimental period, the effect of AT₁a, including significant suppression of glomerular TGF-β1 and ECM levels, did not seem to take advantage of the histologically renoprotective effect in this salt-sensitive hypertension model; however, in the AT₁a group, the reduction of proteinuria and the attenuation of urinary NAG excretion, which also indicates the protection of the interstitial injury, will strongly prevent the further progression of glomerulosclerosis. It is expected that a long study or a high-doses study, whether or not accompanied by antihypertensive action, will make differences in not only the glomerular TGF-β1 and ECM levels but also in the histological changes between the two RAS-inhibitory groups. O’Donnell et al. (24) reported that long-term (22 wk) enalapril treatment (50–200 mg/l drinking water), with reduction of blood pressure, did not affect albuminuria, glomerulosclerosis, and glomerular hemodynamics, suggesting that ACEI may not affect the course of renal disease in a setting of high-salt intake of DS rats (24), whereas there have been no reports of a long-term experiment using AT₁a in high-salt-treated DS rats. The difference of renoprotective action between ACEI and AT₁a may be associated with the blocking manner of ANG II action. AT₁a is able to inhibit the action of ANG II produced via any ANG II synthetic system other than ACE (3). Moreover, the relatively enhanced AT₂ effect by chronic AT₂ blockade (15, 23, 32) may contribute to the renoprotection in the AT₁a group. In the ACEI group, the enhanced bradykinin induced by the blockade of kininase II may play a small role in the reduction of proteinuria in high-salt-treated DS rats.

The glomerular diameters tended to be increased in DS rats receiving a high-salt diet compared with the low-salt-treated DR rats. Interestingly, TCV-116 treatment but not cilazapril treatment tended to further increase the glomerular diameter despite improving the associated glomerulosclerosis and proliferative changes. It is possible that this tendency to increase glomerular size in the TCV-116-treated group is associated with the relaxation of glomeruli induced by the withdrawal from the mesangial contractile actions of ANG II via AT₁a receptors (5); however, in this study, the precise mechanism inducing this tendency was not clarified.

In conclusion, we observed that the RAS blockade significantly attenuated the glomerular injury in high-salt-treated DS rats independently of the antihypertensive effect. In particular, the reduction of the levels of glomerular TGF-β1, PCNA and ECM, and proteinuria was prominent on the TCV-116 treatment. This study indicates that the glomerular-originated TGF-β1 affected this glomerular injury and that the chronic treatment by TCV-116 may be more beneficial for renoprotection in DS hypertensive rats.

Perspectives

The present study demonstrated that the chronic blockade of RAS in high-salt-treated DS rats was effectively protective to the hypertensive nephropathy, especially paying attention to the glomerulopathy. Furthermore, the AT₁a treatment showed more renoprotective effect than ACEI without lowering blood pressure. This fact might indicate not only that the AT₁a has more potent ANG II inhibitory effect but also that relatively enhanced AT₂ action (15, 23, 32) plays a key role in the suppression of glomerulopathy in high-salt-treated DS rats. The reduction of proteinuria by AT₁a treatment might be associated with the decrease of glomerular capillary pressure due to both the morphological repair of glomeruli and the glomerular relaxation. The renoprotective effects of ACEI detected by microscopy but not by molecular technique might be partly associated with
REFERENCES


