Long-Term, High-Dosage Candesartan Suppresses Inflammation and Injury in Chronic Kidney Disease: Nonhemodynamic Renal Protection

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Recent evidence suggests that higher-than-usual antihypertensive dosages of renin-angiotensin-aldosterone system blockers may provide additional protection from progression of chronic renal disease; however, there have been few long-term studies, and the underlying mechanisms remain uncertain. This study examined the effects of long-term (14 mo) administration of ultrahigh dosages of the angiotensin receptor blocker candesartan on the progression of renal injury in spontaneously hypertensive rats (SHR). Beginning 8 wk after birth, SHR underwent unilateral nephrectomy and were given vehicle (control), or candesartan at a standard 5 mg/kg per d (T5), high 25 mg/kg per d (T25), or ultrahigh 75 mg/kg per d dosage (T75). After 2 wk, BP was reduced in all treated groups; however, it was better controlled in the high-dosage groups (T25 and T75). Urinary protein was significantly reduced in T75 after 2 wk of treatment and was also declined in the other two treatment groups but only after 2 mo. Exogenous angiotensin II test showed that complete angiotensin receptor blockade was achieved only in the high-dosage groups. Renal inflammation and macrophage (ED-1) infiltration were significantly ameliorated in both T25 and T75 but not in T5 rats. This was associated with the changes of tubular expression of monocyte chemoattractant protein-1, RANTES (regulated upon expression normal T cell expressed and secreted), and the phosphorylated NF-κB, a marker for activation. Suppression of ED-1, monocyte chemoattractant protein-1, and RANTES expression and NF-κB activation were greater in T75 as compared with T25. These findings suggest that candesartan has dosage-dependent, anti-inflammatory effects that are mediated by suppression of NF-κB activation and chemokine expression. Renal protection with high-dosage therapy may depend on these nonhemodynamic effects.


Systemic and glomerular hypertension, resulting in part from activation of the renin-angiotensin-aldosterone system (RAAS), are believed to be important factors that drive progression of chronic kidney disease (CKD), regardless of the initial insult (1–3). Recent evidence suggests that renal inflammation, characterized by macrophage infiltration, also contributes to progression of renal disease and that angiotensin II (AngII) is a proinflammatory cytokine and contributes to the initiation of inflammation (4–9). In kidney and other organs, AngII stimulates expression of proinflammatory mediators, including growth factors, cytokines and chemokines, and adhesion molecules (10,11). Infusion of AngII into rats causes inflammatory cells to invade the glomeruli and interstitium (12–14). In addition, inflammatory cells can themselves produce AngII, generating a positive feedback loop that perpetuates the inflammatory response and progressive renal injury (15). AngII seems to exert its proinflammatory actions primarily via the AngII type 1 receptor (AT1R) (16), a seven-transmembrane, G protein–coupled receptor that is expressed on many cells, including kidney epithelial cells, endothelial cells, and vascular smooth muscle cells (17).

AT-1 receptor blockers (ARB) have been used extensively in the treatment of various types of CKD (18,19). Although these compounds represent a major therapeutic advance, they fail to arrest completely the progression of disease and tend only to postpone the need for renal replacement therapy. Recently, it was suggested that high dosages of ARB have even greater renoprotective effects than standard-dosage therapy, suppressing extracellular matrix accumulation and even reversing glomerulosclerosis (20–23). However, the mechanisms by which high-dosage therapy reduces injury and, in particular, suppresses renal inflammation have hardly been studied. The aim of this work was to examine the effects of long-term, high-dosage administration of the AT1R antagonist candesartan on injury in a model of chronic progressive kidney damage and to determine the mechanism of its protective effect. Spontaneously hypertensive rats (SHR) that underwent unilateral nephrectomy were treated with standard-, high-, and ultrahigh-dosage candesartan for up to 14 mo. We found that although there was little additional effect of ultrahigh-dosage candesartan on renal hemo-
dynamics, it markedly reduced injury, seemingly by suppressing inflammation.

Materials and Methods

Design of the Animal Experiments

Male SHR were housed according to published guidelines and fed a standard diet. At 8 wk of age, rats underwent unilateral nephrectomy and were randomly assigned to the following groups: (1) Control rats were untreated (n = 32); (2) standard-dosage group (T5), rats received 5 mg/kg per d candesartan in drinking water (n = 32); (3) high-dosage group (T25), rats received 25 mg/kg per d candesartan (n = 32); or (4) ultrahigh-dosage group (T75), rats received 75 mg/kg per d candesartan (n = 32). Treatment was continued until the rats were killed at 2 wk (three rats from each group), 6 wk (eight rats from each group), 9 mo (10 rats from each group), and blood samples were collected for determination of single-nephron protein losses and specimen collection. Rats also received a 0.5-ml bolus and a second 0.5-ml infusion via a tail vein (n = 32). Treatment was continued until the rats were killed at 2 wk (three rats from each group), 6 wk (eight rats from each group), 9 mo (10 rats from each group), and 14 mo (10 rats from each group) after surgery.

Whole-Animal Studies

Systolic BP (SBP) was measured using tail-cuff plethysmography (IITC; Life Science, Woodland Hills, CA). For urine collection, rats were placed in metabolic cages for 24 h, urine volume measured, and urine protein was determined as described previously (27). Blood was obtained from the tail vein. At 2 wk, three rats from each group underwent an exogenous AngII challenge test. At 6 wk, glomerular hemodynamics were assessed in eight rats from each group using micropuncture techniques. At 9 mo (10 rats from each group) and 14 mo (10 rats from each group), rats were anesthetized and mean arterial pressure (MAP), renal plasma flow (RPF), and GFR were measured. GFR was determined by inulin clearance (Cin), and RPF was recorded. Whole-kidney filtration fraction (FF) was calculated using the standard formula. Initial glomerular capillary plasma flow rate was estimated using the measured values for single-nephron GFR and whole-kidney FF.

Exogenous AngII Challenge Test

The completeness of AT1R blockade was assessed 2 wk after initiation of candesartan therapy in three rats from each group. Briefly, rats were anesthetized and prepared for clearance studies. MAP was monitored, GFR was determined by inulin clearance (Cin), and RPF was measured by ultrasonic flow probe as described in the next section. After two 15-min baseline periods, rats received an intravenous infusion of AngII at a rate of 0.2 mg/kg per min. After a 15-min equilibration period, MAP, GFR, and RPF were measured. After kidneys were excised, one portion of the kidney was fixed in 10% formaldehyde for immunohistochemical analysis, and the remainder was snap-frozen in liquid nitrogen and stored at −80°C for RNA and protein extraction.

Immunohistochemical Studies

Immunohistochemical staining was performed with a Vectastain ABC kit (Vector Laboratories, Burlingame, CA). The antibodies for monoclonal chemoattractant protein-1 (MCP-1) and RANTES (regulated upon expression normal T cell expressed and secreted) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA), antibodies for ED-1 were purchased from Serotec (Oxford, UK), and the antibodies for NF-κB p65 and phosphorylated NF-κB p65 were purchased from Cell Signaling (Beverly, MA). The Alexa Fluor goat anti-rabbit or anti-mouse IgG (Molecular Probes, Eugene, OR) was used as secondary antibody. As a negative control, the primary antibody was replaced by nonimmune serum from the same species; no staining occurred. Severity of inflammation was graded by counting the absolute number of ED-1–positive cells in each field and reported as the mean of 20 randomly chosen fields.

Morphologic Analysis

Formalin-fixed kidneys were embedded in paraffin and prepared in 3-μm-thick sections. For general histology, sections were processed for hematoxylin/eosin, periodic acid-Schiff, and Masson-Trichrome staining. A semiquantitative morphometric score index (SI) was used to evaluate the degree of glomerulosclerosis (22,25). Sclerosis was defined as collapse and/or obliteration of glomerular capillary tuft accompanied by hyaline material and/or increase of matrix. Severity of sclerosis for each glomerulus was graded from 0 to 4+ as follows: 0, no lesion; 1+, sclerosis of <25% of the glomerulus; 2+, ≥25% to <50%, >50 to 75%, and >75% of the glomerulus, respectively. A whole-kidney glomerular SI was obtained by averaging scores from 30 randomly selected glomeruli. The percentage value for SI in each individual rat was calculated. Tubulointerstitial fibrosis was assessed semiquantitatively. All sections were examined without knowledge of the treatment protocol. The number of inflammatory cells that infiltrated the renal interstitial area was determined by counting nuclei in hematoxylin/eosin-stained sections. Values for individual rats were determined by averaging of 20 randomly chosen fields.

Micropuncture and Clearance Studies

Six weeks after nephrectomy, eight rats from each group were anesthetized and prepared for micropuncture determination of the pressures, flows, and resistances that govern glomerular ultrafiltration, as described previously (24), with minor modifications. Briefly, polyethylene catheters were inserted into the femoral artery, left and right jugular veins, and the left ureter for infusion of solutions and collection of samples. A tracheostomy was performed. Arterial pressure was continuously measured by a pressure transducer that was connected to a computer that was running WINDAS software (DATAQ Instruments, Akron, OH). All rats received isoncotic rat plasma at a rate of 0.1 ml/min for a total of 10 ml/kg body wt, followed by a sustained infusion of plasma at a rate of 0.5 ml/h to compensate for surgical losses and specimen collection. Rats also received a 0.5-ml bolus and a sustained infusion (0.5 ml/h) of [3H]inulin in saline. Individual tubules were punctured using sharpened glass pipettes. Tubular fluid, urine, and blood samples were collected for determination of single-nephron and whole-kidney GFR by the measurement of [3H] inulin activity. Pressures were measured in cortical tubules and efferent arterioles using the servo-null, micropipette technique. Glomerular capillary pressure was estimated using the stop-flow technique. Whole-kidney RPF was measured after the conclusion of the micropuncture studies using an ultrasonic flow probe (Transonic Systems, Ithaca, NY). In this technique, the left renal artery was dissected away from the perinephric fat and a flow probe was placed around the renal artery near the hilum of the kidney. The area around the probe was infused with a viscous gel. The probe was connected to a flow meter to a computer and RPF was recorded. Whole-kidney filtration fraction (FF) was calculated using the standard formula. Initial glomerular capillary plasma flow rate was estimated using the measured values for single-nephron GFR and whole-kidney FF.

Western Immunoblot Analysis

Rat kidneys were homogenized in RIPA buffer (1% Nonidet P-40, 0.1% SDS, 100 μg/ml PMSF, 0.5% sodium deoxycholate, 1 mM sodium orthovanadate, 2 μg/ml aprotinin, 2 μg/ml leupeptin, and 5 mM EDTA in PBS), and protein concentration was assayed using bicinchoninic acid reagents (Sigma, St. Louis, MO). Tissue homogenates with equal amounts of total protein were processed for immunoblot analysis (26,27).
ELISA of Chemokines

The contents of MCP-1 and RANTES in kidney homogenates with equal amounts of total protein were determined using a specific sandwich enzyme immunometric assay kit for rat MCP-1 (Assay Design, Ann Arbor, MI) and rat RANTES (Biosource Int., Camarillo, CA). The results were normalized for total protein content in kidney homogenates.

Statistical Analyses

One investigator performed counting of ED-1–positive cells as well as histologic scoring in a blinded manner. For immunoblot analysis, bands were scanned and the integrated pixel density was determined using a densitometer and the National Institutes of Health image analysis program. All data are expressed as mean ± SD. Statistical analysis of the data from multiple groups was performed by ANOVA followed by Student-Newman-Keuls tests. Data from two groups were compared by t test. Linear regression analysis was applied to examine relationships between two parameters. P < 0.05 was considered significant.

Results

SBP and Proteinuria

As early as 2 wk after candesartan treatment, SBP was significantly decreased in all treated groups as compared with the control group (P < 0.01). BP was lower with the T25 and T75 groups than with the T5 group, but no significant difference was noted between the T25 and T75 groups (Figure 1). Urinary protein excretion rate was diminished in all treated rats, but the effect was dosage dependent and greatest in the T75 group (Figure 2).

Micropuncture

Glomerular hemodynamics was examined by micropuncture at 6 wk after ablation and therapy. Shown in Table 1, MAP was elevated in control rats and reduced by candesartan in all treatment groups (P < 0.001). In fact, although they seemed well, rats in the high-dosage groups had MAP that were below normal, averaging 88 ± 7 and 83 ± 5 mmHg in T25 and T75, respectively, which was significantly lower than 104 ± 4 mmHg in T5 (P < 0.05). Micropuncture revealed that candesartan also reduced the mean glomerular transcapillary hydraulic pressure difference in all treated groups; however, in contrast to the systemic pressure, no differences in hydraulic pressure were observed between the treated groups, which was approximately 25% lower than control in T5, T25, and T75 (Table 1). Glomerular pressure remained relatively constant because increasing dosages of candesartan were associated with progressive declines in the ratio of afferent arteriole resistance (RA)/efferent arteriole resistance, which was markedly lower in the treated groups, especially in T25 and T75 (P < 0.001). The marked decline in RA that we observed may seem paradox because AngII is believed to constrict preferentially the efferent arteriole. However, a decline in RA is the expected autoregulatory response to the marked reductions in arterial pressure that were associated with drug therapy and may not depend on specific effects of candesartan on the afferent or efferent arterioles. No statistically significant differences were observed in whole-kidney or single-nephron GFR, RPF, or FF among the groups.

Response to Exogenous AngII Challenge

After 2 wk of candesartan therapy, the completeness of AT1R blockade was assessed in three rats from each group by examination of the response to exogenous AngII; results are shown in Figure 3. As predicted, MAP was significantly elevated in control rats and rose further in response to AngII, from 155 ± 5 to 183 ± 2 mmHg (P < 0.05). The increase in BP was associated with a significant decline in RPF, from 2.6 ± 0.7 to 1.4 ± 0.5 ml/min, and in C_inj from 1.24 ± 0.15 to 0.87 ± 0.06 ml/min (both P < 0.05). This represents the typical systemic and renal vasoconstrictor to a relatively high dosage of AngII. It is interesting that although 5 mg/kg is approximately 10 times the
Candesartan Suppresses Inflammation and Injury in CKD

High-Dosage Candesartan Ameliorates Renal Fibrosis

At 9 mo, kidneys from control and T5 rats showed severe renal fibrosis, including glomerulosclerosis and tubulointerstitial fibrosis. Significantly less damage was observed in the T25 and T75 groups, the latter showing the best preservation of kidney structure ($P < 0.05$; data not shown). Fibrosis tended to progress with time. Figure 4 showed renal fibrosis at 14 mo. Compared with 9-mo rats, control rats showed progressive sclerosis both in glomeruli and in interstitium, as evidenced by an average 33% increment in SI from months 9 to 14. Progression was suppressed by candesartan in all treatment groups; however, lesions still progressed in the T5 and T25 groups, with average 17 and 12% increments in SI from 9 to 14 mo, respectively. In contrast, progressive sclerosis was completely halted in the ultrahigh-dosage group (T75), which had the lowest average sclerosis change (3%) from 9 to 14 mo. Also of note, the T75 group had the lowest average sclerosis scores at both 9 and 14 mo.

High-Dosage Candesartan Ameliorates Renal Inflammation in SHR

At month 14, control rats demonstrated severe inflammation in both glomeruli and interstitium. This was significantly attenuated by high-dosage candesartan treatment, especially in the T75 group (Figure 5, A through D). Comparing 9- and 14-mo kidney sections, Control, T5, and T25 rats showed progressive inflammation, as evidenced by average 118 ± 21, 102 ± 18, and 65 ± 19% (control, T5, and T25 groups, respectively) increases in infiltrating inflammatory cell number from 9 to 14 mo. The least progression, only a 17 ± 9% increase, was observed in the ultrahigh-dosage group ($P < 0.01$; Figure 5E).

Immunohistochemistry staining in kidney sections and absolute counting of ED-1–positive cells revealed a marked cellular infiltrate in the control group that was reduced by candesartan, with the lowest number of infiltrating ED-1–positive cells in the T75 group (Figure 6, A through D). For confirmation of these findings and avoidance of possible bias in tissue sectioning, selection, and counting, immunoblot analysis of ED-1 protein was performed on whole-kidney homogenates, which also showed that kidney ED-1 levels were reduced by high-dosage candesartan (Figure 6, E through G).

High-Dose Candesartan Suppresses Renal Expression of MCP-1 and RANTES

Immunohistochemistry staining revealed that renal expression of MCP-1 significantly increased primarily in tubular cells in control rats (Figure 7A). Ultrahigh-dosage candesartan (T75) markedly reduced MCP-1 staining (Figure 7D). The lowest dosage of candesartan (T5; Figure 7B) did not significantly suppress MCP-1 staining, which was greater than that in T25 (Figure 7C) and T75. Absolute kidney content of MCP-1 was determined by ELISA on tissue homogenates (Figure 7E) and was consistent with the immunohistochemistry findings. In addition, renal expression of MCP-1 was found to correlate closely with renal inflammation (Figure 7F), consistent with the chemotactic role of MCP-1 in the generation and persistence of the inflammatory infiltrate.

maximal recommended antihypertensive dosage of candesartan in human, rats on this dosage had a partial response to AngII. MAP increased from 116 ± 11 to 134 ± 22 mmHg, and RPF fell slightly from 2.8 ± 0.4 to 1.9 ± 1.0 ml/min, demonstrating that blockade of AT1R was incomplete in T5 rats. GFR was constant in T5 rats, averaging 0.87 ± 0.32 and 1.02 ± 0.59 before and after AngII, respectively. In contrast, no response to AngII was observed in the T25 and T75 groups. MAP was 80 ± 3 mmHg before AngII and did not change. RPF and $C_{\text{in}}$ also failed to decline in these groups in response to AngII. AngII receptor blockade seemed to be complete at the T25 and T75 dosages. Collectively, these data suggest that AngII receptors were partially blocked in T5 and that complete inhibition was achieved only at and above the 25-mg/kg dosage.
Immunohistochemistry staining revealed abundant renal expression of RANTES that was primarily located in the tubules in untreated rats (Figure 8A). Ultrahigh-dosage candesartan (T75) significantly suppressed RANTES staining, with only sporadic staining noted in a few tubular cells (Figure 8D). The lowest dosage of candesartan (T5) did not suppress RANTES staining, which was greater than that in the T25 and T75 groups (Figure 8, B and C). Absolute kidney content of RANTES was determined by ELISA on tissue homogenates and was consistent with the immunohistochemistry findings (Figure 8E). In addition, renal expression of RANTES was found to correlate closely with renal inflammation, consistent with the chemotactic role of RANTES in the generation and persistence of the inflammatory infiltrate (Figure 8F).

**Table 1. Hemodynamic studies by micropuncture at 6 wk after ablation and therapy**

<table>
<thead>
<tr>
<th>Groups</th>
<th>MAP (mmHg)</th>
<th>ΔP (mmHg)</th>
<th>RA/RE</th>
<th>GFR (ml/min)</th>
<th>SNGFR (nl/min)</th>
<th>FF</th>
<th>RPF (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>157 ± 11</td>
<td>48 ± 6</td>
<td>2.0 ± 0.5</td>
<td>1.32 ± 0.21</td>
<td>46 ± 8.8</td>
<td>0.30 ± 0.13</td>
<td>4.88 ± 1.57</td>
</tr>
<tr>
<td>T5</td>
<td>104 ± 4b</td>
<td>39 ± 4c</td>
<td>1.3 ± 0.3c</td>
<td>1.39 ± 0.27</td>
<td>49 ± 9.1</td>
<td>0.28 ± 0.06</td>
<td>5.03 ± 0.64</td>
</tr>
<tr>
<td>T25</td>
<td>88 ± 7b,c</td>
<td>42 ± 6c</td>
<td>0.7 ± 0.2b</td>
<td>1.23 ± 0.18</td>
<td>43 ± 5.1</td>
<td>0.27 ± 0.05</td>
<td>4.83 ± 0.55</td>
</tr>
<tr>
<td>T75</td>
<td>83 ± 6b,c</td>
<td>39 ± 5c</td>
<td>0.8 ± 0.3b</td>
<td>1.19 ± 0.17</td>
<td>40 ± 7.7</td>
<td>0.21 ± 0.04</td>
<td>5.41 ± 0.95</td>
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</tbody>
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aData are means ± SD. FF, filtration fraction; MAP, mean arterial pressure; ΔP, glomerular transcapillary hydraulic pressure difference; RA/RE, ratio of afferent and efferent resistance; RPF, renal plasma flow; SNGFR, single-nephron GFR; T5, group that was given candesartan at a standard dosage of 5 mg/kg per d; T25, group that was given candesartan at a high dosage of 25 mg/kg per d; T75, group that was given candesartan at an ultrahigh dosage of 75 mg/kg per d.

bP < 0.001 versus control.
cP < 0.05 versus control.

Figure 3. Hemodynamic response of angiotensin II (AngII) injection in the control group and in the groups that were treated with various dosages of candesartan. (A) Mean arterial pressure (MAP) was significantly elevated after AngII infusion in the control and T5 groups. (B) Renal plasma flow (RPF) markedly decreased in the control and T5 groups. (C) Inulin clearance (C, in) was reduced only in the control group. No significant changes of MAP, RPF, and C, in were observed in the T25 and T75 groups. *P < 0.05 preinjection versus postinjection.

Immunohistochemistry staining revealed abundant renal expression of RANTES that was primarily located in the tubules in untreated rats (Figure 8A). Ultrahigh-dosage candesartan (T75) significantly suppressed RANTES staining, with only sporadic staining noted in a few tubular cells (Figure 8D). The lowest dosage of candesartan (T5) did not suppress RANTES staining, which was greater than that in the T25 and T75 groups (Figure 8, B and C). Absolute kidney content of RANTES was determined by ELISA on tissue homogenates and was consistent with the immunohistochemistry findings (Figure 8E). In addition, renal expression of RANTES was found to correlate closely with renal inflammation, consistent with the chemotactic role of RANTES in the generation and persistence of the inflammatory infiltrate (Figure 8F).

Figure 4. Histopathologic changes in kidneys of the control group and of the groups that were treated with various dosages of candesartan after 14 mo treatment. (A) Control rats showed severe glomerulosclerosis and tubulointerstitial fibrosis. Candesartan-treated rats showed amelioration of sclerosis as a dosage dependent in T5 rats (B), T25 rats (C), and T75 rats (D). Comparing month 14 with month 9, control rats showed markedly progressive sclerosis in glomeruli (E) and interstitium (F). Renal fibrosis in the T5 group also showed markedly progressive change, especially in interstitium. The lowest average sclerosis changes occurred in the T75 group. *P < 0.05 control versus all treated group; †P < 0.05 T25 versus control and T5 groups; ‡P < 0.05 T75 versus control, T5, and T25 groups. Magnification, ×100 (Masson-Trichrome).
NF-κB activation and subsequent induction of expression of multiple proinflammatory molecules is considered to be central to the pathogenesis of renal inflammation. NF-κB phosphorylation is a prerequisite and marker for NF-κB activation (28).

We examined NF-κB activation in vivo in SHR kidneys using a specific mAb against phosphorylated NF-κB p65 (p-p65). By immunohistochemistry, kidneys from control rats displayed strongly positive staining for activated NF-κB, mainly located in nuclei of tubular epithelial cells. Candesartan suppressed NF-κB activation in a dosage-dependent manner with the maximal effect in the T75 group (Figure 9, A through D). Immuno- blots on kidney homogenates confirmed the morphologic findings. For better evaluation of the activation status of NF-κB, the ratio of activated p65 (p-p65) to total p65 was calculated and was markedly suppressed by high-dosage candesartan (Figure 9, E and F).

Discussion

The SHR is an animal model of severe systemic hypertension and relative activation of the RAAS (29–31). SHR gradually develop progressive glomerular sclerosis and interstitial fibrosis, preceded by a renal inflammatory infiltrate that is thought to play an important role in the genesis of renal injury. RAAS-blocking drugs reduce renal injury in this model, an effect that most often is attributed to reductions in systemic and glomerular capillary pressures (32–35). It is interesting that data both in animal models and in patients suggest that higher-than-usual antihypertensive dosages of these drugs can have even greater effect to suppress proteinuria and even reverse renal injury in some settings (20–23). However, the effects of very high dosages of RAAS-blocking drugs on renal hemodynamics and injury in SHR have not been previously examined, and the exact mechanism of renal protection with high-dosage therapy remains uncertain.
Similar to previous reports, untreated SHR in this study developed significant hypertension, proteinuria, severe renal inflammation marked by a large number of infiltrating macrophages, and, ultimately, renal fibrosis. Administration of a range of dosages of candesartan, a selective AT1R antagonist, effectively prevented hypertension in all treatment groups. Moreover, high-dosage groups had BP that were reduced as compared with the lowest dosage group ($P < 0.05$) and, in fact, lower than normal for the rat. Nevertheless, despite relative hypotension, rats that received high-dosage candesartan gained weight normally and looked well throughout the entire 14 mo of study. Thus, very-high-dosage therapy, in this case approximately 150 times the maximal recommended dose in human, was safe and well tolerated by SHR, consistent with the previously described favorable adverse effect profile of this class of drugs.

Consistent with the data showing further reductions in BP with dosages $> 5$ mg/kg, administration of exogenous AngII revealed that systemic and renal hemodynamic responses to AngII were only partially blunted but not completed blocked in T5 group. Complete blockade of AngII receptors was achieved at the 25-mg/kg per d dosage and above. That dosages that were much higher than that recommended in human were needed to block completely AngII receptors in SHR may relate to species differences or to the fact that SHR have not only hypertension but also renal injury. In fact, the exact dosage of candesartan that is needed to block completely AT1R in patients with hypertension and renal disease is not well studied, and beneficial effects of higher-than-usual dosages of this agent have been suggested in human as well.

Ultrahigh-dosage (75 mg/kg per d) candesartan was associated with a reduction in protein excretion rate beginning as early as 2 wk after initiation of therapy, which is well before morphologic evidence of renal injury is evident in SHR. High-dosage (25 mg/kg per d) candesartan also reduced proteinuria, although the effect was delayed as compared with the 75-mg/kg group and NS until 2 mo. Consistent with the reduction in protein excretion rate, morphologic studies revealed graded reductions in glomerular sclerosis and tubulointerstitial fibrosis with increasing candesartan dosage, with rats that were in the
Arbitrary units of p-p65 and p65 abundance in immunoblot analysis of p-p65 and total p65 in kidney homogenates. (F) in T25 (C) and T75 (D) groups. (E) Representative Western blot increased in control group (A) and T5 group (B); P-p65 was less p65) staining in kidney section. P-p65 was significantly in-

expression of MCP-1 and RANTES in the tubulointerstitial

the intravascular compartment to the renal parenchyma. Renal

Chemokines are crucial in recruiting inflammatory cells from

example, AngII participates in the recruitment of inflammatory

and regulation of immune and inflammatory responses. For

ics, evidence suggests that the RAAS participates in the genesis

factors that might account for this effect.

In addition to modulating systemic and renal hemodynam-

ics, evidence suggests that the RAAS participates in the genesis

and regulation of immune and inflammatory responses. For

example, AngII participates in the recruitment of inflammatory
cells to a site of injury by direct actions on infiltrating cells and

via regulation of expression of adhesion molecules, cytokines,

and chemokines (12,36,37). In this study, large numbers of

ED-1–positive mononuclear cells were observed in the kidneys of control SHR. Candesartan significantly decreased the extent of inflammation in CKD in general is activation of NF-κB. Binding of AngII to type 1 receptor triggers multiple signaling pathways that phosphorylate and activate NF-κB (38–40), which translocates to the nucleus, where it induces numerous proinflammatory molecules, including MCP-1 and RANTES. As expected, activated NF-κB was evident in nuclei of tubular epithelial cells in untreated SHR, and this was only minimally altered by low-dosage candesartan. In contrast, the level of activated NF-κB in renal tubular cells was obviously reduced in the two high-dosage groups, suggesting that suppression in MCP-1 and RANTES expression in these rats resulted from inhibition of NF-κB. Comparing the results of month 9 with month 14, it was clear that high-dosage candesartan significantly retarded the progression of inflammation in SHR at multiple levels, including NF-κB activation, chemokine expression, and inflammatory infiltration. Moreover, suppression of inflammation was dosage dependent.

It is noteworthy that the beneficial effect of candesartan on renal inflammation was observed only at much higher than standard dosages. The explanation for this dosage dependence is uncertain but may relate to the fact the standard dosage did not completely block AngII receptors. We did not measure the activity or levels of components of the RAAS in our rats, and it is possible that the system is upregulated in this model of renal disease. For example, it has been reported that expression of AT1R is increased in the inflamed renal parenchyma (41–45). Therefore, it is reasonable to speculate that higher dosages of candesartan were needed to block completely the increased numbers of AT1R in the diseased SHR kidney. Further studies are necessary to address this issue.

Long-term, high-dosage candesartan treatment was generally well tolerated, and this is consistent with previous short-term animal experiments that also showed good tolerability (22,23). However, to our knowledge, this is the first study to administer such high dosages of an ARB in an animal model of renal disease for such a long time period. Also of interest is the possibility that high-dosage candesartan conveyed a survival advantage. The long-term study was terminated at 14 mo, by which time two rats had died in the control and T5 groups, whereas no rats were lost in the T25 and T75 groups. Finally, although the these data should not be extrapolated to clinical practice without long-term toxicity studies, a recent short-term clinical trial in 12 patients with various types of chronic renal disease and heavy proteinuria demonstrated good tolerability of candesartan at a dosage that was five times the currently recommended maximal antihypertensive dosage (46).

Conclusion

The AT1R blocker candesartan attenuates proteinuria, ame-

liorates renal fibrosis, and suppresses renal inflammation in a dosage-dependent manner in the SHR model of chronic renal
disease. Standard dosages of candesartan failed to block completely the AngII receptor, but complete inhibition was obtained at very high dosages. The renoprotective actions of high-dosage therapy seem to be independent of the drug’s hemodynamic effects. Instead, high-dosage candesartan suppressed activation of NF-κB, tubular expression of MCP-1 and RANTES, and renal inflammation and injury. Our findings suggest that long-term, high-dosage candesartan is safe and may be superior to regular-dosage therapy in preventing progression of chronic renal disease.

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Disclosures
None.

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