Antihypertensive Effect of Chronic KT3-671, a Structurally New Nonpeptide Angiotensin AT₁-Receptor Antagonist, in Stroke-Prone Spontaneously Hypertensive Rats

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ABSTRACT—KT3-671 (2-propyl-8-oxo-1-[(2'-(1H-tetrazole-5-yl)biphenyl-4-yl)methyl]-4,5,6,7-tetrahydro-cycloheptimidazole), a structurally new nonpeptide angiotensin AT₁-receptor antagonist, was administered orally and repeatedly to 15-week-old stroke-prone spontaneously hypertensive rats for 7 weeks; and its effects on blood pressure, heart rate, renal function, plasma renin concentration (PRC), plasma aldosterone concentration (PAC) and hypertension-related tissue damage in the brain, heart, kidney and mesenteric artery were investigated. KT3-671 at a dose of 3 or 10 mg/kg, p.o. per day prevented development of hypertension and produced a significant and consistent reduction of blood pressure in a dose-dependent manner. Enalapril at a dose of 10 mg/kg per day produced cardiovascular effects similar to those of KT3-671 at 10 mg/kg. Despite marked reduction in blood pressure, neither KT3-671 nor enalapril affected the heart rate. KT3-671 at 10 mg/kg produced a transient and significant reduction of urinary sodium excretion in the second week, but did not affect renal function at any other time during the experimental period. Both KT3-671 at 10 mg/kg and enalapril at 10 mg/kg produced a significant increase in PRC and showed a tendency to decrease PAC. Repeated administration of KT3-671 reduced the severity of the pathological changes in the kidney. These results suggest that KT3-671 is a potentially useful antihypertensive drug.

Keywords: KT3-671, AT₁-receptor antagonist, Stroke-prone spontaneously hypertensive rat, Antihypertensive effect, Renal lesion

KT3-671 is a newly synthesized nonpeptide angiotensin AT₁-receptor antagonist whose detailed pharmacological profile has been reported by Mochizuki et al. (1). It inhibits competitively [¹²⁵I]angiotensin (Ang) II binding to AT₁ receptors in rat liver membranes, but does not interact with AT₂ receptors in bovine cerebellar membranes. In isolated rabbit thoracic aorta, the compound shifts the concentration-contraction curves for Ang II to the right in a parallel fashion. Furthermore, KT3-671 inhibits dose-dependently the Ang II-induced pressor response in normotensive rats. Although KT3-671 has little effect on blood pressure in normotensive rats, oral administration to rats with renal hypertension produces prolonged and dose-dependent hypotension (1), as with other AT₁-receptor antagonists (2–6, and see review by Timmermans et al. (7)). These characteristics suggest that KT3-671 has potential utility for the treatment of hypertension.

In the present study, we investigated the effects of KT3-671 on blood pressure, heart rate, renal function, plasma renin concentration (PRC), and plasma aldosterone concentration (PAC) in stroke-prone spontaneously hypertensive rats. KT3-671 was administered orally and repeatedly at doses of 3 or 10 mg/kg per day for 7 weeks. The results showed that KT3-671 prevented the development of hypertension and produced a significant and consistent reduction of blood pressure in a dose-dependent manner. Despite the marked reduction in blood pressure, neither KT3-671 nor enalapril affected the heart rate. KT3-671 at 10 mg/kg produced a transient and significant reduction of urinary sodium excretion in the second week, but did not affect renal function at any other time during the experimental period. Both KT3-671 at 10 mg/kg and enalapril at 10 mg/kg produced a significant increase in PRC and showed a tendency to decrease PAC. Repeated administration of KT3-671 reduced the severity of the pathological changes in the kidney. These results suggest that KT3-671 is a potentially useful antihypertensive drug.
repeated oral administration of KT3-671 for 7 weeks on blood pressure, heart rate, renal function, plasma renin concentration (PRC), plasma aldosterone concentration (PAC) and hypertension-related tissue damage in the brain, heart, kidney and mesenteric artery in 15-week-old stroke-prone spontaneously hypertensive (SHRSP) rats (8), a hypertensive model with high plasma renin activity (9, 10).

MATERIALS AND METHODS

Chemicals
KT3-671 was synthesized and supplied by Kotobuki Seiyaku Co., Ltd. (Nagano). Enalapril maleate was purchased from Sigma (St. Louis, MO, USA). Solutions of KT3-671 and enalapril were prepared freshly each day just before use by suspension in 0.5% aqueous carboxymethylcellulose sodium (CMC).

Procedures in animals
In the previous study (1), KT3-671 at 0.3 to 10 mg/kg, p.o. produced a dose-dependent inhibition of the pressor response to Ang II with a peak inhibition at 4 hr after oral administration in normotensive conscious rats. The magnitudes of the peak blockade of the pressor response to Ang II by KT3-671 at 3 and 10 mg/kg were about 80% and 100%, respectively (1). Significant inhibition of the response was still observed at 24 hr after the dosing. Thus, we selected KT3-671 at 3 and 10 mg/kg, p.o. for investigating the antihypertensive effect in SHRSP rats and determined the blood pressure between 3 to 5 hr after administration. Enalapril was used as a reference drug that inhibits the renin-angiotensin system. Consecutive dosing of enalapril at 10 mg/kg, p.o. produced a sustained antihypertensive effect in SHRSP rats (11).

Male SHRSP rats from the strain of Okamoto et al. (8) were fed ad libitum with a rat chow containing 0.29% (w/w) sodium and 0.97% (w/w) potassium (CE-2; Clea Japan, Inc., Tokyo) and allowed free access to tap water for at least two weeks before and throughout the experiment. At the age of 15 weeks, the rats were divided into groups and administered orally 3 or 10 mg/kg KT3-671 or 10 mg/kg enalapril once daily for 7 weeks. The control rats received 0.5% aqueous CMC by gavage.

The cardiovascular effects of these drugs were evaluated by the tail-cuff method (PS-200; Riken Kaibatsu, Tokyo) between 3 to 5 hr after drug administration once every week for the first 6 weeks. Briefly, the rats were placed in metal restrainers warmed at 37°C for 15–20 min before the blood pressure determination. Systolic blood pressure and heart rate were determined without anesthesia (12, 13). In the sixth week of the experiment, after determination of renal function and blood pressure by the tail-cuff method, a catheter was inserted into the abdominal aorta through the femoral artery under anesthesia with sodium pentobarbital (9). The rats were allowed to recover from surgery for one week, and daily drug administration was continued during the recovery period. In the seventh week of the experiment, between 3 and 5 hr after the last dose of each drug, mean blood pressure was determined via the aortic catheter using a CP-01 transducer (Century Technology, Inglewood, CA, USA) and an amplifier-recorder system (PAS-401 and PA-011, Star Medical, Tokyo; Recti-Horiz 8K, Sanei, Tokyo) in conscious and freely moving rats (12, 13). A cardiotachometer (HR-001, Star Medical) was triggered by the pulse, and the signal was displayed on the recorder (Recti-Horiz 8K, Sanei) to determine the heart rate. Blood samples of 0.3 ml for determination of PRC and then samples of 1.2 ml for determination of PAC were obtained from the aortic catheter soon after the determination of mean blood pressure.

On the first day and in every week of the experiment, 7 rats from each group were fasted overnight and loaded with 30 ml/kg bicarbonate saline solution (NaCl 110 mM, NaHCO3 30 mM) together with the drug solutions and placed in individual metabolic cages. Urine was collected into a graduated cylinder for 6 hr. Concentrations of urinary sodium, potassium and chloride were determined by ion selective electrodes using an Olympus AU-5200 autoanalyzer (Olympus, Tokyo).

PRC determination
PRC was determined by the method of Carvalho et al. (14) with some modifications (9). Briefly, sample plasma was incubated with the plasma renin substrate pool obtained from 24-hr-nephrectomized rats in the presence of inhibitors of angiotensinase and converting enzyme. The amount of Ang I generated during the incubation was determined by a radioimmunoassay (RIA) with Ang I specific antiserum. PRC was expressed as ng of Ang I formation per ml of sample per hr.

PAC determination
PAC was determined by a RIA (15) using an Aldosterone-Riakit® II (Dinabot, Tokyo).

Pathological determination of the tissues
After blood sampling for PRC and PAC determination, the rats were killed by decapitation. The brain, heart, kidney and mesenteric arteries were dissected out quickly and weighed. These tissues were then fixed in 10% buffered formalin for pathological examination. Routine tissue processing and staining with hematoxylin-eosin, azan, PAS and elastica van Gieson were carried out at Fuji Biomedix Co., Ltd. (Yamanashi). Histological sec-
tions (4-μm thick) of each organ were evaluated in a blind fashion. Each sample was graded for the extent of tissue damage, using a scale of 0–3, where 0 represented normal tissue, 1=mild, 2=moderate and 3=severe damage (12).

**Calculations and statistical analyses**

Means and S.E.M. are presented in the tables and figures. The statistical significance of differences between the control and drug-treated groups was assessed by analysis of variance followed by the Bonferroni method (16). The Mann-Whitney U-test was used for statistical analysis of the tissue lesion scores. All differences at P values of less than 0.05 were considered to be statistically significant.
Fig. 4. Urinary volume (UV), sodium (UNaV), potassium (UKV) and chloride (Uc1V) during repeated administration of KT3-671 in SHRSP rats. On the first day and in every week of the experiment, bicarbonate saline (30 ml/kg) was administered orally together with drug solution, and urine was collected for 6 hr. Values are means ± S.E.M. for 7 rats. *P<0.05, compared with the control. Control (□); enalapril (■); KT3-671: 3 mg/kg (▲), 10 mg/kg (■). wk: week.
RESULTS

Neither dose of KT3-671 or enalapril affected the growth of SHRSP rats (Fig. 2).

KT3-671 had no significant effect on heart rate (Fig. 3). At a dose of 3 mg/kg, KT3-671 prevented the development of hypertension in 15-week-old SHRSP rats, and a significant reduction of blood pressure in comparison with the control was observed at most determinations (P < 0.05 or P < 0.01). KT3-671 at a dose of 10 mg/kg and enalapril at 10 mg/kg produced a significant and consistent reduction in blood pressure from the first week of administration (P < 0.05 or P < 0.01). The antihypertensive effect of KT3-671 and enalapril was confirmed by direct determination of mean blood pressure at week 7.

When bicarbonate saline was loaded on the first day of the experiment, KT3-671 at a dose of 3 or 10 mg/kg did not affect urine volume (UV), and urinary excretion of sodium (U\textsubscript{Na}V), potassium (U\textsubscript{K}V) and chloride (U\textsubscript{Cl}V) (Fig. 4). KT3-671 at a dose of 10 mg/kg produced a significant decrease of U\textsubscript{Na}V only in the second week of therapy. At other determinations, KT3-671 did not produce any significant effects on UV and urinary excretion of electrolytes. No significant effects on renal function were observed in SHRSP rats administered enalapril.

Administration of KT3-671 for 7 weeks increased PRC in a dose-dependent manner, and the increase of PRC at a dose of 10 mg/kg was statistically significant compared with the control (P < 0.01) (Fig. 5). A large and significant increase of PRC was also observed in SHRSP rats administered enalapril (P < 0.01).

KT3-671 given at a dose of 3 mg/kg for 7 weeks did not affect PAC significantly. However, both KT3-671 at a dose of 10 mg/kg and enalapril at a dose of 10 mg/kg produced a slight and insignificant decrease of PAC (Fig. 6).

Administration of KT3-671 at a dose of 3 or 10 mg/kg for 7 weeks produced a slight, but not significant, decrease of heart weight, but did not affect the weights of the brain and the kidney (Table 1). Enalapril at a dose of 10 mg/kg for 7 weeks produced a statistically significant decrease of heart weight in comparison with the control (P < 0.05).

Microscopic examination revealed minimal damage in the brain and the mesenteric artery of the control rats, and thus therapy with KT3-671 and enalapril did not modulate significantly the severity of the pathological changes (data not shown). Mild to moderate fibrosis, coronary artery wall thickening and mononuclear cell infiltration were observed in the heart of some control rats. However, no significant differences in the severity of these changes were observed between the control and drug-treated rats (data not shown). Severe to moderate tubular degeneration and interstitial nephritis and mild to moderate focal tubular atrophy in the kidney were observed in some control rats. KT3-671 and enalapril reduced significantly the severity of these pathological changes in the kidney (Table 2).
Table 1. Brain, heart and kidney weights in SHRSP rats administered KT3-671 and enalapril for 7 weeks

<table>
<thead>
<tr>
<th></th>
<th>Brain (g)</th>
<th>Heart (g/100 g BW)</th>
<th>Kidney (g/100 g BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>right</td>
<td>left</td>
</tr>
<tr>
<td>Control (n=10)</td>
<td>2.025 ± 0.034</td>
<td>0.512 ± 0.009</td>
<td>0.458 ± 0.007</td>
</tr>
<tr>
<td>KT3-671, 3 mg/kg (n=10)</td>
<td>2.009 ± 0.012</td>
<td>0.487 ± 0.015</td>
<td>0.437 ± 0.010</td>
</tr>
<tr>
<td>KT3-671, 10 mg/kg (n=9)</td>
<td>2.008 ± 0.021</td>
<td>0.468 ± 0.015</td>
<td>0.456 ± 0.017</td>
</tr>
<tr>
<td>Enalapril, 10 mg/kg (n=10)</td>
<td>2.007 ± 0.018</td>
<td>0.455 ± 0.013*</td>
<td>0.470 ± 0.025</td>
</tr>
</tbody>
</table>

Values are means±S.E.M. BW=body weight. *P<0.05, compared with the control by the Bonferroni method.

Table 2. Effects of repeated oral administration of KT3-671 and enalapril for 7 weeks on severity of microscopic lesions in the kidney of SHRSP rats

<table>
<thead>
<tr>
<th></th>
<th>Focal tubular atrophy</th>
<th>Tubular degeneration</th>
<th>Interstitial nephritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=10)</td>
<td>1.00 ± 0.30</td>
<td>2.00 ± 0.30</td>
<td>1.00 ± 0.37</td>
</tr>
<tr>
<td>KT3-671, 3 mg/kg (n=10)</td>
<td>0.30 ± 0.15</td>
<td>1.00 ± 0.21**</td>
<td>0.00 ± 0.00*</td>
</tr>
<tr>
<td>KT3-671, 10 mg/kg (n=9)</td>
<td>0.00 ± 0.00**</td>
<td>0.78 ± 0.28*</td>
<td>0.22 ± 0.22</td>
</tr>
<tr>
<td>Enalapril, 10 mg/kg (n=10)</td>
<td>0.10 ± 0.02*</td>
<td>0.70 ± 0.26**</td>
<td>0.10 ± 0.10*</td>
</tr>
</tbody>
</table>

Values are means±S.E.M. of severity scores. *P<0.05, **P<0.01, compared with the control by the Mann-Whitney U-test.

DISCUSSION

The results of the present study demonstrated that daily oral administration of KT3-671, an AT1-receptor antagonist, at a dose of 3 or 10 mg/kg per day for 7 weeks produced a marked and consistent antihypertensive effect in SHRSP rats. No tolerance to the antihypertensive effect of KT3-671 was observed during the period of repeated administration, despite a significant increase in PRC. Furthermore, KT3-671 did not affect growth and heart rate. The major mechanism of the antihypertensive effect of KT3-671 is probably the blockade of the vasoconstrictor action of Ang II through antagonism at AT1-receptor sites in the vascular smooth muscle (1). The release of norepinephrine from sympathetic nerve terminals is facilitated by Ang II through activation of the AT1 receptors at these sites (7, 17). Thus, it is possible that the decrease of norepinephrine release from vascular sympathetic nerve terminals contributes additionally to the acute antihypertensive effect of KT3-671. At the later stage of therapy, the protective effect of KT3-671 against the renal damage induced by Ang II may additionally contribute to its antihypertensive effect. Repeated oral administration of enalapril at a dose of 10 mg/kg per day produced effects similar to those of KT3-671 on blood pressure and heart rate. On the basis of dosage, the degree of reduction in blood pressure by KT3-671 was slightly lower than that by enalapril.

Despite a significant reduction of blood pressure, KT3-671 did not cause reflex tachycardia in the present and previous studies (1), as reported for other AT1-receptor antagonists (2–7, 18, 19). The modulation of sympathetic nerve transmission through antagonism with Ang II at prejunctional AT1 receptors in the heart may be one of the reasons why KT3-671 did not cause reflex tachycardia in the present study.

Keiser et al. (20) described that losartan increased renal blood flow and was mildly natriuretic in anesthetized dogs. In contrast, Wong et al. (18) reported that losartan caused a significant decrease in blood pressure but did not produce an acute diuretic effect in SHR rats. Although KT3-671 at a dose of 10 mg/kg caused a slight and transient reduction of \( U_{\text{Na}}V \) in bicarbonate saline-loaded SHRSP rats at week 2, acute and repeated administration of KT3-671 at 3 or 10 mg/kg did not affect significantly UV and urinary excretion of electrolytes at other points in the experimental period. Thus, despite a marked reduction in blood pressure, KT3-671 does not appear to affect significantly urinary excretion during long-term therapy. Furthermore, no evidence was found suggesting that enhanced UV and \( U_{\text{Na}}V \) contributed to the antihypertensive effect of KT3-671 in SHRSP rats.

It is speculated that intrarenal generation of Ang II constricts the renal efferent arterioles and causes an increase in glomerular hydraulic pressure. Glomerular hyperfiltration, hyperperfusion and hypertension may then initiate and induce glomerular lesions. Amelioration of hypertension-related histological lesions in the kidney of SHRSP rats by repeated administration of KT3-671 can be ascribed to inhibition of the intrarenal effects of
Ang II and reduction of systemic blood pressure.

PRC in the control SHRSP rats was similar to that reported previously from our laboratory (12), and it was about twofold higher than that observed in SHR rats (13). Both KT3-671 and enalapril produced a marked and significant reduction of blood pressure in SHRSP rats in the present study. These observations confirm that the SHRSP is a hypertensive model with high plasma renin activity (9, 10), and they suggest that the extremely high blood pressure observed in the adult SHRSP is largely dependent on Ang II. The dose-dependent increase of PRC produced by KT3-671 can be assumed to be due to inhibition of the negative feedback mechanism of Ang II on renin release in the juxtaglomerular cells, reduction of glomerular perfusion pressure and a compensatory increase of renal sympathetic nerve activity. A similar compensatory rise in PRC has been observed with other AT₁-receptor antagonists in rats (18, 19) and man (21). It has been suggested that the rise in PRC may attenuate the antihypertensive effect of competitive AT₁-receptor antagonists, such as KT3-671, during repeated administration, and may induce a rebound increase of blood pressure after the termination of administration through an increase in Ang II concentration at the receptor sites in vascular smooth muscle. However, KT3-671 at doses of 3 and 10 mg/kg per day produced a consistent decrease in blood pressure throughout the experimental period of 7 weeks.

Ang II has been reported to stimulate aldosterone release from adrenal cortical cells (22). Losartan blocks the Ang II-induced rise in PAC in normotensive and hypertensive rats (23–25). However, little is known about the effect of repeated administration of AT₁-receptor antagonists on physiological PAC. Although statistically not significant, KT3-671 at a dose of 10 mg/kg per day showed a tendency to decrease PAC, which can be explained by inhibition of aldosterone secretion from adrenal cortical cells through antagonist with Ang II. Enalapril caused a degree of PAC reduction similar to that of KT3-671 at 10 mg/kg per day.

Ang II has been reported to have a trophic effect on vascular smooth muscle cells (26) and myocardial cells (27). A tendency for KT3-671 to decrease heart weight can be explained by the direct effect of antagonism with Ang II on myocardial cells and by an indirect effect due to decreased systemic blood pressure, which leads to reduction of cardiac afterload. Repeated administration of KT3-671 and enalapril prevented hypertension-related histological lesions in the kidney. Amelioration of the severity of kidney lesions in SHRSP rats has also been observed after chronic treatment with captopril, enalapril and losartan (28–31). A high incidence of stroke has been reported in SHRSP rats maintained on a high salt intake (29–32). However, we found minimal damage in the brain in the present study, probably because we maintained SHRSP rats on a normal sodium diet and gave tap water for drinking.

In summary, repeated oral administration of KT3-671, an AT₁-receptor antagonist, for 7 weeks to adult SHRSP rats, produced a marked antihypertensive effect without affecting heart rate, and it protected the kidney from damage due to hypertension. Our studies suggest that the antihypertensive effect of KT3-671 can be ascribed to the dilation of vascular smooth muscle, modulation of sympathetic nerve activity, and reduction of aldosterone release through the direct effects of antagonism with Ang II at AT₁-receptors in the respective tissues. It is concluded that KT3-671 has potential utility for the treatment of hypertension.

Acknowledgments

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