Pharmacological Studies on (4S)-1-Methyl-3-{(2S)-2-[N-((1S)-1-Ethoxycarbonyl-3-Phenylpropyl)amino] Propionyl}-2-Oxo-Imidazolidine-4-Carboxylic Acid Hydrochloride (TA-6366), a New ACE Inhibitor:

I. ACE Inhibitory and Anti-Hypertensive Activities†

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Abstract—TA-6366 and its active metabolite 6366A inhibited swine renal angiotensin converting enzyme (ACE) activity with IC50s of 9900 and 2.6 nM, respectively. TA-6366 (0.05–0.5 mg/kg, p.o.) inhibited the angiotensin I (AT-I)-induced pressor response in rats. 6366A augmented bradykinin (BK)-induced contraction of guinea pig ileum more potently than captopril. However, when the augmentation on BK-induced hypotension in rats was used as an indicator, TA-6366 was less active than captopril. TA-6366 increased plasma renin activity and plasma AT-I concentration. Oral administration of TA-6366 lowered the blood pressure in two-kidney one-clip renal hypertensive rats at 0.5 to 2 mg/kg and in spontaneously hypertensive rats (SHRs) at 2 to 10 mg/kg. The antihypertensive effect of TA-6366 was approximately 5 times more potent than that of captopril and almost as potent as that of enalapril. In SHRs, the antihypertensive action of TA-6366 was intensified in potency when administered repeatedly. The duration of action was longer than those of captopril and enalapril. However, TA-6366 had no substantial effect on the blood pressure in DOCA/saline hypertensive rats. These results indicate that TA-6366 is a potent and long lasting antihypertensive agent and that its antihypertensive action is attributable to the inhibition of ACE.

In an earlier study (1), we reported that (4S)-1-methyl-3-{(2S)-2-[N-((1S)-1-ethoxycarbonyl-3-phenylpropyl)amino]propionyl}-2-oxo-imidazolidine-4-carboxylic acid hydrochloride (TA-6366; its chemical structure is shown in Fig. 1), a newly synthesized angiotensin converting enzyme (ACE) inhibitor, has a potent antihypertensive effect in spontaneously hypertensive rats (SHRs) and is the least toxic among the series of derivatives to mice.

In the present study, we investigated more detailed pharmacological properties related to its ACE inhibitory activity.

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Materials and Methods

Animals
Male Wistar rats (either Shizuoka Lab. Animal Ctr. or KBL), male SHRs (Charles River Japan, Inc.), and Hartley guinea pigs (Shizuoka Lab. Animal Ctr.) of either sex were used. They were kept in air conditioned rooms at a temperature of 23±1°C and with a humidity of 55±5% on a 12 hr light-dark cycle with food pellets (CRF-1, Oriental Yeast Industry Co., Ltd.) and water provided ad libitum.

Drugs
TA-6366 and its active metabolite 6366A
(Fig. 1), enalapril and its active metabolite enalaprilat, and captopril were synthetized at the Research Laboratory of Applied Biochemistry, Tanabe Seiyaku Co., Ltd., as described (1–3).

The other drugs used were as follows: angiotensin I and bradykinin (both Protein Research Foundation), hippurylhistidylleucine (Sigma), sodium pentobarbital (Tanabe), deoxycorticosterone acetate (Nacalai Tesque) and EDTA-2Na (Katayama).

**Experimental procedures**

1) **Inhibition of ACE activity**

i) **Inhibitory effect on swine renal cortex ACE activity**: ACE was prepared from swine renal cortex by the method of Oshima et al. (4). The reaction mixture, containing Tris-HCl (60 μmoles, pH 7.4), sodium chloride (60 μmoles), hippurylhistidylleucine (0.5 μmoles), the test drugs and ACE (6 μg of protein) in a final volume of 0.5 ml, was incubated for 20 min at 37°C and then immersed into ice-cold water to stop the enzyme reaction; and histidylleucine was measured microbiologically using *Leuconostac mesenteroides* P-60. Each enzyme reaction was run in duplicate.

The IC50 value, the molar concentration of the test inhibitor causing 50% inhibition of the control ACE activity, was graphically calculated from a linear regression line.

1) **Inhibitory effect on pressor response to angiotensin I (AT-I) in unanesthetized normotensive rats**: Twelve- to 20-week-old Wistar rats anesthetized with sodium pentobarbital (50 mg/kg, i.p.) were cannulated with a polyethylene tube into the abdominal aorta via the femoral artery for blood pressure measurement and into the jugular vein for injection of AT-I. Each catheter was passed subcutaneously, exteriorized on the neck, and filled with saline containing heparin. One to two days after the operation, the catheter was connected to a pressure transducer (Gould Statham, P50, P10EZ; Nihon Kohden, MPU-0.5), and blood pressure was recorded on a polygraph (Nihon Kohden, RM-6000).

AT-I (300 ng/kg) dissolved in physiological saline was injected into the jugular vein at fixed intervals after oral administration of the drugs. The mean±S.E.M. of the pressor response to AT-I before oral administration of the drugs was 36.1±0.8 mmHg (n=76). The inhibitory activity of the drug was expressed as the ratio of AT-I-induced increases in mean blood pressure between pre- and post-administration.

2) **Inhibition of bradykinin (BK) inactivation**

i) **Augmentative effect on BK-induced contraction of guinea pig ileum**: The isolated guinea pig ileum preparation was suspended in an organ bath filled with Tyrode's solution (30±1°C) saturated with a gas mixture of 95% O2 and 5% CO2. Contractile response to BK (5 ng/ml) was measured using an isotonic transducer (Nihon Kohden, TD-112S) in the presence of a 1.0 g load and recorded on an analogue recorder (Watanabe Sokki, SR-6511). The drugs were added 2 min before BK treatment. The augmentative activity of the drug was determined from the ratio of contractile amplitude before and after drug treatments.
The AC50 value, the molar concentration of the test inhibitor causing 50% augmentation of the control contraction, was graphically calculated from a linear regression line.

ii) Augmentative effect on depressor response to BK in unanesthetized normotensive rats: Twelve- to 18-week-old Wistar rats were used. The cannulation into the abdominal aorta for blood pressure measurement and into the jugular vein for the injection of BK was performed as described above. BK (3 μg/kg) dissolved in physiological saline was injected into the jugular vein at fixed intervals after oral administration of the drugs. The mean±S.E.M. of the depressor response to BK before oral administration of the drugs was -22.7±0.7 mmHg (n=32). Augmentative activity of the drug was expressed as the ratio of BK-induced decreases in diastolic blood pressure between pre- and post-administration.

3) Effect on plasma renin activity (PRA) and plasma AT-I and aldosterone concentrations in SHRs

Seven- to 8-month-old SHRs were used. Six hours after oral administration of the drugs, renal arteries were occluded bilaterally with hemostatic clamps under light ether anesthesia. Blood samples were collected from the abdominal aorta and transferred to polyethylene tubes with EDTA-2Na salt. PRA, plasma AT-I and aldosterone concentrations were measured radioimmunologically with commercial assay kits (Midori Juji and Dainabot).

4) Antihypertensive effect in various hypertension model rats and normotensive rats

a) Experimental animals

i) Two-kidney, one-clip renal hypertensive rats (2K,1C-RHRs): Six- to 7-week-old Wistar rats were used. Under sodium pentobarbital (50 mg/kg, i.p.) anesthesia, a piece of stainless-steel bar (0.25 mm in diameter, 20 mm in length) as a splint was attached to the left renal artery, and the artery together with the bar was tied with cotton string. Then, the bar was pulled out. Four to 7 weeks later, rats whose systolic blood pressures were higher than 160 mmHg were used as a group of 6 to 10.

ii) SHRs: Sixteen- to 22-week-old male SHRs were used as a group of 5 to 7.

iii) Deoxycorticosterone acetate (DOCA)/saline hypertensive rats (DOCA/s-HRs: Seven week-old male Wistar rats were used. Under sodium pentobarbital (50 mg/kg, i.p.) anesthesia, the left kidney of the rat was removed by surgical operation. From one week after nephrectomy, 5 mg/kg of DOCA suspended in 0.5% sodium carboxymethyl cellulose solution was injected subcutaneously twice a week, at fixed intervals, for 10 weeks. Simultaneously, drinking water was replaced by a 1% NaCl solution during the period of DOCA administration. At 10 to 11 weeks after nephrectomy, rats whose systolic blood pressures were higher than 185 mmHg were used as a group of 6.

iv) Normotensive rats (NRs): Ten- to 14-week-old male Wistar rats were used as a group of 5 to 9.

b) Experimental protocol

i) Single administration in 2K,1C-RHRs, SHRs, DOCA/s-HRs and NRs: Systolic blood pressure was measured by the tail cuff method (38°C, about 10 min) at fixed intervals after the administration of the drugs to rats fasted for 16 to 18 hr.

ii) Repeated administration in SHRs: SHRs were given the drugs orally once a day for 2 weeks. Blood pressure and heart rate were measured by the tail cuff method as mentioned above and with a pulse rate meter, respectively, at fixed intervals on days 1, 8 and 15.

Data analysis

All the results were expressed as the mean±S.E.M. In the case of uniform variance, the significance of difference was determined by Dunnett’s or Scheff’s multiple comparison following ANOVA. Otherwise, it was done by a Scheffe-type multiple comparison following Kruskal–Wallis analysis.

Results

1) Inhibition of ACE activity

i) Inhibitory effect on swine renal cortex ACE activity: TA-6366 was almost as potent as enalapril and about 1500 times less potent than captopril (Table 1). On the other hand, 6366A, an active form of TA-6366, and enalaprilat, an active form of enalapril, both were about 2 times more potent than captopril.
Table 1. In vitro potencies of TA-6366 and 6366A

<table>
<thead>
<tr>
<th>Compound</th>
<th>Converting enzyme (IC50 nM)</th>
<th>Guinea pig ileum&lt;sup&gt;a&lt;/sup&gt; BK (AC50 nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA-6366</td>
<td>9900</td>
<td>1.7</td>
</tr>
<tr>
<td>6366A</td>
<td>2.6</td>
<td>1.7</td>
</tr>
<tr>
<td>Enalapril</td>
<td>8900</td>
<td>2.4</td>
</tr>
<tr>
<td>Enalaprilat</td>
<td>3.1</td>
<td>2.4</td>
</tr>
<tr>
<td>Captopril</td>
<td>6.9</td>
<td>26</td>
</tr>
</tbody>
</table>

Values were calculated from the linear regression lines of the dose-response curves. <sup>a</sup>: Each value was obtained from 4 or 5 separate experiments. IC50 and AC50 are concentrations of compounds producing 50% inhibition and augmentation, respectively.

ii) Inhibitory effect on pressor response to AT-I: TA-6366 dose-dependently inhibited the AT-I-induced pressor response at 0.05–0.5 mg/kg, p.o., whereas 6366A did so only at 0.2 mg/kg or more (Fig. 2).

Enalapril and captopril also showed inhibitory effects on the response in the same dosage range as that of TA-6366.

The onset of the effect of TA-6366 was slower than those of enalapril and captopril at doses of 0.05 and 0.1 mg/kg, p.o.

When the time courses of the inhibitory effects of each drug at a dose of 0.5 mg/kg were compared, the effects of TA-6366, 6366A and enalapril reached maximum at 1, 3 and 1 hr after administration, respectively, and lasted for over 7 hr, whereas that of captopril reached a maximum at 30 min and disappeared within 4 hr after dosing. The duration of the effect of TA-6366 was longer than those of enalapril and captopril at this dosage.

2) Inhibition of bradykinin (BK) inactivation

i) Augmentative effect on BK-induced contraction of guinea pig ileum: 6366A and enalaprilat concentration-dependently augmented the BK-induced contraction at concentrations of 10<sup>-9</sup> M or more, whereas captopril did so at concentrations of 10<sup>-8</sup> M or more (Table 1).

6366A was almost as potent as enalaprilat and about 10 times more potent than captopril.

ii) Augmentative effect on depressor re-
response to BK: TA-6366 augmented the BK-induced depressor response at oral doses of 0.02 and 0.1 mg/kg (Fig. 3). The augmentative effect of TA-6366 reached a maximum 1 to 2 hr after administration and lasted for over 4 hr.

Enalapril and captopril augmented the response in the same dosage as that of TA-6366. TA-6366 was almost as potent as enalapril and less potent than captopril with respect to the maximum augmentative activity.

3) Effect on PRA, plasma AT-I and aldosterone concentrations in SHRs

TA-6366 significantly increased PRA and plasma AT-I concentration at doses of 2 and 5 mg/kg, p.o. (Table 2). Enalapril and captopril slightly but not significantly increased these concentrations at doses of 5 and 20 mg/kg, p.o., respectively. However, none of the drugs caused significant effects on plasma aldosterone concentration.

4) Antihypertensive effect of TA-6366

a) Single administration

i) Antihypertensive effect in 2K1C-RHRs: TA-6366 and enalapril almost dose-dependently decreased the blood pressure at doses of 0.5 to 2.0 mg/kg, p.o. (Figs. 4 and 5). Captopril significantly decreased blood pressure at 2 mg/kg, but not at 1 mg/kg, p.o. TA-6366 was almost as potent enalapril and approximately 4 to 5 times more potent than captopril as judged by the dose-response relation.

TA-6366, enalapril and captopril showed maximum antihypertensive effects 6, 6 and 1 hr after administration, respectively. The decreased blood pressure caused by 0.5 and 1 mg/kg of both TA-6366 and enalapril returned to the pre-administration level within 24 hr after administration, whereas at a dose of 2 mg/kg of both drugs, a significant decrease in blood pressure was still observed even 24 hr after administration.

On the other hand, at a dosage of 2 mg/kg of captopril, the blood pressure returned to the pre-administration level within 24 hr after administration.

ii) Antihypertensive effect in SHRs: TA-6366 and enalapril significantly and dose-dependently decreased the blood pressure at oral doses of 2 to 10 mg/kg, whereas it was significantly decreased by captopril at an oral dose of 10 mg/kg (Fig. 5). The antihypertensive effects of TA-6366 or enalapril alone

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Table 2. Plasma renin activity (PRA) and plasma angiotensin I (AT-I) and aldosterone (Ald) concentrations 6 hr after treatment with TA-6366, enalapril and captopril in SHRs

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (mg/kg, p.o.)</th>
<th>No. of rats</th>
<th>PRA (ng/ml/hr)</th>
<th>AT-I (ng/ml)</th>
<th>Ald (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>0.5</td>
<td>7</td>
<td>8.6±0.77</td>
<td>2.53±0.35</td>
<td>625.5±39.0</td>
</tr>
<tr>
<td>TA-6366</td>
<td>2</td>
<td>7</td>
<td>30.9±4.0*</td>
<td>15.51±2.58*</td>
<td>703.4±44.4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>7</td>
<td>42.0±6.9**</td>
<td>22.02±2.04**</td>
<td>674.6±34.0</td>
</tr>
<tr>
<td>Enalapril</td>
<td>5</td>
<td>7</td>
<td>25.1±2.9</td>
<td>13.48±1.30</td>
<td>645.6±26.1</td>
</tr>
<tr>
<td>Captopril</td>
<td>20</td>
<td>6</td>
<td>24.0±4.5</td>
<td>9.94±1.37</td>
<td>644.4±30.9</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E.M. Significantly different from the distilled water-treated group: *P<0.05, **P<0.01 (Schefte type multiple comparison).
at doses of 2 and 5 mg/kg, and at a dose of 10 mg/kg were observed 3 and 1 hr after the
dosing, respectively, and lasted for over 6 hr. On the other hand, the antihypertensive effect
of captopril at a dose of 10 mg/kg was observed 1 hr after the dosing, but disappeared
within 6 hr after the dosing (data not shown). TA-6366 was almost as potent as enalapril
and about 5 times more potent than captopril as judged by the dose-response relation.

iii) Antihypertensive effect in DOCA/s-
HRs: TA-6366 and enalapril at an oral dose of
20 mg/kg and captopril at an oral dose of 100
mg/kg had no significant effect on the blood
pressure when the maximum change in the
blood pressure occurred (Fig. 5).

iv) Hypotensive effect in NRs: TA-6366,
enalapril or captopril had no significant effect
on the blood pressure even at doses of 5, 5 or
20 mg/kg, p.o., respectively (Fig. 5). TA-
6366, enalapril and captopril, however, signifi-
cantly decreased the blood pressure at doses
of 10, 10 and 50 mg/kg, p.o., respectively.
The hypotensive effects of TA-6366 and
enalapril were observed 3 hr after adminis-
tration and reached a maximum 6 hr after the
dosing, whereas that of captopril was sus-
tained at about the same magnitude from 1 to
6 hr after administration (data not shown).

Repeated administration in SHRs
TA-6366 dose-dependently and signifi-
cantly reduced the blood pressure at doses of

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Fig. 4. Time courses of antihypertensive effects of
TA-6366, enalapril and captopril in two-kidney, one-
clip renal hypertensive rats. Each value represents the
mean±S.E.M. Some of the standard errors are
within the symbols. Data are expressed as the per-
centage of change in systolic blood pressure relative to
pretreatment with the drugs. O: distilled water
(N=10), ●: TA-6366 (N=6–7), □: enalapril (N=7).
▲: captopril (N=7–8). Significantly different from
the distilled water treated group: *P<0.05, **P<0.01
(Scheffe's multiple comparison).

Fig. 5. Antihypertensive effects of TA-6366, enalapril and captopril in various hypertension model rats
and normotensive rats. Each column represents the mean±S.E.M. N=5–10. 2K,1C-RHRs: Two-kidney,
one-clip renal hypertensive rats. SHRs: spontaneously hypertensive rats, DOCA/saline HRS: DOCA/saline
hypertensive rats, NRs: normotensive rats. Data in the treatment groups with TA-6366 and enalapril
and in the treatment group with captopril are shown with values 6 hr and 1 hr after the dosing, respectively.
Data are expressed as the percentage of change in systolic blood pressure relative to pretreatment with
the drugs. Significantly different from the distilled water treated group: *P<0.05, **P<0.01 (Scheffe's
multiple comparison).
2, 5 and 10 mg/kg/day, p.o., during the period of administration (Fig. 6). On day 1, TA-6366 showed a maximum antihypertensive effect 6 hr after administration. The blood pressure at a dose of 2 mg/kg/day almost returned to the pre-administration level within 24 hr after administration, whereas at doses of 5 and 10 mg/kg/day, a significant decrease in blood pressure was still observed even 24 hr after administration. On day 8, the antihypertensive activity of TA-6366 was intensified in potency at doses of 2 and 10 mg/kg/day. Thereafter, no further intensification of potency of action was observed in spite of an increase in the number of administrations. Enalapril, at doses of 2 and 10 mg/kg/day, and captopril, at the dose of 20 mg/kg/day, also reduced the blood pressure during the period of administration.

The durations of the antihypertensive activities of enalapril and captopril, like that of TA-6366, were intensified as the number of administrations increased.

Fig. 6. Effects of repeated administrations of TA-6366, enalapril and captopril on blood pressure in spontaneously hypertensive rats. Each value represents the mean±S.E.M. N=5. Data are expressed as the percentage of change in systolic blood pressure relative to pretreatment with the drugs on Day 1. ○: distilled water (4 ml/kg); ●: 2 mg/kg, p.o.; □: 5 mg/kg, p.o.; ▲: 10 mg/kg, p.o.; △: 20 mg/kg, p.o. Significantly different from the distilled water treated group. *P<0.05, **P<0.01 (Dunnett's multiple comparison).

The significant antihypertensive effects of TA-6366 at doses of 5 and 10 mg/kg/day, but not 2 mg/kg/day, were still observed even 24 hr after the dosing during the period of administration. On the other hand, no significant decrease in blood pressure was caused 24 hr after the dosing of either 10 mg/kg/day of enalapril or 20 mg/kg/day of captopril during the period of each administration. The duration of the antihypertensive effect of TA-6366 was longer than that of enalapril or captopril.

TA-6366, like captopril or enalapril, had no significant effect on the heart rate during the period of administration (Table 3).

Discussion

Although TA-6366 was less potent than 6366A, an active metabolite of TA-6366, in its inhibitory effect on in vitro ACE activity in swine renal cortex, TA-6366 was more potent than 6366A in its inhibitory effect on AT-I-induced pressor response in rats when administered orally. This indicates that TA-6366 is a prodrug that inhibits ACE after it is hydrolyzed into the more active metabolite 6366A in vivo.

TA-6366 inhibited the AT-I-induced pressor response more persistently than enalapril or captopril, when compared at the same dosage of 0.5 mg/kg, p.o. Furthermore, in SHRs, increases in PRA and plasma AT-I concentration 6 hr after the oral administration of TA-6366 were more prominent than those of enalapril or captopril. These results suggest that the duration of the ACE inhibitory activity of TA-6366 in vivo is longer than that of enalapril or captopril.

It is well-known that ACE catalyzes the inactivation of BK (5). 6366A augmented the BK-induced contraction of isolated guinea pig ileum more potently than captopril. However, TA-6366, when given orally, augmented the BK-induced depressor response in rats less potently than captopril.

It is reported that a metabolite of captopril, namely captopril disulphide dimer, is as effective as captopril in augmenting the BK-induced depressor response in urethane anesthetized rats, but is much less potent than captopril in augmenting the BK-induced contraction of isolated guinea pig ileum (6). The difference between TA-6366 and captopril in
Table 3. Effects of repeated administration of TA-6366, enalapril and captopril on heart rate in spontaneously hypertensive rats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose</th>
<th>Day 1</th>
<th>Day 8</th>
<th>Day 15</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>0</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Distilled water</td>
<td></td>
<td>451±25</td>
<td>436±22</td>
<td>437±35</td>
</tr>
<tr>
<td>TA-6366</td>
<td>2</td>
<td>420±8</td>
<td>425±21</td>
<td>426±27</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>450±9</td>
<td>446±16</td>
<td>437±10</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>446±7</td>
<td>446±11</td>
<td>462±9</td>
</tr>
<tr>
<td>Enalapril</td>
<td>2</td>
<td>413±17</td>
<td>436±17</td>
<td>434±4</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>446±17</td>
<td>457±25</td>
<td>435±13</td>
</tr>
<tr>
<td>Captopril</td>
<td>20</td>
<td>456±16</td>
<td>452±20</td>
<td>429±10</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E.M. (n=5). *: Time after drug administration.
BK augmenting effect in vitro and in vivo is probably due to the BK augmenting effect of captopril disulphide dimer. Considering our result that the duration of the augmentative effect of captopril on the depressor response to BK was longer than that of the inhibitory effect on the pressor response to AT-I, captopril has a more selective effect on the response to BK than to AT-I. However, in the case of TA-6366, there was no dissociation between the augmentative effect on the depressor response to BK and the inhibitory effect on the pressor response to AT-I.

Thus, these results suggest that captopril probably acts more prominently on the kallikrein-kinin system than on the renin-angiotensin system, whereas TA-6366 does not have such a selectivity.

It has been reported that an increase in plasma renin level is a contributory factor in developing and maintaining hypertension in 2K-RHRs (7, 8). On the other hand, it is known that PRA is suppressed in DOCA/s-HRs (9). Considering that TA-6366 showed a remarkable antihypertensive effect in 2K,1C-RHRs, but exhibited little effect in DOCA/s-HRs, the effect in 2K,1C-RHRs seems to be mainly attributable to a decrease in circulating AT-II levels via ACE inhibition.

TA-6366 also showed an antihypertensive effect in SHRs whose PRA is known to be either equal to or lower than that of NRs (10, 11). Therefore, the antihypertensive effect of TA-6366 in SHRs is thought to result from the inhibition of ACE activity not only in the plasma but also in tissues, including vascular walls. As it is known that the local formation of AT-II in the vasculature is elevated in SHR (12) and that renin concentration and ACE activity in the arteries are significantly higher in SHRs than in normotensive WKY rats (13), the reduction in blood pressure caused by TA-6366 in SHRs is probably due to inhibition of the local production of AT-II in the vasculature. The inhibitory effect of TA-6366 on ACE activity of the serum and the aorta in Wistar rats and SHRs was more persistent than that of enalapril (T. Watanabe et al., unpublished data). Therefore, the superior persistence of the antihypertensive effect of TA-6366 by repeated administration is probably related to this result.

It has been reported that renin activity in the arteries is lower in DOCA/s-HRs than in NRs (13). Therefore, the suppressed renin activity in the vasculature as well as plasma in DOCA/s-HRs may contribute to the result that TA-6366 caused little antihypertensive effect in this model.

In NRs, TA-6366 showed hypotensive effects only at doses approximately 5 to 20 times higher than those producing antihypertensive effects in 2-K,1C-RHRs and SHRs, indicating that the role of the renin-angiotensin system on blood pressure regulation in NRs may be less important than that in 2K,1C-RHRs or SHRs.

TA-6366 had no effect on heart rate despite its remarkable antihypertensive effect in SHRs. Considering that AT-II facilitates transmission at sympathetic neuro-effector sites (14) and that the inhibition of AT-II formation by blockade of ACE suppresses the response to sympathetic activation in animals (15, 16), this result is attributable to a decrease in noradrenaline release from the sympathetic nerve endings resulting from the inhibition of AT-II formation.

These results indicate that TA-6366 is a potent and long-lasting antihypertensive agent and that its antihypertensive action is mainly attributable to suppression of the renin-angiotensin system and augmentation of the kallikrein-kinin system.

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References


