Effects of Long-Term Administration of (4S)-1-Methyl-3-[(2S)-2-[N-((1S)-1-ethoxycarbonyl-3-phenylpropyl)amino]propionyl]-2-oximidazolidine-4-carboxylic Acid Hydrochloride (TA-6366), a New Angiotensin I Converting Enzyme (ACE) Inhibitor, from the Pre-hypertensive Stage on Morphological Change and Mechanical Property Related to Sodium Ion Permeability in Aorta of Spontaneously Hypertensive Rats (SHRs)

Masami KUBO,* Kinji KOBAYASHI, ** and Ryuichi ISHIDA*

Pharmacological Research Laboratory* and Safety Research Laboratory, ** Tanabe Seiyaku Co., Ltd., 3-16-89, Kashima, Yodogawa-ku, Osaka, 532, Japan

(Received June 24, 1992)

Effects of (4S)-1-methyl-3-[(2S)-2-[N-((1S)-1-ethoxycarbonyl-3-phenylpropyl)amino]propionyl]-2-oximidazolidine-4-carboxylic acid hydrochloride (TA-6366) on morphological change and mechanical property related to sodium ion permeability in the aorta of spontaneously hypertensive rats (SHRs) were examined, as compared with those of enalapril and captopril. Ten-week oral administration of TA-6366 (1 and 5 mg/kg/d) from 4 weeks of age impeded aortic media-thickening together with a rise in blood pressure in SHRs. Concomitantly, aorta weights in both groups were markedly decreased. The higher dose of TA-6366 almost fully suppressed the accelerated tension development induced by K+-free medium and decreased total sodium ion content in the aorta. These vascular effects of TA-6366 was more prominent than those of enalapril and captopril at 5 mg/kg/d. The difference in potencies on the above vascular parameters between TA-6366 and these drugs seemed to be mainly related to the difference in their antihypertensive activities. These results suggest that TA-6366 has preventive effects against progression of vascular diseases, particularly atherosclerosis, accompanied with hypertension.

Keywords — (4S)-1-methyl-3-[(2S)-2-[N-((1S)-1-ethoxycarbonyl-3-phenylpropyl)amino]propionyl]-2-oximidazolidine-4-carboxylic acid hydrochloride (TA-6366); angiotensin I converting enzyme (ACE) inhibitor; aortic hypertrophy; media thickness; hypertension; spontaneously hypertensive rat (SHR); sodium ion permeability

Introduction

The altered structure in the cardiovascular system accompanying hypertension has been considered to be a secondary adaptation to the elevated blood pressure.1-3 However, various antihypertensive treatments have revealed differences in their ability to cause regression of these structure changes.4-7 This suggests that factors other than blood pressure are involved in the determination of the cardiovascular structure. For example, an abnormal electrolytes metabolism in the vasculature is thought to cause structure changes, such as swelling of the vessel wall and narrowing of the vascular lumen.8-11

As another candidate, angiotensin II (Ang II) is well known to stimulate hypertrophy of the smooth muscle cells in culture.12 In addition, since recent findings demonstrate the existence of an intrinsic renin-angiotensin system (RAS) within the vascular wall,13,14 there is considerable interest in the involvement of locally generated Ang II in the regulation of local vascular function and structure. Indeed, many studies have shown that angiotensin I converting enzyme (ACE) inhibitors are effective on these vascular changes associated with hypertension, but there are variations in their abilities.15-18

TA-6366, (4S)-1-Methyl-3-[(2S)-2-[N-((1S)-1-ethoxycarbonyl-3-phenylpropyl)amino]propionyl]-2-oximidazolidine-4-carboxylic acid hydrochloride, a newly developed ACE inhibi-

Fig. 1. Chemical Structure of TA-6366
tor (Fig. 1), potently impeded hypertension development in young spontaneously hypertensive rats (SHRs); and its long-term effect on blood pressure was confirmed even after withdrawal. Concomitant evidence that TA-6366 inhibited the aortic ACE more persistently than enalapril and captopril suggests a pronounced ability to cause regression of vascular structure changes in young SHRs.

The purpose of the present study was to examine the effect of long-term treatment with TA-6366 on morphological change and mechanical property related to Na+ metabolism in SHR aorta and to compare its ability with that of enalapril and captopril.

**Materials and Methods**

**Animals and Drug Treatmeent** — One hundred and eleven 4-week old male SHRs (from Charles River Japan, Inc.), each weighing 65—94 g, and 20 age-matched Wistar-Kyoto rats (WKVs), each weighing 87—97 g (from Charles River Japan, Inc.), were used. The animals were maintained under identical conditions of temperature (23 ± 1 °C), humidity (55 ± 5%) and photoperiod (12 h on, 12 h off); and they were allowed normal rat chow (CRF-1, Oriental Yeast Industry Co., Ltd.) and water ad libitum.

One hundred and five SHRs and 14 WKVs were randomly divided into the following 4 groups: group I (35 SHRs) for the measurements of aorta weight and electrolyte contents 24 h after the final dosing; group II (26 SHRs and 8 WKVs) for the measurement of the aortic contraction induced by K+-free medium to examine the mechanical properties 24 h after the final dosing; groups III (27 SHRs and 6 WKVs) and IV (17 SHRs) for the measurements of aortic media thickness and heart weight 24 h and 5 weeks after the final dosing, respectively. SHRs in groups I and III were then assigned the following treatment groups: control (10 ml distilled water/kg/d) group, TA-6366, 1 mg (2.3 μmol)/kg/d group, TA-6366, 5 mg (11.3 μmol/kg/d group, enalapril, 5 mg (10.0 μmol)/kg/d group and captopril 5 mg (23.0 μmol)/kg/d group. SHRs in group II and IV were also divided into the following 3 treatment groups: control (10 ml distilled water/kg/d) group, TA-6366, 5 mg/kg/d group and enalapril, 5 mg/kg/d group. The drugs and vehicle were administered by gavage once a day at a fixed time (10:00—11.00 A.M.) from 4 weeks of age for 10 weeks in groups I, III and IV, and for 14 weeks in group II.

The remaining 6 rats each of SHRs and WKVs were used to measure aortic media thickness at 4 weeks of age (group V).

**Measurement**

**Blood Pressure** — Systolic blood pressure was measured by the tail cuff method after the rats were put into a cabinet (MK-1000, Muromachi Kikai) kept at 38 °C for about 8 min. The measurement was performed using animals selected randomly among the corresponding treatment groups before long-term treatment with the drugs, 24 h after the final dosing, and 5 weeks after withdrawal.

**Aortic Media Thickness and Heart Weight** — Immediately after the animals in groups III, IV and V were killed by bleeding under sodium pentobarbital (30 mg/kg, i.v.) anesthesia, the thoracic aorta was removed and fixed with 10% formalin for 3 d. Thereafter, the tissue was embedded in O.C.T. compound (Miles) and cut into coronal sections at a thickness of 6 μm with a cryostat at −20 °C. After micrographs of these slices stained with van Gieson stain, magnified to ×50, were taken, each film was analyzed by a Computerized Image Processing (MCID-II, Imaging Research, Canada). Media thickness was expressed as the distance between the two edges of the medial layer. The measurement of each aorta was performed at 3 randomly different points; and their average was calculated. As to the animals in groups III and IV, the heart together with the aorta was isolated. After the blood on the organ was wiped off with filter paper, the organ was weighed.

**Aorta Weight and Aortic Electrolyte Contents** — Twenty-four hours after the final dosing, the animals in group I were bled to death under ether anesthesia. Immediately after the thoracic aortas (about 30 mm in length from the branch of the left subclavian artery) were isolated, the connective tissue and the coagulated blood attached to the aorta were removed, and
then their wet weights were measured. After their tissues dried at 105 °C for 24 h were weighed, they were immersed in 5% nitric acid at a room temperature for 5 d to extract sodium and potassium. The concentration of both cations in the supernatants of the extracts was analyzed by flame photometry (205D, Hitachi).

**Contractile Response of the Aorta to K+-Free Medium** — Twenty-four hours after the final dosing, the animals in group II were bled to death under ether anesthesia. The thoracic aorta was quickly isolated, cleaned of adherent connective tissues and cut into a helical strip of 25 mm in length and 4 mm in width. The aortic strips were suspended between a fixed post and a force-displacement transducer (SB-IT, Nihon-Kohden Kogyo Co., Ltd.). The preparations were immersed in organ baths containing 20 ml of Tyrode's solution, which was maintained at 37 °C and aerated with a mixture of 95% O₂ and 5% CO₂. The resting tension was adjusted to 1 g. The strips were equilibrated for about 90 min in the bathing media, during which time the solution was replaced about every 20 min, and thereafter conditioned by three applications of hypertonic 60 mM KCl solution.

After being conditioned with 60 mM KCl, the muscles were exposed to K+-free Tyrode's solution for 2 h in the presence of 10⁻⁶ M phenolamine to exclude the influence of endogenous catecholamine. The developed isometric force was written on a recorder (SR-6211, Graphtec) through an amplifier (EF-601G or AP-620G, Nihon-Kohden Kogyo Co.). The constituents of Tyrode's solution were as follows (mM): NaCl, 136.8; KCl, 5.4; CaCl₂, 2.5; MgCl₂, 1.0; NaHCO₃, 11.9 and glucose, 5.5. K+-Free Tyrode's solution was made by omitting KCl from the above solution. Contractile responses to K+-free solution were expressed as relative value of the maximal contraction induced by 60 mM KCl.

**Drugs** — TA-6366, enalapril and captopril were synthesized at the Research Laboratory of Applied Biochemistry, Tanabe Seiyaku Co., Ltd., as previously reported. The other drugs used were as follows: phenolamine mesylate (Ciba-geigy) and sodium pentobarbital (Abbott).

**Statistics** — All the results were expressed as the mean ± S.E.M. When comparing two groups, statistical analysis was performed by Student’s 𝑡-test. When comparing multiple groups, statistical analysis was performed as follows: In the case of an equal variability, the significant difference was calculated by Scheffe’s or Dunnett’s multiple comparison following ANOVA. If an equal variability was absent, statistical analysis was calculated by a Scheffe’s type or Dunnett’s type multiple comparison following Kruskal-Wallis analysis.

**Results**

### Effects of Long-Term Treatment

#### Effects on Blood Pressure and Body Weight

<table>
<thead>
<tr>
<th>Group</th>
<th>Drugs (mg/kg/d)</th>
<th>Before treatment</th>
<th>24 h after 10-week treatment</th>
<th>5 weeks after withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBP (mmHg)</td>
<td>BW (g)</td>
<td>SBP (mmHg)</td>
<td>BW (g)</td>
</tr>
<tr>
<td>SHRs</td>
<td>Distilled water</td>
<td>137.0±1.9</td>
<td>76.9±1.9 (18)</td>
<td>202.4±2.1</td>
</tr>
<tr>
<td></td>
<td>TA-6366</td>
<td>1.375±2.6</td>
<td>71.2±2.1 (12)</td>
<td>181.3±4.6</td>
</tr>
<tr>
<td></td>
<td>TA-6366</td>
<td>5.380±1.9</td>
<td>73.2±1.4 (16)</td>
<td>155.2±1.8</td>
</tr>
<tr>
<td></td>
<td>Enalapril</td>
<td>137.4±2.0</td>
<td>73.1±1.4 (15)</td>
<td>165.3±1.6</td>
</tr>
<tr>
<td></td>
<td>Captopril</td>
<td>135.2±3.0</td>
<td>72.6±2.3 (10)</td>
<td>183.1±3.2</td>
</tr>
<tr>
<td>WKYs</td>
<td>Distilled water</td>
<td>121.7±2.0</td>
<td>80.5±3.4 (12)</td>
<td>147.0±4.0</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. Measurements were performed using animals selected randomly among the corresponding treatment groups. Number of rats are shown in parentheses. Significantly different from the distilled water-treated group in SHRs: a) 𝑝<0.05, b) 𝑝<0.01. Significantly different from the enalapril-treated group: c) 𝑝<0.01.
TABLE II. Aortic Media Thickness and Relative Heart Weight (HW) 24 h and 5 Weeks after 10-Week Oral Treatment with TA-6366, Enalapril and Captopril in SHRs

<table>
<thead>
<tr>
<th>Group</th>
<th>Drugs</th>
<th>Dose (mg/kg/d)</th>
<th>24 h after 10-week treatment</th>
<th>5 weeks after withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Media thickness (μm)</td>
<td>Relative HW (mg/100 g BW)</td>
<td>Media thickness (μm)</td>
</tr>
<tr>
<td>SHRs</td>
<td>Distilled water</td>
<td>114.2 ± 1.4</td>
<td>319.3 ± 2.0 (6)</td>
<td>114.9 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>TA-6366</td>
<td>94.5 ± 3.0</td>
<td>289.7 ± 4.5 (6)</td>
<td>105.1 ± 6.3</td>
</tr>
<tr>
<td></td>
<td>TA-6366</td>
<td>87.1 ± 4.1</td>
<td>280.1 ± 3.2 (5)</td>
<td>107.7 ± 4.8</td>
</tr>
<tr>
<td></td>
<td>Enalapril</td>
<td>98.7 ± 4.1</td>
<td>290.3 ± 2.9 (5)</td>
<td>107.7 ± 4.8</td>
</tr>
<tr>
<td></td>
<td>Captopril</td>
<td>103.9 ± 4.4</td>
<td>315.2 ± 4.9 (5)</td>
<td>107.7 ± 4.8</td>
</tr>
<tr>
<td>WKYs</td>
<td>Distilled water</td>
<td>86.8 ± 4.0</td>
<td>272.3 ± 3.6 (6)</td>
<td>114.9 ± 3.3</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. HW, heart weight; BW, body weight. Number of rats are shown in parentheses. Significantly different from the distilled water-treated group in SHRs: a) p < 0.05, b) p < 0.01. Significantly different from the enalapril-treated group: c) p < 0.01.

Gain — Systolic blood pressure of the control group in SHRs was significantly higher (about 15 mmHg) than that in WKYs at 4 weeks of age (Table I). Thereafter, it rose up to the level of 202.4 ± 2.1 mmHg at 14 weeks of age, the level being 50—60 mmHg higher than that in age-matched WKYs. Before treatment with the drugs, no significant difference in blood pressure was observed between the control group and all the drug-treated groups in SHRs. After 10 weeks of treatment, blood pressure of both the TA-6366 groups, like that of the enalapril and captopril groups, was significantly lower than that of the control group, and the extent of reduction depended on the dose.

When blood pressure was compared among all the drug-treated groups, TA-6366 at 1 mg/kg/d lowered it as much as captopril, whereas TA-6366 at 5 mg/kg/d reduced it about 10 mmHg lower (p < 0.01) than enalapril did. In addition, there was no significant change in blood pressure between 10 and 14 weeks of treatment with TA-6366 and enalapril (data not shown).

After 10 weeks treatment, there was no significant difference in heart rate between the control group in SHRs and all the drug-treated groups (data not shown). TA-6366 and captopril did not affect the body weight increase, whereas enalapril slightly but significantly impeded it (Table I).

Effect on Aortic Media Thickness and Heart Weight — No significant difference in aortic media thickness was observed between SHRs and WKYs at 4 weeks of age (93.8 ± 1.6, N = 6; 91.4 ± 2.6 μm, N = 6, respectively). After that, the media of the control group significantly thickened in SHRs at 14 weeks of age, as compared with that of the age-matched WKY (Table II, Fig. 2). In both of the TA-6366 groups, media-thickening was significantly impeded, the effect being dose-dependent. On the other hand, the media of both enalapril and captopril groups was slightly, but not significantly thinner than that of the control group. When the correlation between blood pressure and media thickness was compared among all the drug-treated groups after 10 weeks treatment, the media thickness almost depended on blood pressure in the TA-6366 (5 mg/kg/d), enalapril and captopril groups, whereas that in the TA-6366 (1 mg/kg/d) group was decreased below the level predicted by its blood pressure-lowering effect (Fig. 3).

Relative heart weights of both the TA-6366 groups significantly decreased as compared with those of the control group in SHRs (Table II). Enalapril also significantly reduced relative heart weight, whereas captopril showed no significant difference.

Effect on Aorta Weight and Aortic Electrolyte Contents — In both the TA-6366 groups, wet and dry weights in the aorta were significantly smaller than those of the control group (Table III). Both dry and wet weights of the enalapril group also decreased, but not significantly on the latter parameter. On the other hand, there was
Effect of TA-6366 on Aortic Hypertrophy

Fig. 2. Typical Micrographs of Cross-Sections of Aorta after 10-Week Oral Treatment with TA-6366, Enalapril and Captopril in SHRs

A, SHR treated with distilled water (10 ml/kg); B, SHR treated with TA-6366 (1 mg/kg/d); C, SHR treated with TA-6366 (5 mg/kg/d); D, SHR treated with enalapril (5 mg/kg/d); E, SHR treated with captopril (5 mg/kg/d); F, WKY treated with distilled water (10 ml/kg).

no significant decrease in the aorta weights of the captopril group.

TA-6366 significantly reduced Na⁺ in the aorta at 5 mg/kg/d, but not 1 mg/kg/d, whereas it caused no significant change in K⁺ at both doses. On the other hand, neither enalapril nor captopril affected either electrolyte contents.

Effect on Aortic Contraction Induced by K⁺-Free Medium — K⁺-Free solution induced a persistent contraction which developed slowly after some delay (Fig. 4). The contractile tension of SHR aorta increased faster than that of the WKY aorta and reached the maximal level at about 90 min after exposure to the solution. TA-6366 at 5 mg/kg/d significantly delayed the tension development during the exposure. The development of the TA-6366 group proceeded at almost the same degree as that of WKY until
about 70 min after the exposure, but thereafter it was slower than that of WKY. Enalapril also suppressed the development slightly, but not significantly. As judged by the time, at which contraction reached the half level of the maximal contraction due to 60 mM KCl, TA-6366 delayed the development more strongly than enalapril (Table IV).

Changes in Blood Pressure, Body Weight, Media Thickness and Heart Weight after Withdrawal

Five weeks following cessation after 10-week treatment, blood pressure of the control group rose to about 17 mmHg higher level than that after the final dosing (Table I). Blood pressure of the TA-6366 5 mg/kg/d group was elevated by about 30 mmHg as compared with that after the final dosing, but it remained significantly lower (about 35 mmHg) than that of the control group. In the enalapril group, it was still at lower level than that of the control group, but about 10 mmHg higher ($p<0.01$) than that of the TA-6366 group.

Media thickness of the TA-6366 group, like that of the enalapril group, was slightly, but not significantly, thinner than that of the control group (Table II). Concomitantly, relative heart

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**TABLE III. Wet and Dry Weights, and Electrolytes Contents in Thoracic Aorta 24 h after 10-Week Oral Treatment with TA-6366, Enalapril and Captopril in SHRs**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (mg/kg/d)</th>
<th>No. of rats</th>
<th>Wet weight (mg/cm)</th>
<th>Dry weight (mg/cm)</th>
<th>Na⁺ (mEq/kg dry weight)</th>
<th>K⁺ (mEq/kg dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td></td>
<td>7</td>
<td>8.10±0.25</td>
<td>3.18±0.07</td>
<td>223.7±4.0ᵇᵇ</td>
<td>147.5±2.9ᵇᵇ</td>
</tr>
<tr>
<td>TA-6366</td>
<td>1</td>
<td>7</td>
<td>7.42±0.11ᵇ</td>
<td>2.96±0.03ᵇᵇ</td>
<td>217.5±5.8</td>
<td>148.3±3.6</td>
</tr>
<tr>
<td>TA-6366</td>
<td>5</td>
<td>7</td>
<td>7.21±0.20ᵇᵇ</td>
<td>2.94±0.05ᵇᵇ</td>
<td>202.1±4.5ᵇᵇ</td>
<td>140.6±3.0</td>
</tr>
<tr>
<td>Enalapril</td>
<td>5</td>
<td>7</td>
<td>7.54±0.20ᵇᵇ</td>
<td>2.93±0.06ᵇᵇ</td>
<td>212.3±3.5</td>
<td>142.1±2.2</td>
</tr>
<tr>
<td>Captopril</td>
<td>5</td>
<td>7</td>
<td>8.01±0.11</td>
<td>3.11±0.05</td>
<td>213.4±3.3</td>
<td>144.9±1.5</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. a) $N=6$. Significantly different from the control: b) $p<0.05$. c) $p<0.01$.
TABLE IV. Contractile Response Time during Exposure to 
K⁺ -Free Solution in Aorta 24 h after 14-Week Oral Treat-
ment with TA-6366 and Enalapril in SHRs

<table>
<thead>
<tr>
<th>Group</th>
<th>Drugs</th>
<th>Dose (mg/kg/d)</th>
<th>No. of rats</th>
<th>Timea (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHRs</td>
<td>Distilled</td>
<td>9</td>
<td>57.4 ± 1.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA-6366</td>
<td>5</td>
<td>8</td>
<td>78.2 ± 5.0*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Enalapril</td>
<td>5</td>
<td>66.3 ± 4.3</td>
<td></td>
</tr>
<tr>
<td>WKYs</td>
<td>Distilled</td>
<td>8</td>
<td>72.4 ± 5.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>water</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. a) Response time at which contraction reached the half level of the maximal contraction induced by 60 mM KCl. Significantly different from the distilled water-treated group in SHRs: b) p < 0.05.

weight of the TA-6366 group still decreased significantly as compared with that of the control group and the enalapril group. On the other hand, in the enalapril group, it was slightly, but not significantly, lighter than that of the control group.

No significant difference in body weight was observed between the control group and the TA-6366 group (Table I). On the other hand, body weight of the enalapril group was significantly lower than that of the control group.

Discussion

The long-term administration of TA-6366 (1 and 5 mg/kg/d) from 4 weeks of age, when aortic media-thickening was not yet initiated, imped ed the thickening and decreased the aorta weight in SHRs. These findings suggest that long-term treatment with TA-6366 prevents smooth muscle hypertrophy characteristic of SHRs.

The antihypertensive activity of TA-6366 by single administration has been found to be approximately 5 times more potent than that of captopril and almost as potent as that of enalapril in SHRs.20) Although we decided the dose used in the present study, based on the above evidence, blood pressure 24 h and 5 weeks after the final dosing of TA-6366 was lower than that of enalapril. The possible cause of this seems to be related to the difference in duration of antihypertensive action between TA-6366 and enalapril.

Elevated blood pressure is well known to alter the vascular structure.1-3,29) Thus, the vascular effect of TA-6366 seems to be attributable to the reduced pressure stress due to its blood pressure-lowering effect. Media thickness-reducing effect of TA-6366 was more pronounced than that of enalapril and captopril. Captopril has already been reported to cause regression of the vascular hypertrophy, though the reported effective dose is 5 to 20 times higher than that in the present study.5,18,30) When the correlation between blood pressure and media thickness was compared among all the drug-treated groups, TA-6366 at the higher dose, like enalapril and captopril, decreased media thickness, almost dependently of the degree of the reduced blood pressure. Thus, the difference in the potencies on medial hypertrophy among TA-6366, enalapril and captopril may correlate with the difference in their antihypertensive activities.

TA-6366 at the lower dose tended to be a little more effective in inhibiting media thickening than in impeding a rise in blood pressure. When aortic ACE activity was compared among all the drug-treated groups after the same treatment as that in the present study, TA-6366 at the lower dose inhibited it as potently as the drug at the higher dose and more potently than enalapril and captopril.19) Considering that Ang II has been reported to cause vascular hypertrophy in part by a non-pressor mechanism,31) the effect of TA-6366 against aortic hypertrophy may be partly related to suppression of Ang II production due to its ACE inhibitory action in the aorta. However, to discuss whether the inhibitory effect of TA-6366 on medial hypertrophy closely correlates to its inhibition of Ang II production in the aorta, further experiments are needed.

Long-term treatment with TA-6366 persistent ly decreased relative heart weight. The effect of TA-6366 on heart weight was more prominent than that on media thickness after withdrawal. We have previously confirmed that the duration of ACE inhibition of TA-6366 in the heart was shorter than that in the aorta (Hashimoto et al., unpublished data). Thus, the long-lasting effect of TA-6366 on heart weight cannot be explained only by its ACE inhibitory action in the heart. Regression of cardiac hypertrophy has been reported to be achieved by various antihypertensive drugs, despite different modes of action.32)
Therefore, relative heart weight-reducing effect of TA-6366 may be simply dependent on the reduced cardiac afterload that is seen as a consequence of persistent blood pressure fall.

TA-6366 decreased aortic Na\(^+\) content, which may provide another evidence for the lack of muscle hypertrophy. Tissue Na\(^+\) content is known to vary with the movement of the fluid volume. Although TA-6366 had no significant effect on urine volume and urinary electrolytes after the same treatment as that in this study,\(^{19}\) acute study with the drug has been found to induce the natriuretic action in SHRs (Hashimoto et al., unpublished data). Thus, the above mentioned effect of TA-6366 may be due to its possible diuretic action at the early stage during the long-term treatment.

TA-6366 delayed the accelerated tension development of the aorta from SHRs induced by K\(^+\)-free medium. As to the smooth muscle contraction induced by K\(^+\)-free environment, there are the following two possible mechanisms: The first mechanism involves the possibility that an increase in intracellular Na\(^+\) concentration, resulting from inhibition of Na\(^+\)-K\(^+\)-ATPase, may promote an influx of extracellular Ca\(^{2+}\).\(^{33,34}\) In addition, the rate of the rise of this contraction has been known to well correlate with Na\(^+\) permeability in the aortic membrane of SHRs.\(^{35}\) The second mechanism concerns noradrenaline release from adrenergic nerve following inhibition of the sodium pump.\(^{20,21}\) In the present experimental condition treated with phenolamine, however, the involvement of the latter mechanism seems to be denied. Thus, the delay effect of TA-6366 on the tension development may be attributable to its inhibitory action on the excessive Na\(^+\) permeability in the aorta of SHRs. Ang II enhances vascular permeability to Na\(^+\),\(^{36}\) which suggests the involvement of RAS in the genesis of the abnormal permeability. In addition, elevated blood pressure is known to cause the abnormal ion permeability in the vasculature. Thus, the above mentioned effect of TA-6366 may be partly attributable to the inhibition of Ang II production in addition to the reduced blood pressure, and consequently TA-6366 seems to prevent structure change, such as narrowing of the vascular lumen, resulting from an abnormal electrolytes metabolism. Considering that effect of enalapril on Na\(^+\) flux was less potent than that of TA-6366, difference in antihypertensive activities between TA-6366 and enalapril may determine their abilities to alter membrane Na\(^+\) permeability in the aorta.

In conclusion, long-term treatment with TA-6366 impeded cardiovascular hypertrophy characteristic of SHRs, dependently of its potent and long-lasting antihypertensive action. These results suggest that TA-6366 has a preventive effect on progression of vascular diseases, particularly atherosclerosis, accompanied with hypertension.

Acknowledgements We thank Mr. M. Nakao for his excellent technical assistance.

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