Effects of YM358, an Angiotensin II Type 1 (AT_1) Receptor Antagonist, and Enalapril on Blood Pressure and Vasoconstriction in Two Renal Hypertension Models

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The effects of the angiotensin II receptor antagonist YM358 on blood pressure were compared to those of the angiotensin converting enzyme inhibitor enalapril in one-kidney, one-clip renal hypertensive rats (1K1C RHR), two-kidney, one-clip renal hypertensive rats (2K1C RHR) and normotensive rats (NTR). Additionally, the local drug actions in peripheral tissues were investigated using isolated mesenteric arteries from these rats. In 2K1C RHR, YM358 and enalapril produced a long-lasting hypotensive effect in a dose-dependent manner. In 1K1C RHR, YM358 (30 mg/kg) also produced an antihypertensive effect, whereas enalapril (30 mg/kg) had no effect. Administration of YM358, but not enalapril, to 1K1C RHR, 2K1C RHR and NTR did not affect heart rate. In isolated mesenteric arteries from 1K1C RHR and 2K1C RHR, angiotensin II (Ang II), angiotensin I (Ang I) and tetradecapeptide (TDP), a physiologically active renin substrate, produced concentration-dependent vasoconstriction. YM358 (10^{-7} m) inhibited the vasoconstricting responses to Ang II, Ang I and TDP in isolated mesenteric arteries. In contrast, enalaprilat (10^{-7} m), an active metabolite of enalapril, did not completely inhibit the response to Ang I and TDP. These results indicate that YM358 has higher efficacy than enalapril for the treatment of hypertension.

Key words angiotensin II type 1 receptor antagonist; YM358; enalapril; enalaprilat; renovascular hypertension

The renin-angiotensin system (RAS) plays an important role in the regulation of systemic blood pressure. The renin-angiotensin cascade starts with the cleavage of angiotensinogen by renin to form angiotensin I (Ang I) and culminates in the conversion of the inactive decapetide Ang I to the vasoconstrictor hormone angiotensin II (Ang II). Ang II influences vascular tone by several mechanisms.2) Traditionally, the RAS has been viewed as an endocrine system, however, a number of studies have shown multiple pathways of Ang II production in peripheral tissues, and suggested that Ang II generated locally may contribute to a sustained elevation in blood pressure.3–5)

Angiotensin converting enzyme (ACE) inhibitors and Ang II type 1 (AT_1) receptor antagonists both block the RAS. Although these two types of drugs have very similar overall effects, the manifestation of their actions may be different. Since ACE is also involved in the cleavage of bradykinin, neurotensin, met-enkephalin and substance P as well as Ang I,6) the use of ACE inhibitors could lead to a potentiation of the effects of these peptides. It is reported that some of the therapeutic features of ACE inhibitors and some of their side effects may be attributed to angiotensin independent mechanisms.7,8) In contrast, AT_1 receptor antagonists specifically block the RAS. Such higher specificity may reduce the incidence of side effects.

Several clinical studies have been conducted to compare the efficacy of Ang II antagonists with that of ACE inhibitors. In double-blind clinical trials, hypertensive patients were treated with losartan, the first orally active AT_1 receptor antagonist. The antihypertensive efficacy of losartan was comparable to that of ACE inhibitors such as captopril, enalapril or lisinopril.9,10) In a randomized trial in patients with heart failure, losartan treatment reduced mortality compared with captopril treatment.11) These reports indicate the usefulness of AT_1 receptor antagonist in patients with hypertension or heart failure. Comparison of outcome between AT_1 receptor antagonists and ACE inhibitors in chronic hypertension treatment and heart failure is ongoing.

YM358 is a nonpeptide AT_1 receptor antagonist which specifically inhibits Ang II-induced vasoconstriction in rabbit aorta.12) In several rat models of hypertension such as spontaneously hypertensive rats (SHR), stroke-prone spontaneously hypertensive rats and two kidney, one-clip renal hypertensive rats (2K1C RHR), YM358 decreased blood pressure significantly and dose-dependently without affecting heart rate (HR).13,14)

The present study was conducted to compare the hypertensive effects of YM358 with that of the ACE inhibitor enalapril in two kinds of hypertension model: one-kidney, one-clip renal hypertensive rats (1K1C RHR), a non-renin-dependent hypertensive model and 2K1C RHR, a renin-dependent hypertensive model. The vasodilatory effects of YM358 and enalaprilat, the active metabolite of enalapril, were also evaluated using mesenteric arteries isolated from 1K1C RHR and 2K1C RHR.

MATERIALS AND METHODS

Animals Five-week-old male Wistar rats (body weight 100–120 g) were assigned to one of two groups, 1K1C RHR or 2K1C RHR. 2K1C RHR was used as a renin-dependent hypertension while 1K1C RHR, a non-renin-dependent hypertension. The rats were housed under identical conditions with free access to regular rat food (Clea Japan; Tokyo, Japan) and water until the measurement of the blood pressure.

To induce 1K1C hypertension, a silver clip (0.22 mm i.d.) was placed around the left renal artery, and right nephrec-

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tomography was performed. To induce 2K1C hypertension, a silver clip (0.22 mm i.d.) was placed around the left renal artery while the right renal artery was left intact.

Three days before the measurement of blood pressure, the rats were anesthetized with pentobarbital sodium (60 mg/kg body weight intraperitoneally), and a polyethylene catheter (PE-50; Clay-Adams; Parsippany, NJ) was inserted into the femoral artery to measure blood pressure. Blood pressure and HR were recorded in unrestrained conscious rats using a pressure transducer (TP-400T; Nihon Kohden Kogyo; Japan) connected to a polygraph (RM-6000; Nihon Kohden Kogyo; Japan). Only animals with a mean blood pressure (MBP) greater than 150 mmHg 4 weeks after clip implantation were used. About 80% of 2K1C RHR and 60—80% of 1K1C RHR developed hypertension. Age-matched normotensive rats (NTR) were used as control groups.

All experiments were performed in accordance with the regulations of the Animal Experimentation Ethics Committee of Yamanouchi Pharmaceutical Co., Ltd.

**Plasma ACE Activity** Blood samples were collected through the aorta with heparinized syringes while the animals were anesthetized with ether. The samples were centrifuged at 800×g for 15 min at 4°C, and the plasma was stored at −20°C until the assay. Plasma ACE activity was determined by a modification of the method of Friedland et al. (1986) as described in detail by Santos et al. (1987). Rat plasma (10 μl) was incubated with 5 μM hippuryl-histidyl-leucine (H-His-Leu), an artificial substrate, in 500 μl of 0.4 M sodium borate buffer, pH 8.3, containing 0.9 M sodium chloride. The enzyme reaction was stopped by the addition of 1.2 ml of 0.34 M NaOH. Blank samples were prepared by reversing the order of addition of enzyme and NaOH. One hundred microliters of o-phthaldialdehyde in methanol (20 mg/ml) were added to each tube, and 10 min later the reaction was terminated by addition of 200 μl of 3 N HCl. The reaction mixture was then centrifuged at 800×g for 5 min at room temperature. The histidyl-leucine (His-Leu) product was measured fluorometrically (365 nm excitation and 495 nm emission wavelengths). Standard curves for His-Leu (1—60 nmol) were prepared under the same conditions. All measurements were made in duplicate. ACE activity was expressed in nanomoles His-Leu per min per ml plasma.

**Plasma Renin Activity (PRA)** Blood samples (1 ml) were collected through the femoral catheter into tubes containing EDTA (final concentration 4 mm) as an anticoagulant and centrifuged at 800×g for 15 min at 4°C. The plasma was stored frozen (−20°C) until PRA was measured by radioimmunoassay using a renin activity assay kit (Sorin Bio-medica; Saluggia, Italy).

**Concentration of Ang II in Plasma and Tissue** Blood samples were collected through the abdominal aorta with heparinized syringes while the animals were anesthetized with ether. Plasma Ang II concentration was measured by radioimmunoassay after purification using the following process. A 2-ml sample was passed through a Sep-Pak C18 cartridge (Waters; Milford, MA) which had been pretreated with 0.1% trifluoroacetic acid (TFA) followed by 5 ml of 50% acetonitrile in 0.1% TFA. After washing with 10 ml of 0.1% TFA, trapped peptides were eluted with 3 ml acetonitrile/water/TFA (50:49.9:0.1, v/v/v). The eluate was dried in a vacuum centrifuge. Angiotensins were then separated by reverse-phase high performance liquid chromatography (HPLC). Samples were loaded on a Shim-pack CLC-ODS (M column (4.6×250 mm; Shimadzu; Tokyo) and eluted with a linear gradient of acetonitrile from 11% to 35% in 25 mm sodium phosphate buffer, pH 7.6, over a period of 35 min at a flow rate of 1 ml/min. Fractions of 1 to 2 ml were collected and dried in a vacuum centrifuge. Samples were re-dissolved in 0.1 M sodium phosphate buffer, pH 7.4, containing 0.05 M NaCl, 0.1% bovine serum albumin, 0.1% Triton X-100, and 0.01% NaN3 for radi immunoassay. The samples were preincubated with commercial Ang II antiserum (Peninsula Lab; Belmont, CA) at 4°C for 10 to 12 h. The reaction mixture was then incubated with [3-125I]iodotyrosyl4] Ang II (Amersham, U.K.) for an additional 36 to 48 h at 4°C. The antibody-bound antigen was separated from free antigens by double antibody precipitation. The Ang II antiserum showed less than 1% cross-reactivity with Ang I and renin substrate but 100% cross-reaction with angiotensin III. The detection limit for Ang II was 1 pg/tube.

**Tissue Ang II Concentration** was measured after purification, as follows. The superior mesenteric artery was rapidly removed by cutting the intestinal border of the mesentery and dissecting away the adhering connective tissue. Blood was carefully washed off from the tissue with cold saline. The mesenteric vascular beds were weighed and homogenized using a Polytron homogenizer in 0.1 mol/l HCl. The homogenate was centrifuged at 20000×g for 30 min at 4°C and the supernatant was applied to a Sep-Pak C18 cartridge. The eluate was applied to the HPLC column and eluted Ang II was measured as described previously.

**Antihypertensive Effects of YM358 and Enalapril in 2K1C RHR, 1K1C RHR and NTR** Blood pressure and HR of rats were monitored as described previously while the animals were conscious and unrestrained. The rats were administered a single bolus dose of drugs once a day (between 09:00 and 10:00) in a 0.5% methylcellulose solution by oral gavage in a volume of 5 ml/kg. 2K1C RHR received YM358 (3, 10, 30 mg/kg), enalapril (3, 10, 30 mg/kg) or the vehicle. 1K1C RHR received YM358 (10, 30 mg/kg), enalapril (10, 30 mg/kg) or the vehicle. NTR received YM358 (10, 30 mg/kg), enalapril (30 mg/kg) or the vehicle.

**In Vitro Measurement of Mesenteric Artery Perfusion Pressure** Mesenteric arterial beds isolated from 2K1C RHR, 1K1C RHR and NTR were prepared for in vitro perfusion experiments as described by McGregor. (1988) The rats were sacrificed by decapitation, and the abdominal cavity opened and a cannula (PE-50) inserted into the superior mesenteric artery. The ileocolic branch of the superior mesenteric artery was then tied off. After the superior mesenteric artery was perfused with 10 ml of a modified Krebs solution (mM: NaCl 118.4, KCl 4.7, CaCl2 2.5, MgCl2 1.2, NaHCO3 25.0, KH2PO4 1.2, and glucose 11.1) to remove the blood, the intestine was dissected from the mesentery by cutting close to the intestinal border. The mesentery was removed from the rat and placed in a water-jacketed organ bath maintained at 37°C. The mesenteric arteries were perfused at a constant rate of 4 ml/min with the Krebs solution equilibrated with 95%O2—5%CO2 at 37°C using a peristaltic pump (MP-3 microtub pump; Tokyo Rikakikai; Tokyo, Japan). Perfusion pressure was monitored via a side branch of the perfusion cannula by a pressure transducer (TP-400T; Nihon Kohden Kogyo; Japan).
Kogyo; Tokyo, Japan) connected to a recorder (Rikadenki; Tokyo, Japan). After a 1 h stabilization period, the perfusion solution was exchanged for that containing 20 mM K+ and phenylephrine. Phenylephrine concentration was adjusted in the range of 0.2 to 20 mg/ml to achieve a stable perfusion pressure (approximately 100 mm Hg). Previous experiments indicated the tachyphylaxis did not occur when Ang II, Ang I or tetradecapeptide (TDP: Asp-Ang-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser; the renin substrate) was injected into the perfusion solution every 30 min for 3 h.

Concentration response curves for Ang II (2—200 pmol), Ang I (10—700 pmol) and TDP (70—4000 pmol) were obtained by measuring the response to a bolus injection of 10—40 μl of each peptide solution into the perfusion flow. Injection of the same volumes of the Krebs solution did not affect the perfusion pressure. A single mesenteric preparation was used to obtain one set of concentration response data for each peptide. $E_{max}$ (the maximum contractile response) and EC50 (the concentration eliciting 50% of $E_{max}$) values were derived from the curve.

Next, the effect of RAS inhibitors on the pressor responses to Ang II, Ang I and TDP were evaluated. Ang II, Ang I and TDP concentrations that produced approximately 50-mmHg increase in perfusion pressure were used. The responses to Ang II, Ang I and TDP were determined before and after addition of YM358 (10−8—10−7 m) or enalaprilat (10−7—10−6 m). Each dose of YM358 or enalaprilat was tested using separate mesenteric preparations, and the results are expressed as percentages of the Ang II, Ang I or TDP response in the absence of YM358 or enalaprilat in the modified Krebs solution, which served as control. As a time matched control, the vehicle (H2O) was tested. Both YM358 and enalaprilat were freely soluble in the perfusion solution. The preparations were allowed to equilibrate for 1 h to stabilize after changing the perfusion solution containing YM358 or enalaprilat.

Drugs Both YM358 and enalaprilat were prepared by Yamanouchi Pharmaceutical Co., Ltd., Japan. Ang I and Ang II were purchased from Peptide Institute (Osaka, Japan). N-Acetyl tetradecapeptide (porcine) (TDP), enalapril and Hip-His-Leu were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Statistical Analysis All results are expressed as the means±S.E.M. Differences in MBP, HR and changes in perfusion pressure were assessed by two-way repeated measures ANOVA, followed by Dunnett’s t-test or Student’s t-test. Differences in changes of perfusion pressure by YM358 or enalaprilat were assessed by Dunnett’s t-test. The other data were assessed by Tukey’s t-test. Differences between groups were considered to be significant at p<0.05.

RESULTS

PRA, ACE Activity in Plasma and Ang II Concentration in Plasma and Tissue PRA was significantly higher in 2K1C RHR than in 1K1C RHR or NTR (Table 1). Plasma Ang II concentrations in 2K1C RHR and 1K1C RHR were significantly higher than those in NTR. In contrast, there was no significant difference in plasma ACE activity among the 3 groups. Tissue Ang II concentration (346±35 pg/g tissue) in 2K1C RHR was significantly higher than that in 1K1C RHR (228±25 pg/g tissue) or NTR (168±15 pg/g tissue).

Ephapses of YM358 and Enalapril on MBP in 2K1C RHR, 1K1C RHR and NTR Four weeks after clip installation, pretreatment values of MBP and HR were significantly higher in 2K1C RHR and 1K1C RHR than in NTR (Table 2).

2K1C RHR: Oral administration of YM358 (3, 10 and 30 mg/kg) significantly decreased MBP in a dose-dependent manner, but there was no significant change in HR (Fig. 1). The maximal decrease in MBP was observed 9 h after YM358 (30 mg/kg) administration. Enalapril (3, 10 and 30 mg/kg p.o.) also produced a significant and a dose-dependent decrease in MBP, but unlike YM358, it showed a slow onset of the effect. Enalapril (10 mg/kg) decreased significantly HR 24 h after administration.

1K1C RHR: Oral administration of YM358 (10 and 30 mg/kg) produced a significant and dose-dependent decrease in MBP without any change in HR (Fig. 2). YM358 (30 mg/kg p.o.) gradually lowered MBP, showing maximal decrease 24 h after the administration. Enalapril (10 and 30 mg/kg p.o.) did not affect MBP. Enalapril (30 mg/kg p.o.) increased HR significantly 3 and 5 h after the administration.

NTR: YM358 (10 and 30 mg/kg p.o.) slightly but significantly decreased MBP without affecting HR (Fig. 3). Enalapril (30 mg/kg p.o.) slightly decreased MBP but significantly increased HR for up to 8 h after the administration.

Comparison of the Increases in Perfusion Pressure Induced by Ang II, Ang I and TDP The concentration response curves of the increases in perfusion pressure elicited by Ang II, Ang I and TDP are shown in Fig. 4. Ang II, Ang I and TDP elicited concentration-dependent increases in the perfusion pressure of mesenteric artery isolated from 2K1C RHR, 2K1C RHR and NTR. The EC50 and $E_{max}$ values are shown in Table 3. EC50 values of Ang I and TDP were significantly greater in 2K1C RHR and 1K1C RHR than in NTR. EC50 values of Ang II, however, did not show significant differences among 2K1C RHR, 1K1C RHR and NTR. $E_{max}$ values of Ang II, Ang I and TDP in 2K1C RHR and 1K1C RHR

| Table 1. Renin Activity, ACE Activity and Ang II Concentration (Ang II conc.) in Plasma of 2K1C RHR, 1K1C RHR and Age-Matched NTR |
|-----------------|-----------------|-----------------|
| Renin activity (Ang I ng/ml/h) | ACE activity (His-Leu nmol/ml/min) | Ang II conc. (pg/ml) |
| 2K1C RHR | 28.6±7.0 (15)* | 175.8±10.0 (9) | 23.17±5.75 (6)* |
| 1K1C RHR | 8.5±2.0 (11) | 183.0±7.4 (8) | 25.30±5.1 (5)* |
| NTR | 7.0±1.1 (16) | 204.6±16.9 (6) | 5.12±0.26 (6) |
| Values are means±S.E.M. Numbers of rats are shown in parentheses. * p<0.05 compared with the NTR group using Tukey’s t-test. |

| Table 2. Pretreatment Values of MBP and HR in 2K1C RHR, 1K1C RHR and Age-Matched NTR |
|-----------------|-----------------|
| n | HR (beats/min) | MBP (mmHg) |
| 2K1C RHR | 36 | 380±5* | 166±3* |
| 1K1C RHR | 19 | 374±8* | 186±8* |
| NTR | 13 | 343±6 | 106±2 |
| Values are means±S.E.M. * p<0.05, significantly different from the NTR values using Tukey’s t-test. ** p<0.05, significantly different from the 2K1C RHR values using Tukey’s t-test. |

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Fig. 1. Effects of Orally Administered YM358 and Enalapril on MBP and HR in 2K1C RHR
Rats were treated with a single p.o. dose of vehicle (C), 3 mg/kg of YM358 or enalapril (●), 10 mg/kg of YM358 or enalapril (▲) or 30 mg/kg of YM358 or enalapril (◆). Values are expressed as means±S.E.M. (n=4 to 5). *p<0.05, compared with the vehicle-treated groups using two-way repeated measures ANOVA, followed by Dunnett's t-test. Pretreatment values (means±S.E.M.) of MBP and HR in 2K1C RHR were as follows: vehicle, 177±10 mmHg and 367±23 beats/min (n=5); YM358, 3 mg/kg: 173±6 mmHg and 362±16 beats/min (n=4); 10 mg/kg: 171±9 mmHg and 379±13 beats/min (n=4); 30 mg/kg: 171±14 mmHg and 383±9 beats/min (n=4); enalapril, 3 mg/kg: 144±3 mmHg and 389±13 beats/min (n=4); 10 mg/kg: 156±6 mmHg and 406±6 beats/min (n=4); 30 mg/kg: 161±6 mmHg and 395±4 beats/min (n=4). There was no difference among them (Tukey's t-test).

Fig. 2. Effects of Orally Administered YM358 and Enalapril on MBP and HR in 1K1C RHR
Rats were treated with a single p.o. dose of vehicle (C), 10 mg/kg of YM358 or enalapril (▲), 30 mg/kg of YM358 or enalapril (◆). Values are expressed as the means±S.E.M. (n=4 to 5). *p<0.05, compared with the vehicle-treated groups using two-way repeated measures ANOVA, followed by Dunnett's t-test. Pretreatment values (means±S.E.M.) of MBP and HR in 1K1C RHR were as follows: vehicle, 200±13 mmHg and 383±18 beats/min (n=5); YM358, 10 mg/kg: 189±6 mmHg and 352±16 beats/min (n=4); 30 mg/kg: 194±12 mmHg and 403±9 beats/min (n=5); enalapril, 10 mg/kg: 189±12 mmHg and 388±12 beats/min (n=4); 30 mg/kg: 177±3 mmHg and 354±13 beats/min (n=5). There was no difference among them (Tukey's t-test).
were significantly greater than those in NTR.

**Effects of YM358 and Enalaprilat on Perfusion Pressure** In vessels isolated from 2K1C RHR, 1K1C RHR and NTR, YM358 (10^{-9}–10^{-7} M) (left panels) blocked the pressor responses to Ang II, Ang I and TDP in a dose-dependent manner (Figs. 5—7). Enalaprilat (10^{-9}–10^{-7} M) (right panels) produced a dose-dependent inhibition of Ang I-induced pressor response (Figs. 5—7). In the presence of enalaprilat (10^{-7} M), the pressor response elicited by Ang I was significantly reduced, whereas responses to TDP were unaffected in isolated mesenteric arteries of 2K1C RHR, 1K1C RHR and NTR (Figs. 5—7).

**DISCUSSION**

In this paper, the hypotensive and vasodilatory effects of the new AT1 receptor antagonist YM358 and the ACE inhibitor enalapril were compared in two kinds of rat renal hypertensive models, 2K1C RHR and 1K1C RHR. The 2K1C RHR is thought to accurately replicate renin-dependent hypertension based on the data that plasma renin activity is elevated compared with that in NTR. Additionally, the 1K1C RHR is thought to be a non-renin-dependent hypertension model except in the very early stages, because PRA at the chronic hypertension phase is reported to be at
Table 3. EC$_{50}$ and $E_{\text{max}}$ of Contractile Responses to Ang II, Ang I and TDP of Mesenteric Arteries from 2K1C RHR, 1K1C RHR and Age-Matched NTR

<table>
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<th>Ang II</th>
<th>Ang I</th>
<th>TDP</th>
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<tr>
<td>EC$_{50}$ (pmol)</td>
<td></td>
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<tr>
<td>2K1C RHR</td>
<td>22±3</td>
<td>68±5*</td>
<td>143±15*</td>
</tr>
<tr>
<td>1K1C RHR</td>
<td>17±1</td>
<td>59±4*</td>
<td>301±35*</td>
</tr>
<tr>
<td>NTR</td>
<td>20±2</td>
<td>147±16</td>
<td>759±16</td>
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<tr>
<td>$E_{\text{max}}$ (mmHg)</td>
<td></td>
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<tr>
<td>2K1C RHR</td>
<td>106±13*</td>
<td>115±21*</td>
<td>93±8*</td>
</tr>
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<td>1K1C RHR</td>
<td>90±9*</td>
<td>113±8*</td>
<td>98±10*</td>
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<tr>
<td>NTR</td>
<td>54±8</td>
<td>59±7</td>
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Values are means±S.E.M. EC$_{50}$=concentration of agonist producing 50% of the maximal contractile response, $E_{\text{max}}$=maximal responses to agonist. * p<0.05, significantly different from the NTR values using Tukey's t-test.

2K1C RHR

![Graph showing contractile responses to Ang II, Ang I, and TDP in 2K1C RHR arteries.](image)

Fig. 5. Effects of YM358 and Enalaprilat on Perfusion Pressure of Mesenteric Arterial Beds Isolated from 2K1C RHR

Changes in perfusion pressure in mesenteric arterial beds isolated from 2K1C RHR induced by TDP (300 pmol), Ang I (70 pmol) and Ang II (30 pmol) were measured in the presence and absence of YM358 (left panel) and enalaprilat (right panel). Results are expressed as percentages of contraction evoked by TDP, Ang I or Ang II in the absence of YM358 and enalapril for each mesenteric preparation (%). Means±S.E.M. are shown for vessel specimens from different individuals; the numbers of specimens are shown in parentheses. * p<0.05, significantly different from the vehicle-treated groups. In experiments on the effect of enalapril on perfusion pressure induced by TDP, Student's t-test was used to compare with the vehicle-treated group. For comparison among other groups, Dunnett's t-test was used to compare with the vehicle-treated groups.

1K1C RHR

![Graph showing contractile responses to Ang II, Ang I, and TDP in 1K1C RHR arteries.](image)

Fig. 6. Effects of YM358 and Enalaprilat on Perfusion Pressure of Mesenteric Arterial Beds Isolated from 1K1C RHR

Changes in perfusion pressure in mesenteric arterial beds isolated from 1K1C RHR induced by TDP (400 pmol), Ang I (40 pmol) and Ang II (20 pmol) were measured in the presence and absence of YM358 (left panel) and enalaprilat (right panel). Results are expressed as percentages of contraction evoked by TDP, Ang I or Ang II in the absence of YM358 and enalapril for each mesenteric preparation (%). Means±S.E.M. are shown for vessel specimens from different individuals; the numbers of specimens are shown in parentheses. * p<0.05, significantly different from the vehicle-treated groups. In experiments on the effect of enalapril on perfusion pressure induced by TDP, Student's t-test was used to compare with the vehicle-treated group. For comparison among other groups, Dunnett's t-test was used to compare with the vehicle-treated groups.

normal levels. In this experiment, PRA of 2K1C RHR was about four times higher than that of NTR whereas that of 1K1C RHR was the same level as that of NTR. These results are consistent with those reported in the literature. An increase in plasma Ang II concentration was observed in both 2K1C RHR and 1K1C RHR, suggesting that elevation of circulating Ang II may, at least in part, contribute to the development and maintenance of hypertension in these models.

A single administration of enalapril (10, 30 mg/kg p.o.) to 2K1C RHR produced a dose-dependent decrease in MBP, whereas the same dosage of enalapril fail to reduce MBP in 1K1C RHR. These results are consistent with previous reports. In contrast, YM358 lowered MBP both in 2K1C RHR and in 1K1C RHR. Thus, the antihypertensive effect of YM358 seems to be different from that of enalapril, and
YM358 showed a broader spectrum against rat hypertensive models than enalapril.

In addition, tachycardia was observed after the administration of enalapril to 1K1C RHR and NTR, but was not noted with YM358. There are many reports that enalapril did not affect HR in 2K1C renal hypertensive dog, 211 SHR, 220 rabbit211 and hypertensive patients,24 hence we can not explain the cause of tachycardia with enalapril in the present study.

YM358 lowered MBP more potently in 2K1C RHR than in 1K1C RHR. However, plasma Ang II concentrations were almost at the same level in these two animal models. In 1K1C RHR, not only activation of RAS but also volume expansion are believed to play important roles in the development and maintenance of hypertension.25,26 The participation of plasma Ang II in maintenance of hypertension in 1K1C RHR may be less than that in 2K1C RHR, and as a result, the hypertensive activity of YM358 in 1K1C RHR may be weak.

The difference between 1K1C RHR and 2K1C RHR was also observed in the time-course changes of the hypertensive activities after administration of YM358 to the rats. The onset of the decrease in MBP was gradual after administration of YM358 to 1K1C RHR whereas that in 2K1C RHR was rapid. The difference in the onset of the hypertensive effects between 2K1C RHR and 1K1C RHR was also reported with other AT1 receptor antagonists such as losartan and TCV-116.27 In this experiment, vascular Ang II concentration was higher in 2K1C RHR than in 1K1C RHR. Vascular Ang II is expected to contribute to the maintenance of hypertension in renal hypertensive rats.28 Thus, the antagonism of the AT1 receptor might cause the rapid decrease in blood pressure in 2K1C RHR.

A perfusion study was undertaken to assess the effects of YM358 and enalapril on the vasoconstriction caused by the vascular RAS activities. Ang II and its precursors (Ang I and TDP) were administered into a perfusion system consisting of the mesenteric arteries isolated from 2K1C RHR, 1K1C RHR and NTR, while the perfusion pressure was continuously measured. All these agonists produced a concentration-dependent increase in the perfusion pressure, indicating that local RAS activity exists in the isolated mesenteric arteries of these rats. The vasoconstriction by these agonists was more dominant in these hypertensive rats than in NTR. Therefore it could be speculated that the local RAS activity is increased in hypertensive rats. The advanced contractile response of mesenteric arteries from 2K1C RHR and 1K1C RHR may participate in maintenance of the hypertension. YM358 inhibited the vasoconstriction produced by Ang II, Ang I and TDP in a concentration-dependent manner. It is reported that vascular tissues contain all components of the RAS necessary to generate Ang II locally.29 In a previous experiment, YM358 up to $10^{-5}$ M did not show any relaxant activity in isolated rabbit aorta which was pre-contracted with prostaglandin E2, norepinephrine, [Arg9]-vasopressin, endothelin-1, histamine, 5-hydroxytryptamine or KCl.30 Thus, YM358 is considered not to have direct vasodilatory activity. Therefore, the inhibitory effect of YM358 is caused by its antagonistic activity against the action of Ang II, and Ang II generated from Ang I or TDP in the arteries, or against the direct action on the Ang II receptors.

Although enalapril showed concentration-dependent inhibition in the pressor response induced by Ang I, it did not inhibit completely, and 20—40% of the responses remained even at $10^{-5}$ M of enalapril. Enalapril induced no attenuation of the pressor response by TDP. Juul et al.29 reported that the response to TDP (3×$10^{-10}$—$10^{-8}$ M) was abolished by 0.1 µM saralasin and 600 kIU/ml aprotinin, but not by $10^{-3}$ M captopril in isolated rat femoral beds. It was reported that enalapril did not affect vasoconstriction produced by Ang II.31 Considering these reports and the different effects between YM358 and enalapril to the pressor responses by Ang I or TDP, a local Ang II generating system involving an ACE-independent pathway and renin- and ACE-independent pathway may exist in rats.

The present findings indicate that YM358 is more potent than enalapril in reducing perfusion pressure induced by Ang I and TDP in mesenteric resistant arteries and in reducing
blood pressure in 1K1C RHR and NTR. Therefore, the AT₁ receptor antagonist, YM358, has potential as an antihypertensive agent.

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REFERENCES AND NOTES

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