Significance of the Vascular Concentration of Angiotensin II–Receptor Blockers on the Mechanism of Lowering Blood Pressure in Spontaneously Hypertensive Rats

Shinji Takai1,*, Denan Jin1, Hiroshi Sakonjo2, Takayuki Takubo3, and Toyofumi Nakanishi3

1Department of Pharmacology, 2Department of Clinical and Laboratory Medicine, Osaka Medical College, 3Department of Clinical and Laboratory Medicine, Osaka Medical College, 2-7 Daigaku-cho, Takatsuki City, Osaka 569-8686, Japan

Received September 10, 2013; Accepted October 16, 2013

Abstract. To clarify the hypotensive mechanism of angiotensin II receptor–blockers (ARBs), drug concentrations in plasma and vascular tissues were measured using matrix-assisted laser desorption ionization time-of-flight mass spectrometry and imaging mass spectrometry. In spontaneously hypertensive rats, systolic blood pressure (SBP) was measured 2 and 24 h after administration of candesartan cilexetil (0.3, 1, or 3 mg/kg) or azilsartan (0.3, 1, or 3 mg/kg). SBP was similarly lowered 2 h after administration of azilsartan or candesartan cilexetil, but it was significantly lower in the azilsartan-treated group than in the candesartan cilexetil-treated group at 24 h. Angiotensin II–induced vascular contractions were similarly attenuated 2 h after administration of these drugs, and the contractions were significantly lower in the azilsartan-treated group at 24 h. Although plasma concentration was significantly lower in the azilsartan-treated group at 24 h, vascular concentration of azilsartan was significantly greater than that of candesartan. Significant correlations between SBP and vascular concentrations were observed both at 2 and 24 h, while no significant correlation was observed between plasma and vascular concentrations. In conclusion, the mechanism of ARB-induced hypotension is likely to depend on vascular concentrations rather than plasma concentrations.

Keywords: angiotensin II, angiotensin II receptor blocker, hypertension, imaging mass spectrometry, vascular contraction

Introduction

The incidence of stroke and myocardial infarction are elevated in patients with hypertension (1 – 3). A variety of drugs have been developed to lower blood pressure, including sympathetic nerve blockers, renin-angiotensin blockers, calcium channel blockers, and diuretics; and medication-induced hypotension has resulted in decreased rates of stroke and myocardial infarction (1 – 3). In a review by Kario et al. (4), it was suggested that an ambulatory blood pressure profile-based strategy would help ‘perfect 24-h blood pressure control’ and help to protect high-risk patients with atherosclerotic vascular events. Numerous comparisons of various types of anti-hypertensive agents targeting cardiovascular events have been conducted in clinical studies, with significant differences between drugs having been observed in patients with hypertension (5, 6). However, certain clinical studies have failed to report significant differences among different drug types (7, 8). Although some agents may be particularly useful in preventing cardiovascular events, a more important effect for patients with hypertension may be the production of lower blood pressure that is stable for 24 h after administration of medication (1 – 4).

Angiotensin II plays an important role in regulating blood pressure, with the inhibition of angiotensin II formation, by angiotensin-converting enzyme (ACE) inhibitors, and the blockade of angiotensin II type 1 (AT1) receptors, by angiotensin II–receptor blockers.

*Corresponding author. pha010@art.osaka-med.ac.jp
Published online in J-STAGE on November 29, 2013
doi: 10.1254/jphs.13167FP
(ARBs), having been widely used for lowering blood pressure in patients with hypertension (1–3). In spontaneously hypertensive rats (SHR), elevated angiotensin II levels, via the induction of ACE, are observed in arteries but not in plasma (9, 10). Furthermore, it has been reported that there were no significant differences among ACE inhibitors in their ability to inhibit plasma ACE activity, but significant differences were observed in the inhibition of vascular ACE activity (9, 10). The potency of vascular ACE inhibition by ACE inhibitors may be associated with their lipophilic properties (9, 10). Highly lipophilic ACE inhibitors have been demonstrated to produce more potent and longer-lasting vascular ACE inhibition than lower-lipophilicity ACE inhibitors (9, 10). Additionally, ARBs were also more potent than low lipophilicity ARBs for vascular protection (11). However, it is unclear if there is a direct relationship between the potency of vascular AT₁-receptor blockade and ARB hypotensive efficacy because it has been difficult to detect AT₁ receptor–bound ARBs in vascular tissues.

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS) and imaging mass spectrometry (IMS) are powerful tools for clarifying spatial distribution, as well as quantification of drugs and their metabolites, lipids, peptides, and proteins, in tissue sections (12, 13). Recently, we demonstrated that the concentrations of an ARB (candesartan) could be determined in plasma and aorta sections after oral administration of candesartan cilexetil, which is a prodrug of candesartan, in mice using MALDI-TOFMS and IMS in the selected reaction monitoring (SRM) mode (14). The ARB azilsartan is structurally similar to candesartan (15). Candesartan has a tetrazol ring, while azilsartan has a 5-oxo-1,2,4-oxadiazole structure, with the remainder of their structures being identical (15). Due to their structural differences, azilsartan has a higher affinity than candesartan toward AT₁ receptors, as well as higher lipophilicity (16). In the present study, to clarify the relationship between the blocking potency of vascular AT₁ receptors and hypotensive efficacy by ARBs, we evaluated the relationship between plasma and vascular concentrations and the hypotensive effects observed after oral administration of candesartan cilexetil and azilsartan in SHR.

Materials and Methods

Materials

Candesartan (candesartan cilexetil, the prodrug of candesartan), and azilsartan were obtained from Takeda Pharmaceutical Co., Ltd. (Osaka). α-Cyano-4-hydroxycinnamic acid (α-CHCA) was obtained from Sigma-Aldrich Japan (Tokyo). An indium–tin–oxide (ITO)-coated glass slide (Bruker Daltonics Inc., Billerica, MA, USA) was used for IMS.

Animals

Eighteen-week-old male spontaneously hypertensive rats (SHR) were obtained from Japan SLC, Inc. (Shizuoka). All rats were given normal rat chow (F-2; Funabashi Farm Co., Chiba). The experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (Animal Research Laboratory, Osaka Medical College, Osaka).

The animals were orally administered candesartan cilexetil (0.3, 1.0, and 3.0 mg/kg; each dose: n = 5) and azilsartan (0.3, 1.0, and 3.0 mg/kg; each dose: n = 5) by gavage. Untreated animals were used as the vehicle group (n = 5). Systolic blood pressure (SBP) and heart rate were monitored by tail-cuff plethysmography (BP-98; Softron Co., Tokyo) 2 and 24 h after oral drug administration. After measurements of SBP and heart rate, the rats were anesthetized with 35 mg/kg of sodium pentobarbital to obtain blood and tissues.

Vascular responses in the isolated artery

Isolated rat carotid arteries were cut into 10 × 1.0-mm helical strips and placed on a myograph under a resting tension of 1.0 g in Tyrode’s solution (137 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl₂, 1.1 mM MgCl₂, 0.42 mM NaH₂PO₄, 12 mM NaHCO₃, and 5.7 mM glucose, pH 7.4) at 37°C and continuously bubbled with 5% CO₂ in O₂. The strips were initially vasocnstricted with 50 mM KCl, and the response of each strip served as the reference for the agonist-induced contraction of the corresponding strip. After the bathing medium was washed out, vascular contractions were assessed by angiotensin II (0.1–100 μM) treatment (17). Contractions were expressed as a percentage of 50 mM KCl–induced contractions.

Renin and ACE activities and angiotensin II concentration in blood

Plasma renin activity (PRA) was determined using an SRL renin kit (TFB Co., Tokyo). Serum ACE activity was measured by incubating plasma for 30 min at 37°C with 5 mM of the substrate hippuryl-His-Leu (Peptide Institute Inc., Osaka) 100 mM phosphate buffer (pH 8.3) containing 300 mM NaCl (18). The reaction was terminated by addition of 3% metaphosphoric acid, and the mixture was subsequently centrifuged at 8,000 × g for 5 min at 4°C. The supernatant was applied to a reversed-phase column (4.6 mm i.d. × 250 mm, CAPCELL PAK C18 MG S-5; Shiseido, Tokyo), which had been equilibrated with 10 mM KH₂PO₄·CH₃OH
Vascular Concentration of ARBs

(1:1, pH 3.0), and eluted with the same solution. Hippuric acid was detected by ultraviolet absorbance at 228 nm. One unit of ACE activity was defined as the amount of enzyme required to cleave 1 μmol hippuric acid/min. Serum angiotensin II concentrations were measured using an enzyme immunoassay kit (Peninsula Laboratories Inc., Belmont, CA, USA).

Analysis of MALDI-TOFMS and MALDI-IMS

For MALDI-TOFMS analysis, an aliquot of plasma (diluted 200 × with distilled water) was added to an equivalent volume of methanol, as previously reported (14). A 5-μL aliquot of the MALDI matrix, consisting of 7 mg/mL of a-CHCA in 60% HPLC-grade acetonitrile and 0.2% TFA, was directly mixed with 5 μL of the diluted plasma sample; and 2 μL of the mixture was then placed on the MALDI plate (Bruker Daltonics, Billerica, MA, USA) and dried at room temperature.

Aorta tissue was frozen in TissueTech™ (Sakura Finetec, Osaka) and each block was sectioned at a thickness of 12 μm (14). The sections were mounted on ITO-coated glass slides (Bruker Daltonics), and a-CHCA matrix was coated onto the sections using Image Prep™ (Bruker Daltonics) and dried at room temperature.

For the quantification of candesartan and azilsartan, MALDI-TOFMS and IMS with the SRM mode were performed on a TOFMS Autoflex speed (Bruker Daltonics), which utilized a Smartbeam™-II solid-state laser (wavelength, 355 nm; focused diameter, 20 μm; repetition rate, 500 Hz) (14). Methanol solutions of candesartan and azilsartan at each concentration were used as standards (14).

Statistical analyses

Data are expressed as means ± S.E.M. Significant differences between mean values of the two groups were evaluated using Student’s t-test for unpaired data. Significant differences among the mean values of multiple groups were evaluated using a one-way analysis of variance followed by Fisher’s test. The statistical correlations were assessed using linear regression analysis (Pearson’s correlation coefficient). Differences were considered significant when the P-value was < 0.05.

Results

SBP and heart rate

SBP was dose-dependently lowered 2 h after administration of candesartan cilexetil and azilsartan. In the 3.0 mg/kg azilsartan–treated group, SBP tended to be lower than in the group receiving the same dose of candesartan cilexetil; however, no significant differences in SBP were detected at equivalent doses of the drugs (Fig. 1A). SBP was also dose-dependently lowered 24 h after administration of candesartan cilexetil and azilsartan; however, SBP at 1.0 and 3.0 mg/kg was significantly lower in the azilsartan–treated group than in the candesartan cilexetil–treated group (Fig. 1A). No significant differences in heart rate were detected between candesartan cilexetil and azilsartan at the same doses (Fig. 1B).

Renin–angiotensin system parameters in plasma

PRA was significantly higher in all groups at 2 h after administration of candesartan cilexetil and azilsartan; however, SBP at 1.0 and 3.0 mg/kg was significantly lower in the azilsartan–treated group than in the candesartan cilexetil–treated group (Fig. 1A). No significant differences in heart rate were detected between candesartan cilexetil and azilsartan at the same doses (Fig. 1B).

Fig. 1. SBP (A) and heart rate (B) 2 and 24 h administration of candesartan cilexetil and azilsartan at 0.3, 1.0, and 3.0 mg/kg. Open, hatched, and filled columns show vehicle, candesartan cilexetil– and azilsartan–treated groups, respectively. **P < 0.01 vs. control group. #P < 0.05 vs. candesartan cilexetil–treated group at the same dose.
after drug administration, the angiotensin II concentration was significantly higher in the azilsartan-treated group compared to the candesartan cilexetil–treated group at 3.0 mg/kg (Table 1).

Plasma drug concentrations
Plasma concentrations of candesartan and azilsartan increased in a dose-dependent manner 2 h after administration, with no significant differences observed between drugs at each dose (Fig. 2A). However, plasma concentrations of candesartan were significantly higher than those of azilsartan at the same dose 24 h after drug administration (Fig. 2B).

Effects of drugs on angiotensin II–induced vascular contraction
In isolated arteries, angiotensin II–induced vascular contraction was significantly reduced 2 h after administration of candesartan cilexetil at 0.3 mg/kg compared to vehicle (Fig. 3A). Angiotensin II–induced vascular contraction was further reduced at 1.0 mg/kg of candesartan cilexetil, compared to 0.3 mg/kg, but a further reduction was not observed between 1.0 and 3.0 mg/kg (Fig. 3A). In comparison, angiotensin II–induced vascular contraction was dose-dependently reduced 2 h after administration of azilsartan up to 3.0 mg/kg (Fig. 3B).

Twenty-four hours after administrations of both candesartan cilexetil and azilsartan, angiotensin II–induced vascular contractions were dose-dependently

---

**Table 1.** Renin activity, ACE activity, and angiotensin II level in plasma

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>PRA (ng AI/mL per h)</th>
<th>ACE activity (mU/mL)</th>
<th>Angiotensin II (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 h</td>
<td>24 h</td>
<td>2 h</td>
</tr>
<tr>
<td>Vehicle</td>
<td>7.02 ± 0.83</td>
<td></td>
<td>41.8 ± 3.30</td>
</tr>
<tr>
<td>CAND CIL (0.3)</td>
<td>62.8 ± 8.73**</td>
<td>16.4 ± 2.04**</td>
<td>38.8 ± 1.96</td>
</tr>
<tr>
<td>CAND CIL (1.0)</td>
<td>67.8 ± 4.19**</td>
<td>23.2 ± 4.22**</td>
<td>41.8 ± 2.17</td>
</tr>
<tr>
<td>CAND CIL (3.0)</td>
<td>61.8 ± 8.49**</td>
<td>21.6 ± 3.59**</td>
<td>41.8 ± 2.85</td>
</tr>
<tr>
<td>AZIL (0.3)</td>
<td>63.6 ± 7.03**</td>
<td>23.6 ± 3.41**</td>
<td>39.6 ± 1.63</td>
</tr>
<tr>
<td>AZIL (1.0)</td>
<td>72.8 ± 4.08**</td>
<td>29.8 ± 2.73**</td>
<td>40.8 ± 2.35</td>
</tr>
<tr>
<td>AZIL (3.0)</td>
<td>73.4 ± 8.05**</td>
<td>33.4 ± 2.69**</td>
<td>41.4 ± 2.33</td>
</tr>
</tbody>
</table>

AZIL: azilsartan. CAND CIL: candesartan cilexetil. **P < 0.01 vs. Vehicle. *P < 0.05 vs. 3 mg/kg candesartan cilexetil.
Vascular Concentration of ARBs

reduced (Fig. 3: C and D). Overall, the effect of candesartan cilexetil tended to be weaker 24 h compared to 2 h after administration (Fig. 3: A and C). However, the attenuating effect of azilsartan was continued from 2 to 24 h after the administration (Fig. 3: B and D).

Effects of drugs on the maximum response of angiotensin II–induced vascular contraction

In the present study, we compared the vascular maximal contractions, elicited by 100 μM angiotensin II, in all treatment groups. Maximal contractions were significantly reduced 2 h after administration of candesartan cilexetil and azilsartan at all doses (Fig. 4). However, no significant differences between these drugs were observed for each dose (Fig. 4).

In the candesartan cilexetil–treated groups, the maximum contraction was dose-dependently reduced at 24 h after drug administration, but the contraction at the same dose was weaker at 24 h compared to 2 h (Fig. 4). The maximal contractions were also dose-dependently reduced in the azilsartan-treated groups at 24 h after drug administration, whereas the contractions were stronger at 24 h compared to 2 h (Fig. 4). At the same dose of these two drugs, significant reductions of the maximum contraction were observed in the azilsartan-treated group compared with in the candesartan cilexetil–treated group at 24 h after administration (Fig. 4).

Correlations among SBP, plasma drug concentrations, and angiotensin II–induced vascular contraction

A significant negative correlation between SBP and plasma drug concentrations was observed in the drug-treated groups 2 h after administration (Fig. 5A). However, there was no significant correlation between SBP and plasma drug concentration in the drug-treated groups 24 h after administration (Fig. 5B).

Fig. 4. The maximum response of angiotensin II–induced vascular contraction in isolated arteries in the control group (open column), as well as 2 and 24 h after administration of candesartan cilexetil (hashed columns) and azilsartan (filled column) at 0.3, 1.0, and 3.0 mg/kg. **P < 0.01 vs. control. #P < 0.05 and ##P < 0.01 vs. the candesartan cilexetil-treated group at equivalent doses.

Fig. 5. Correlations among SBP, plasma concentration and the maximum angiotensin II–induced vascular contraction. Correlations between SBP and plasma concentrations of drugs 2 h (A) and 24 h (B) after administration of candesartan cilexetil and azilsartan. Correlations between SBP and the maximum angiotensin II–induced vascular contraction 2 h (C) and 24 h (D) after administration of candesartan cilexetil and azilsartan. Significant correlations were observed between SBP and plasma concentration at 2 h ($r = -0.672, P < 0.01$) and between SBP and the maximum angiotensin II–induced vascular contraction at 2 h ($r = 0.601, P < 0.01$) and at 24 h ($r = 0.694, P < 0.01$).
A significant positive correlation between SBP and the maximal angiotensin II–induced vascular contraction was observed in the drug-treated groups, both at 2 h and 24 h after administration (Fig. 5: C and D).

**Vascular drug concentrations**

We measured drug concentrations in vascular slices from all groups. We found that the drugs were not detected in the vehicle group as well as at doses under 1.0 mg/kg in the candesartan cilexetil–treated groups 24 h after administration, which were below the detection limit (0.5 pmol/mm²). Therefore, we evaluated vascular concentrations of candesartan and azilsartan in the 3.0 mg/kg–treated groups. Two hours after administration, vascular concentrations were significantly higher in the azilsartan–treated group than in the candesartan cilexetil–treated group (Fig. 6A).

Twenty-four hours after administration, the vascular concentration of candesartan was reduced compared with 2 h, but that of azilsartan was maintained (Fig. 6: A and B). The vascular concentration of azilsartan was significantly higher than that of candesartan at 24 h (Fig. 6B).

**Correlations among vascular drug concentrations, SBP, angiotensin II–induced vascular contraction, and plasma drug concentrations**

A significant negative correlation between SBP and vascular drug concentrations was observed at 3.0 mg/kg in the candesartan cilexetil– and azilsartan–treated groups,

![Vascular drug concentrations after administration of candesartan cilexetil and azilsartan.](image)

Fig. 6. Vascular drug concentrations after administration of candesartan cilexetil and azilsartan. Typical spatial distribution images in aortas from 3 mg/kg of candesartan cilexetil– and 3 mg/kg of azilsartan–treated SHR 2 h and 24 h after administration (A). Vascular drug concentrations 2 h (B) and 24 h (C) post-administration of 3.0 mg/kg candesartan cilexetil and azilsartan. *P < 0.05 and **P < 0.01 vs. the candesartan cilexetil–treated group.

![Correlations among SBP, vascular concentration and the maximum angiotensin II–induced vascular contraction.](image)

Fig. 7. Correlations among SBP, vascular concentration and the maximum angiotensin II–induced vascular contraction. Correlations between SBP and vascular concentrations after administration of candesartan cilexetil and azilsartan at 3.0 mg/kg (A). Correlations between the maximum angiotensin II–induced vascular contraction and vascular concentrations after administration of candesartan cilexetil and azilsartan at 3.0 mg/kg (B). Correlations between plasma and vascular concentrations after administration of candesartan cilexetil and azilsartan at 3.0 mg/kg (C). Significant correlations were observed between SBP and vascular concentration (r = −0.712, P < 0.01) and between the maximum angiotensin II–induced vascular contraction and vascular concentration (r = −0.656, P < 0.01).
both at 2 h and 24 h after administration (Fig. 7A). Similarly, a significant negative correlation between the maximal angiotensin II–induced vascular contractions and vascular concentrations was also observed at 3.0 mg/kg in the candesartan cilexetil– and azilsartan-treated groups, both at 2 and 24 h after administration (Fig. 7B). Conversely, there was no significant correlation between plasma and vascular concentrations at 2 and 24 h after administration (Fig. 7C).

**Discussion**

In the present study, we initially compared the hypotensive effects of candesartan cilexetil with that of azilsartan 2 h after administration. At 0.3 and 1.0 mg/kg, the SBP-lowering effect tended to be stronger in the candesartan cilexetil–treated group than in the azilsartan-treated group, whereas it tended to be weaker in the candesartan cilexetil–treated group at 3.0 mg/kg. The effect of candesartan cilexetil at 1.0 mg/kg may be nearing a plateau, whereas the dose-dependent effects of azilsartan were observed until 3.0 mg/kg. The differences in SBP-lowering effect between candesartan cilexetil and azilsartan may be unassociated with plasma drug concentrations. The plasma concentration of candesartan, which is the active form of candesartan cilexetil, tended to be higher than that of azilsartan 2 h after administration of the same dose (3.0 mg/kg). In general, plasma drug concentrations are thought to reflect the SBP-lowering effect. The plasma concentration of candesartan 2 h after 3.0 mg/kg was significantly higher (approximately 3-fold, \( P < 0.01 \)) than following the 1.0 mg/kg dose; however, there were no differences in SBP observed following the 3.0 and 1.0 mg/kg doses. In Fig. 5A, a significant negative correlation between SBP and plasma drug concentration was observed in an analysis using all drug-treated rats; however, the effect of candesartan cilexetil 2 h after the 3.0 mg/kg dose appears to be contrary to this trend.

Similar to SBP, angiotensin II–induced vascular contraction 2 h after administration of candesartan cilexetil was dose-dependently reduced at 0.3 and 1.0 mg/kg, with no further reduction observed at 3.0 mg/kg. Conversely, angiotensin II–induced vascular contraction 2 h after administration of azilsartan was dose-dependently reduced from 0.3 to 3.0 mg/kg, which reflects the effects observed in SBP. A significant positive correlation between SBP and angiotensin II–induced vascular contraction was observed, with SBP closely reflecting angiotensin II–induced vascular contraction.

Recently, we demonstrated that we could determine the concentrations of candesartan in plasma and aorta-sections after oral administration of candesartan cilexetil in mice using MALD-TOFMS and IMS with SRM mode (14). Although plasma concentrations of candesartan and azilsartan were detectable even 24 h after administration of 0.3 mg/kg (the lowest dose used in the present study), vascular concentrations were not detectable 24 h after administration of candesartan cilexetil at doses below 1.0 mg/kg. Therefore, we compared the vascular concentration of candesartan with that of azilsartan only 24 h after administration of each drug at 3.0 mg/kg. In the analysis of vascular drug concentrations, it was found that levels of azilsartan were significantly higher than those of candesartan 2 h after administration at 3.0 mg/kg. Two hours after administration, SBP and angiotensin II–induced vascular contractions tended to be weaker in the azilsartan-treated group than in the candesartan cilexetil–treated group (3.0 mg/kg). In contrast, plasma azilsartan concentrations tended to be lower than plasma candesartan concentrations. Both SBP and angiotensin II–induced vascular contraction might be influenced more strongly by vascular drug concentrations than by plasma concentrations.

The structure of azilsartan includes a 5-oxo-1,2,4-oxadiazole, in place of the tetrazole ring that is present in ARBs such as losartan, candesartan, and irbesartan (15). This structural modification results in azilsartan being more lipophilic than ARBs with a tetrazole ring (15). In the present study, the plasma concentration after administration of 3.0 mg/kg of candesartan cilexetil did not produce enhanced angiotensin II–induced vascular contraction and SBP in spite of the elevated plasma concentration. Unlike ARBs, the inhibitory effects of ACE inhibitors are easily determined in plasma as well as in tissues by measuring ACE activity (19). In SHR, ACE inhibitors with higher lipophilicity showed stronger ACE inhibitory effects in vascular tissues and exhibited significant correlations between vascular ACE activity and SBP, but not between plasma ACE activity and SBP (20). In the present study, the highly lipophilic ARB azilsartan showed a significant augmentation of vascular concentration compared with candesartan, despite plasma azilsartan concentrations being the same or lower at 2 and 24 h. Similar findings have been observed using ACE inhibitors. For example, the ACE inhibitors trandolapril (lipophilic) and enalapril (hydrophobic) completely inhibit plasma ACE activity 3 h after administration, but trandolapril reduces ACE activity significantly more than enalapril (20). Highly lipophilic ACE inhibitors may penetrate vascular tissues more readily (10). Superior penetration of azilsartan into vascular tissues may result in augmentation of vascular concentrations, and efficacy may be dependent on its lipophilicity.

Twenty-four hours after administration, SBP was significantly lower in the azilsartan-treated group than
in the candesartan cilexetil-treated group at 1.0 and 3.0 mg/kg. Angiotensin II–induced vascular contractions were also significantly attenuated in the azilsartan-treated group at all doses. On the other hand, plasma candesartan concentrations were significantly augmented at 24 h compared to azilsartan at all doses. Both SBP and angiotensin II–induced vascular contractions partially recovered 24 h following candesartan cilexetil treatment compared to 2 h after administration. However, the effects of azilsartan treatment were maintained for 24 h. Such long-term angiotensin II blockade by azilsartan may be associated with its high affinity toward AT1 receptors (16). Miura et al. (21) demonstrated that candesartan and azilsartan interact with Tyr^{113}, Lys^{199}, and Gln^{257} in the AT1 receptor, and molecular docking models indicated that the hydrogen bonding between the oxadiazole of azilsartan-Gln^{257} is stronger than between the tetrazole of candesartan-Gln^{257}. Thus, the high affinity toward AT1 receptors of azilsartan is likely to be associated with the long-term blockade.

In the present study, both PRA and plasma angiotensin II concentrations were significantly elevated in all drug-treated groups compared with the control group. Elevation of PRA is a well-known consequence of ARB administration, which resulted in increasing plasma angiotensin II concentrations. These phenomena are thought to be due to AT1-receptor blockade by ARBs. However, no significant differences in plasma ACE activity were observed among the treatment groups. Overall, both PRA and angiotensin II were reduced at 24 h compared to 2 h post-administration. However, PRA and angiotensin II tended to be higher in the azilsartan-treated group than in the candesartan cilexetil-treated group, with a significant difference between these drugs at 3.0 mg/kg. The upregulated PRA may be a reflection of long-term blockade of AT1 receptors by azilsartan.

In conclusion, vascular concentrations of ARBs, rather than plasma concentrations, may elicit the lowering effect of SBP via vascular blockade of AT1 receptors.

References

8. ALLHAT officers and coordinators for the ALLHAT collaborative research group. The antihypertensive and lipid-lowering treatment to prevent heart attack trial. Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs diuretic: the antihypertensive and lipid-lowering treatment to prevent heart attack trial (ALLHAT). JAMA. 2002;288:2981–2997.


