Contribution of Angiotensin Converting Enzyme Gene Polymorphism to the Action of Angiotensin II Receptor Antagonist (CS-866)

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An insertion/deletion polymorphism of the angiotensin converting enzyme (ACE) gene has been reported to affect the activity of ACE. This study was conducted to investigate the relationship between the action of the angiotensin II receptor antagonist CS-866 and the insertion/deletion (I/D) polymorphism of the ACE gene in Japanese healthy subjects.

The effects of a single oral dose of CS-866 16 mg on blood pressure (BP) and pulse rate in relation to the ACE genotype were studied in 12 healthy men, 7 with the II genotype and 5 with the DD genotype. The pharmacodynamics of CS-866 in relation to the ACE genotype was evaluated. The effect of CS-866 on the plasma renin-angiotensin system was determined.

The plasma levels of angiotensin II were significantly higher in the DD genotype than the II genotype before the administration of CS-866. The insertion/deletion polymorphism did not change any pharmacokinetic parameters of CS-866 in plasma.

BP in the supine position decreased with the administration of CS-866, while the pulse rate increased. The increase in plasma levels of angiotensin II following administration of CS-866 was associated with the potency of the action of CS-866. The BP-lowering action of CS-866 was greater in the DD genotype subjects than in II genotype subjects. Postural change amplified this genetic difference.

The increase in the levels of angiotensin II following administration of CS-866 appears to be closely related to the antihypertensive action of CS-866. Higher activity of ACE in the subjects with the DD genotype might potentiate the action of CS-866, probably intensifying the rise in the levels of angiotensin II.

Key words : angiotensin converting enzyme, polymorphism, angiotensin receptor, blood pressure, angiotensin II

Introduction

An angiotensin-converting enzyme (ACE) is a component of the renin-angiotensin system and plays important roles in circulatory homeostasis through generation of vasoconstrictor angiotensin II (Ang II) and degradation of vasodilator kinins1). An insertion/deletion (I/D) polymorphism in intron 16 of the human ACE gene, the presence or absence of a 287-bp Alu repeat sequence, is associated with high ACE activity. Subjects with the DD genotype have higher ACE activity than the II genotype...
subjects, which in turn produces higher Ang II levels\(^2-^4\). An enhanced response to Ang I infusion in subjects with the DD genotype has also been reported\(^5,^6\). Ang II receptor antagonists have been developed and have become available for the treatment of hypertension\(^7\). Several large clinical trials are now under way to demonstrate their effectiveness for treating cardiovascular-renal diseases. All Ang II receptor antagonists undergoing clinical application inhibit the renin-angiotensin system by selectively blocking the type 1 angiotensin II receptor (AT1R). AT1R antagonists have been shown to inhibit the pressor response to exogenous Ang II in a dose dependent manner\(^8\). The ACE polymorphism, thus, is thought to affect the inhibitory effect of the AT1R antagonists on BP. However, there is little information whether the ACE polymorphism affects the antihypertensive action of AT1R antagonists.

CS-866, (5-methyl-2-oxo-1,3-dioxolen-4-yl) methoxy-4-(1-hydroxy-1-methylethyl)-2-propyl-1-(4-[2-(tetrazol-5-yl)-phenyl] phenyl) methylimidazol-5-carboxylate, a prodrug type AT1R antagonist, is deesterified to the active acid, RNH-6270. CS-866 lowered BP and increased plasma renin (PRA) and Ang II concentrations at low doses and has a long elimination half-life\(^9\). The first purpose of this study was to indicate that the response of the AT1R antagonist, CS-866 is, in part, genetically determined.

There are two main subtypes of Ang II receptors: AT1R and AT2R. The AT1R antagonist selectively blocks AT1R, which is a dominant subtype in the cardiovascular system, and mediates virtually all the previously known actions of Ang II, including vasoconstriction, production of growth factors, hypertrophy of smooth muscle and cardiomyocyte\(^9\). It has been reported that the blockade of AT1R caused an increase in plasma levels of Arg II mediated through a negative-feedback mechanism\(^10\). The blockade of AT1R is, thus, assumed to follow alternative activation of AT2R. The clinical significance of AT2R is still under investigation\(^11\). Recent evidence, however, suggests that AT2R appears to down-modulate actions mediated by AT1R, resulting in decreased vasoconstrictor responses\(^12-^15\). It has been proposed that blockade of AT1R may possibly cause the stimulation of AT2R by the excess of Ang II through a negative-feedback system\(^16\). The second purpose of this study was to show that the activation of AT2R by the AT1R antagonists might explain why the I/D polymorphism of the ACE gene affected the blood pressure-lowering action of CS-866.

**Methods**

1. **Subjects**

   Twelve healthy normotensive males (with aged 20 to 43) were selected to provide the two genotypes, 7 of the II genotype and 5 of the DD genotype. Laboratory tests were performed on each subject prior to the study. Subjects were excluded from the study if they were unable to follow the study protocol, in which they had to stand up from the supine position within 30 seconds. The subjects did not take any drugs for at least 7 days prior to the administration of CS-866. Alcohol intake was prohibited for 2 days prior to the experiments. Subjects were given about 10 g/day supplemental sodium during the trial. On the day of drug administration, subjects were given about 8 g/day supplemental sodium due to overnight fasting.

   All subjects provided written informed consent for the study after review of the protocol and consent form by the Institutional Review Board of Clinical Pharmacology Center, LTA Medical Co. (Fukuoka, Japan). The study was conducted in agreement with the Declaration of Helsinki (Somerset West Amendment, 1996).

   The subjects were hospitalized in the Osaki Clinic, a division of the Clinical Pharmacology Center, LTA Medical Co. (Tokyo, Japan) during the study.

2. **Study design**

   Each subject was given a single oral dose of CS-866 (16 mg) at 9:00 a.m. on the study day. From midnight before the study day until 1.5 hours after the first measurement of blood pressure, subjects
were under fasting conditions and remained in bed except to go to the toilet and for scheduled measurements of SBP, DBP and pulse rate in the standing position. One hour before administration, plasma samples for predose PRA, and levels of Ang I, Ang II and aldosterone were obtained. The measurements of SBP, DBP and pulse rate were recorded using a BP monitor (BP-1001S, Nippon Colin Co.). Supine SBP and DBP measurements were made after subjects had rested for at least 10 minutes in the supine position. Standing SBP and DBP measurements were made after 2 minutes in the upright position. SBP, DBP and pulse rate were measured before and at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 hours after administration in the supine position and before and at 2, 4, 8, 12 and 24 hours after administration in the standing position. Posture-related change in SBP, DBP or pulse rate (ΔSBP, ΔDBP or Δpulse rate) was defined as the difference between the standing and supine position values.

The blood samples for measuring plasma levels of RNH-6270 were obtained at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 hours after administration. The blood samples for measuring PRA, and plasma levels of aldosterone, Ang I and Ang II were obtained before and at either 4 or 6 hours after administration. Blood samples for biochemical parameters were collected after at least 30 min of supine rest. Plasma was stored −20°C until analyzed.

3. Analytical methods

1) Determination of ACE genotype

Whole blood samples (10 ml) were obtained from all subjects, and genomic DNA was isolated from peripheral lymphocytes with an extraction kit (GENOMIX, Talent, Italy). A 287-bp insertion/deletion polymorphism in intron 16 of the ACE gene was identified by polymerase chain reaction. The allele-specific primers were from the protocol of Zee et al17).

2) Pharmacokinetic analysis

Concentrations of RNH-6270 in plasma and urine were assayed by column-switching high performance liquid chromatography with fluorescence detection (excitation and emission wavelengths were 260 nm and 370 nm, respectively) according to the method of Muth et al18). Peak concentration (Cmax) and the time to Cmax (Tmax) were determined from observed values. The area under the plasma concentration-time curve (AUC) from 0 to infinite was calculated according to the equation: AUC = AUCt+Cpt/ƒÀ where Cpt and ƒÀ represent the observed plasma concentration at the last sampling time (t) and the elimination rate constant at the terminal phase obtained by a least-squares linear regression, respectively.

4. Data analysis

Data are expressed as means±SD and were compared between the two genotypes by one-way or two-way ANOVA. Ninety-five percent confidence intervals were calculated for the differences between two genotypes. The Expert StatView 4.0 for the Macintosh system was used to calculate the association.

Results

1. Subject characteristics

We analyzed the clinical variables of subjects subdivided by genotypes. The subject characteristics are summarized in Table 1. Average weight of the subjects with the DD genotype was slightly heavier than the subjects with the II genotype. However, no clinically important difference between the two genotypes was observed except for

| Table 1 Baseline Characteristics of Healthy Male Volunteers in Relation to ACE Genotype |
|-------------------------------------------------|-----------------|-----------------|
| age (years) | II (n=7) | 29.1±4.6 | 28.8±9.5 |
| weight (kg) | II (n=7) | 60.1±7.8 | 71.2±3.4* |
| SBP (mg) | II (n=7) | 114.3±6.7 | 114.4±3.9 |
| DBP (mg) | II (n=7) | 65.6±4.7 | 62.6±3.2 |
| pulse rate (beats/min) | II (n=7) | 57.4±6.2 | 51.4±7.2 |
| plasma angiotensin I (pg/ml) | II (n=7) | 124.7±10.5 | 136.0±84.7 |
| plasma angiotensin II (pg/ml) | II (n=7) | 9.0±7.1 | 28.0±10.8** |
| plasma renin (ng/ml/hr) | II (n=7) | 2.0±1.3 | 2.0±1.5 |
| plasma aldosterone (pg/ml) | II (n=7) | 93.3±22.6 | 77.8±14.3 |

Values are expressed as means±SD. *p<0.05, **p<0.01 for difference between genotypes. ACE: angiotensin-converting enzyme, SBP: systolic blood pressure, DBP: diastolic blood pressure.

DD and II: genotypes.
plasma levels of Ang II. The plasma levels of Ang II in the subjects with the DD genotype were significantly higher than in the subjects with the II genotype. This finding is consistent with previous investigations.2-4,19)

2. Plasma drug concentrations
The I/D polymorphism of the ACE gene did not change the plasma concentration of the active metabolite RNH-6270 to a clinically important range. The pharmacokinetic parameters of the C_{max}, AUC and T_{max} values of RNH-6270 in relation to the II and DD genotypes are shown in Table 2.

3. SBP, DBP and pulse rate in the supine position
First, we examined whether the I/D polymorphism of the ACE gene was able to affect the response of CS-866 to SBP, DBP and pulse rate in the supine position. The time courses of SBP, DBP and pulse rate after administration are presented in Fig. 1. Mean SBP declined significantly after administration of CS-866. There was no significant difference between the II and DD genotypes [F(1,100) = 3.449, p = 0.0662]. Mean DBP also declined significantly after administration of CS-866. The decrease in DBP of the DD genotype was significantly greater than that of the II genotype [F(1,100) = 14.009, p = 0.0003]. Mean pulse rate increased with the administration of CS-866. The increase in pulse rate in the subjects with the DD genotype was significantly greater than in the subjects with the II genotype [F(1,100) = 9.748, p = 0.023]. These results suggested that the administration of CS-866 appears to be more relevant for the subjects with the DD genotype.

4. Posture-related changes in SBP, DBP and pulse rate
The change from the supine to upright posture causes a shift of blood volume from the intrathoracic compartment towards the lower body regions. The shift induces increases in pulse rate and peripheral vascular resistance to avoid a drop in arterial BP.20) As shown in Table 3, mean SBP, DBP and pulse rate increased with postural change. Before the administration of CS-866, there was no significant difference in SBP, DBP or pulse rate with postural changes (ΔSBP, ΔDBP and Δpulse rate) between two genotypes.

The changes in mean SBP, DBP and pulse rate in the standing position after the administration of CS-866 are shown in Fig. 2.

Mean SBP in the standing position tended to decrease with the administration of CS-866 [F(5,60) = 3.756, p = 0.057], while the mean DBP greatly decreased [F(5,60) = 2.737, p = 0.027]. The mean pulse rate increased slightly after administration [F(5,60) = 2.357, p = 0.051]. Mean SBP after the administration of CS-866 in the standing position tended to be lower in the subjects with the DD genotype than in the subjects with the II genotype [F(1,60) = 3.756, p = 0.057], while mean DBP in the subjects with the DD genotype was considerably lower than in the subjects with the II genotype [F(1,60) = 14.461, p = 0.0003]. There was no significant difference in pulse rate in the standing position between the two genotypes.

The posture-related changes in SBP, DBP and pulse rate (ΔSBP, ΔDBP, and Δpulse rate) are shown in Fig. 3.

ΔDBP was significantly attenuated by administration of CS-866 [F(5,60) = 3.111, p = 0.015]. This attenuation was more evident in the subjects with the DD genotype than in the subjects with the II genotype [F(1,60) = 5.177, p = 0.027]. Neither ΔSBP nor Δpulse rate after the administration of CS-866 differed between two genotypes.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Pharmacokinetic Parameters of CS-866 in Relation to ACE Genotype</th>
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<tr>
<td></td>
<td>II (n=7)</td>
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<tr>
<td>T_{max} (hrs)</td>
<td>1.7±0.5</td>
</tr>
<tr>
<td>C_{max} (ng/ml)</td>
<td>496.8±111.7</td>
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<tr>
<td>AUC_{0-∞} (ng·hrs/ml)</td>
<td>2822.1±725.1</td>
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</table>

Values are expressed as means±SD.
ACE : angiotensin-converting enzyme, DD and II : genotypes, T_{max} : time to reach peak plasma concentration, C_{max} : peak plasma concentration, AUC_{0-∞} : area under the plasma concentration-time curve.
5. Biochemical parameters

The PRA, plasma levels of Ang I and Ang II were markedly increased by the administration of CS-866, while the plasma levels of aldosterone were decreased (data not shown). These results were consistent with previous investigations according to the actions of AT1R antagonists on the renin-angiotensin system\(^{20,21}\). The relationship between biochemical parameters (PRA, plasma levels of aldosterone, Ang I, and Ang II) and cardiovascular responses (SBP, DBP and pulse rate) in the supine position were examined. As shown in Fig. 4, the decrease in DBP after administration of CS-866 showed a negative association with plasma levels of Ang I \((r = -0.5064, \ p = 0.0179)\) and Ang II \((r = -0.4344, \ p = 0.0484)\). The increase in HR after administration of CS-866 showed a positive association with the increase in PRA \((r = 0.5734, \ p = 0.0056)\) and plasma levels of Ang II \((r = 0.4775, \ p = 0.0275)\). These results suggest that the enhancement of the renin-angiotensin system may be positively related to the action of CS-866, which is inconsistent with the inhibitory action of CS-866 on AT1R. On the other hand, the decrease in the plasma levels of aldosterone, which seems to be caused by the blockade of AT1R, did not correlate with any parameters of cardiovascular responses to CS-866. There was a good correlation among the increase in PRA and the plasma levels of Ang I

![Fig. 1](image_url) Time-course alterations in SBP (A), DBP (B) and pulse rate (C) in the supine position after administration of CS-866 in relation to ACE genotype (open circle, II genotype; closed circle, DD genotype).

Data are expressed as means±SD. 16 mg of CS-866 was administered. P values indicate the level of significance for the difference between the two curves.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Posture-related Changes of SBP, DBP and Pulse Rate in Relation to ACE Genotypes</th>
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<tr>
<td></td>
<td>II ( (n=7) )</td>
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<tr>
<td>SBP (mmHg)</td>
<td>supine</td>
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<tr>
<td></td>
<td>standing</td>
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<td>ΔSBP</td>
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<td>DBP (mmHg)</td>
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<td>ΔDBP</td>
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<td></td>
<td>standing</td>
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<td>Δpulse rate</td>
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</table>

Data are expressed as means±SD. Significance was determined by one-way ANOVA between subjects with II and DD genotypes.
Fig. 2  Time-course alterations in SBP (A), DBP (B) and pulse rate (C) in the supine position after administration of CS-866 in relation to ACE genotype (open circle, II genotype; closed circle, DD genotype).

Data are expressed as means±SD. 16 mg of CS-866 was administered. P values indicate the level of significance for the difference between two curves.

Fig. 3  Time-course alterations in posture-related changes in SBP (ΔSBP, A), DBP (ΔDBP, B) and pulse rate (Δpulse rate, C) after administration of CS-866 in relation to ACE genotype (open circle, II genotype; closed circle, DD genotype).

Posture-related change was defined as the difference between the standing position and supine position values. Data are expressed as means±SD. 16 mg of CS-866 was administered. P values indicate the level of significance for the difference between the two curves.
and Ang II. The decrease in plasma levels of aldosterone, however, did not correlate with any other biochemical parameters of the renin-angiotensin system (data not shown). The blockade of AT1R could not, thus, fully explain the blood pressure-lowering action of CS-866.

**Discussion**

The principal objective of this study was to indicate that the cardiovascular responses to the AT1R antagonist, CS-866, differed between the II and DD genotypes of the ACE gene. The plasma concentration versus time profiles for CS-866 and its active metabolite RNH-6700 were nearly identical for the II and DD genotypes, indicating that any changes in pharmacodynamic effects in this study were unlikely the result of a change in the circulating levels of the AT1R antagonist.

It has been considered that the D allele of the ACE gene might be a new risk factor for several cardiovascular-renal diseases. The associations between the D allele and an increased risk of hypertension, left ventricular hypertrophy, myocardial infarction, or renal failure have been reported\(^\text{17,23–25}\). However, some studies did not find such associations\(^\text{26–28}\). In the present study, the I/D polymorphism of the ACE gene did not affect any cardiovascular responses or biochemical parameters with the exception of plasma levels of Ang II before administration of CS-866. In the present study, the I/D polymorphism of the ACE gene had no affect on the cardiovascular response or biochemical parameters with the exception of plasma levels in regard to Ang II before administration of
CS-866. It has been reported that I/D polymorphism of the ACE gene influences serum ACE concentrations and ACE activity\(^2\-\text{4})\). However, it remains controversial as to whether or not the basal levels of angiotensin II in subjects with DD genotype are higher than those with the II genotype\(^2\-\text{6,29})\). Due to the small sample size, further study is necessary to confirm the influence of I/D polymorphism on basal Ang II levels.

The administration of CS-866 decreased SBP and DBP and increased the pulse rate in the supine position. The drop in DBP and the rise in pulse rate in the supine position after administration of CS-866 were clearly larger in the DD genotype. Furthermore, the posture change amplified this different response in DBP to CS-866 between the two genotypes. O'Donnell et al.\(^24\) indicated that there is evidence for association and genetic linkage of the ACE locus with DBP in men. The change in the levels of Ang II, a reflex of the genetic change in ACE activity, is, thus, likely to be more crucial in the regulation of DBP via peripheral vascular resistance rather than SBP or pulse rate.

Our data indicates that the increases in PRA and levels of plasma Ang I or Ang II after administration of CS-866 were associated with the blood pressure-lowering action of CS-866. This finding suggests that the action of CS-866 may be greatly influenced by plasma levels of Ang II. Because the biosynthesis of aldosterone is mediated primarily via AT1R\(^21 \)\), the decrease in plasma levels of aldosterone is thought to reflect the amplitude of the blockade of AT1R. Our data showed that the decrease in plasma levels of aldosterone did not correlate with any other parameter of the plasma renin-angiotensin system (data not shown). Gigante et al.\(^22 \) suggested that the biosynthesis of aldosterone, which is modulated by Ang II formed locally in the adrenal medulla, is independent of the influence of the circulating renin-angiotensin system.

The increased plasma levels of Ang II are, thus, assumed to have little connection with the potency of the blockade of AT1R. Therefore, the blood pressure-lowering action of CS-866 seems to be not only via the blockade of AT1R. Recently, the alternative activation of AT2R caused by excess Ang II after the blockade of AT1R has been proposed\(^19 \). The rise in the plasma levels of Ang II may be important to determine the amplitude of the stimulation of AT2R. It is well-known that the activity of ACE is higher in subjects with the DD genotype\(^2\-\text{4,19})\). The activation of AT2 receptors by AT1 antagonists may explain the difference in the action of CS-866 between the subjects with II and DD genotypes.

The change in arterial pressure induced by a postural change immediately activates the sympathetic baroreflex, an important feedback system in the stabilization of arterial pressure\(^30,31 \). Ang II may play an important role in the modulation of sympathetic outflow and baroreflex function\(^30 \). Our data showed that the difference in the action of CS-866 on DBP between the two genotypes was more evident after postural change. However, ΔSBP and Δpulse rate, the better indices of the change in baroreflex function, did not change after administration of CS-866, suggesting the maintenance of baroreflex control\(^30,32 \). Postural change by standing up resulted in an increase in PRA\(^33,34 \). The increase in PRA caused by postural change might accentuate the difference in the rise in levels of Ang II between the two genotypes. Our data showed that the increase in the levels of Ang II after the administration of the AT1R antagonist was correlated with the decrease in DBP. Postural change, thus, might cause the greater reduction in vascular tone in the subjects with the DD genotype probably through the activation of AT2R by higher levels of Ang II.

In conclusion, the blood pressure-lowering action of CS-866 was greater in the subjects with the DD genotype than in the subjects with the II genotype. The increase in the levels of Ang II after the blockade of AT1R appears to be closely related to the action of CS-866 probably through the activation of AT2R. Higher activity of ACE in the subjects with the DD genotype might amplify the rise in the levels of Ang II.
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References


