Angiotensin I-Converting Enzyme Gene Polymorphism and Acute Response to Captopril in Essential Hypertension

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Insertion/deletion (I/D) polymorphism of the angiotensin converting enzyme (ACE) gene has been shown to be a determinant for serum ACE level and a marker for several cardiovascular diseases. We investigated whether the ACE gene can predict the therapeutic efficacy of ACE inhibitors in essential hypertensive patients. The response of blood pressure and plasma renin activity (PRA) 1 h after 50 mg captopril administration were evaluated in 82 inpatients with untreated essential hypertension (42 men, 40 women; mean age ± SD: 52 ± 13 years; range: 27 to 79 years) in relation to ACE genotypes. There were no differences in age, gender, blood pressure, and PRA in the basal conditions, among essential hypertensive patients with the II, ID, and DD genotypes (n = 36, 34, and 12, respectively). The acute responses of PRA and blood pressure to an ACE inhibitor were similar in the three groups. The blood pressure response was negatively correlated with baseline PRA (r = 0.497). These data suggest that PRA but not the I/D polymorphism of the ACE gene is a useful predictor of the short-term antihypertensive effects of ACE inhibitors. Am J Hypertens 1997; 10:1064–1068 © 1997 American Journal of Hypertension, Ltd.

KEY WORDS: Essential hypertension, angiotensin converting enzyme, polymerase chain reaction, genotype, angiotensin converting enzyme inhibitor, genetics.

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ment of left ventricular hypertrophy (LVH),\(^2,3\) and proliferation of vascular smooth muscle cells.\(^4,5\) Therefore, ACE inhibitors have many advantages other than lowering the blood pressure and are recommended for initial therapy of hypertension.\(^6–8\)

Previous studies have reported that insertion/deletion (I/D) polymorphism of the ACE gene, which is mapped to human chromosome 17q23, influenced the serum level of ACE.\(^9,10\) Additionally, the D allele of the ACE gene has been shown to be associated with myocardial infarction,\(^11\) LVH,\(^12,13\) and diabetic mellitus.\(^14\) However, no association has been found between patients with hypertension and ACE gene polymorphism.\(^15,16\)

In about 70% of patients given ACE inhibitor captopril, an immediate fall in blood pressure occurs, but
the response is variable in each patient. It is therefore valuable to predict the response to treatment in different individuals. There are many factors that have been reported to influence the response to ACE inhibitors, such as race, plasma renin activity, and circulating blood volume. ACE gene polymorphism may be a candidate for a predictor, because its relation to plasma ACE activity has been reported.

In order to reveal whether the ACE gene is a useful predictor of the antihypertensive effects of ACE inhibitors, we investigated the relationship between ACE genotypes and the acute responses of blood pressure and PRA to the ACE inhibitor captopril in Japanese patients with essential hypertension.

**METHODS**

**Patients** We studied 82 inpatients with essential hypertension (42 men, 40 women; mean age ± SD: 52 ± 13 years; range: 27 to 79 years). Hypertension was defined as a systolic blood pressure > 160 mm Hg or a diastolic blood pressure > 95 mm Hg on at least three different occasions at the outpatient clinic in the First Department of Internal Medicine in our hospital. Secondary causes of hypertension and heart, liver, and kidney diseases were excluded by history and by physical and laboratory examinations.

**Protocol** Patients were maintained on a regular salt diet (10 g/day NaCl) for 1 week in the inpatient setting to allow stabilization of the systemic sodium balance and blood pressure. No diuretics or antihypertensive medications were permitted for at least 4 weeks before the study. After they had fasted overnight and rested in the supine position for 1 h, blood samples were obtained from all subjects and blood pressure was determined with a mercury sphygmomanometer every min for 10 min by a single physician. The mean of consecutive blood pressure readings was used for analysis. Blood pressure was again measured and a venous blood sample for measurement of PRA was obtained 1 h after administration of 50 mg of oral captopril. In preliminary study (n = 69), a rise of PRA and a fall of mean blood pressure (MBP) after captopril administration reached the maximum 1 h after the administration. Therefore, we measured the blood pressure and PRA 1 h after captopril administration and estimated short-term antihypertensive effects of the ACE inhibitor. The PRA and plasma aldosterone concentration (PAC) were assayed by a radioimmunoassay. Serum electrolyte concentrations were measured with an auto analyzer. Body mass index (BMI) was calculated as body weight (in kilograms)/height² (in meters squared).

**DNA Studies** Genomic DNA was isolated from peripheral leukocytes. The genotype of the ACE gene was determined by the polymerase chain reaction (PCR), according to the method of Rigat et al. The sense nucleotide primer was 5’-CTGGAGACCACCTCCATCTTCTTCT-3’ and the antisense primer was 5’-GATGTGGCCATCACATTGTCAGAT-3’. These primers allowed detection of a genomic DNA segment of 490 bp corresponding to the I allele and a 190 bp segment corresponding to the D allele. ACE genotypes were classified as II, ID, or DD. PCR was performed with 50 µL of the reaction solution containing 50 pmol/L of each primer, 1.5 mmol/L of MgCl₂, 50 mmol/L of KCl, 10 mmol/L of Tris-HCl (pH 8.3), 200 µmol/L of each dNTP, and 2.5 U Taq DNA polymerase (Takara Shuzo Co., Kyoto, Japan). Amplification was performed as follows: initial denaturation at 95°C for 2 min followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 2 min, and extension at
72°C for 3 min. The PCR products were electrophoresed on 2% agarose gels, and DNA was visualized with ethidium bromide staining.

**Statistical Analysis** Values are expressed as the mean ± SD. Differences between groups were analyzed by one-way analysis of variance (ANOVA). Linear regression analysis was used to study the relationship between the variables. A P of <.05 was accepted as statistically significant.

**RESULTS**

The ACE genotype distribution and their clinical characteristics are shown in Table 1. There were no significant differences in age, sex, body mass index, or routine biochemical indices such as serum concentrations of creatinine and electrolytes among patients with three ACE genotypes.

The blood pressure and PRA in the basal conditions on the regular salt diet were similar in different genotype groups. The responses of blood pressure and the PRA to captopril were not influenced by the genotype of ACE gene (Table 1).

In single regression analysis, the fall in MBP to captopril was significantly correlated with the PRA in the basal conditions (r = 0.497, P < .05, Figure 1b). The age, MBP, BMI, and PAC in the basal conditions were not correlated with the change in the MBP after captopril administration.

**DISCUSSION**

Because the renin-angiotensin system is related not only to blood pressure regulation but also to the development of complications of hypertension such as LVH and atherosclerosis, the ACE inhibitor, which reduces plasma angiotensin II concentration, is one of the most ideal antihypertensive drugs. However, as the response to the ACE inhibitor varies patient by patient, it is highly beneficial to be able to predict efficacy of this medication for each individual. Recently, the ACE gene polymorphism has been shown to be a marker of various cardiovascular diseases such as myocardial infarction, hypertrophic cardiomyopathy, and LVH. Such a role of the ACE gene can be explained by its effect on serum levels of ACE. The D allele is associated with an increased ACE level. Given this assumption, we tested the hypothesis that the genotype of ACE gene can predict the antihypertensive effect of an ACE inhibitor. However, we could not find the association of the ACE gene polymorphism with the acute responses of blood pressure and of PRA to captopril. This lack of association may be due to the following reason. Renin is the rate-limiting factor of angiotensin formation under normal conditions, and its production is regulated by a feedback mechanism involving plasma renin activity (PRA) and blood pressure. In the basal state, the PRA is not increased in the ACE D allele, and thus, the renin-angiotensin system does not play a major role in the regulation of blood pressure. However, when the renin-angiotensin system is activated, such as in the presence of a salt-rich diet, the ACE D allele may be associated with an increased risk of hypertension. Therefore, the association of the ACE gene polymorphism with the acute responses of blood pressure and PRA to captopril may be dependent on the activation state of the renin-angiotensin system.

**TABLE 1. CLINICAL CHARACTERISTICS AND RESPONSES TO CAPTOPRIL IN HYPERTENSIVE PATIENTS CLASSIFIED BY ACE GENOTYPES**

<table>
<thead>
<tr>
<th></th>
<th>II</th>
<th>ID</th>
<th>DD</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>36</td>
<td>34</td>
<td>12</td>
</tr>
<tr>
<td>Sex (men/women)</td>
<td>19/17</td>
<td>16/18</td>
<td>7/5</td>
</tr>
<tr>
<td>Age (years)</td>
<td>52 ± 12</td>
<td>54 ± 13</td>
<td>48 ± 14</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.0 ± 0.5</td>
<td>25.0 ± 0.6</td>
<td>23.7 ± 1.2</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>0.91 ± 0.11</td>
<td>0.80 ± 0.03</td>
<td>0.93 ± 0.15</td>
</tr>
<tr>
<td>Serum Na (mmol/L)</td>
<td>141.81 ± 0.32</td>
<td>142.70 ± 0.42</td>
<td>141.54 ± 0.53</td>
</tr>
<tr>
<td>Serum K (mmol/L)</td>
<td>3.99 ± 0.07</td>
<td>4.07 ± 0.07</td>
<td>4.01 ± 0.13</td>
</tr>
<tr>
<td>Serum total Ca (mmol/L)</td>
<td>4.50 ± 0.04</td>
<td>4.55 ± 0.04</td>
<td>4.48 ± 0.06</td>
</tr>
<tr>
<td>Responses of blood pressure and PRA to captopril</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRA pre (ng/mL/h)</td>
<td>1.49 ± 0.29</td>
<td>1.97 ± 0.52</td>
<td>1.73 ± 0.52</td>
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<tr>
<td>PRA post (ng/mL/h)</td>
<td>6.20 ± 2.20</td>
<td>6.30 ± 1.90</td>
<td>7.05 ± 3.41</td>
</tr>
<tr>
<td>ΔPRA (ng/mL/h)</td>
<td>4.68 ± 1.91</td>
<td>4.30 ± 1.42</td>
<td>5.30 ± 3.20</td>
</tr>
<tr>
<td>SBP pre (mm Hg)</td>
<td>158.8 ± 4.3</td>
<td>151.5 ± 3.4</td>
<td>166.5 ± 8.7</td>
</tr>
<tr>
<td>SBP post (mm Hg)</td>
<td>145.7 ± 4.7</td>
<td>138.7 ± 4.2</td>
<td>153.0 ± 6.7</td>
</tr>
<tr>
<td>ΔSBP (mm Hg)</td>
<td>12.8 ± 2.3</td>
<td>12.6 ± 2.1</td>
<td>13.5 ± 3.8</td>
</tr>
<tr>
<td>DBP pre (mm Hg)</td>
<td>94.0 ± 2.1</td>
<td>96.4 ± 2.1</td>
<td>96.8 ± 4.8</td>
</tr>
<tr>
<td>DBP post (mm Hg)</td>
<td>85.9 ± 2.2</td>
<td>89.7 ± 2.7</td>
<td>92.8 ± 5.6</td>
</tr>
<tr>
<td>ΔDBP (mm Hg)</td>
<td>7.2 ± 1.4</td>
<td>6.7 ± 2.2</td>
<td>4.0 ± 3.6</td>
</tr>
<tr>
<td>MBP pre (mm Hg)</td>
<td>115.6 ± 2.2</td>
<td>114.7 ± 2.1</td>
<td>120.1 ± 5.9</td>
</tr>
<tr>
<td>MBP post (mm Hg)</td>
<td>106.0 ± 2.5</td>
<td>106.0 ± 2.9</td>
<td>112.9 ± 5.6</td>
</tr>
<tr>
<td>ΔMBP (mm Hg)</td>
<td>9.0 ± 1.5</td>
<td>8.7 ± 2.0</td>
<td>7.2 ± 3.1</td>
</tr>
</tbody>
</table>

PRA, plasma renin activity; Δ, change by captopril administration; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, DBP + 1/3 (SBP − DBP); Pre, before captopril administration; Post, 1 h after captopril administration.
circumstances. The effect of ACE inhibitors on blood pressure requires inhibition of the majority of ACE activity. Based on the above, the small differences in ACE activity associated with ACE gene polymorphisms would not be expected to alter the blood pressure responses to ACE inhibition. In addition, age, mean blood pressure, BMI, and PAC were not correlated with the antihypertensive effect of captopril in the present study. Among the variables studied, only PRA could predict the blood pressure response to captopril. This finding is consistent with the previous reports. We could not detect any new predictor other than PRA.

The renin-angiotensin system and the response to ACE inhibitors are changed by the systemic sodium balance. Thus, NaCl intake was kept constant as 10 g/day in the present study. As PRA, PAC, and the response to the ACE inhibitor were similar in patients with II, ID, and DD in this study condition, we can safely deny the association between the renin-angiotensin activity and ACE gene polymorphism.

However, the mechanisms by which ACE inhibitors modulate blood pressure and development of atherosclerosis are multifactorial. Direct vasodilation as well as chronic responses through the renal system and sympathetic nervous system, as well as the antiproliferative actions of endothelium are also important in the effects of ACE inhibitors. The acute blood pressure response to captopril is mainly due to vasodilation induced by the reduction of plasma concentration of angiotensin II. However, chronic antihypertensive effects of an ACE inhibitor may result from not only direct vasodilation but also the modifications in the sodium balance, renal function, vascular structure, endothelial mediated vasodilation, and sympathetic nervous system. It is possible that there is a discrepancy between acute and chronic effects of an ACE inhibitor. Therefore, we cannot completely rule out the possibility of a relationship between the ACE gene polymorphism and the chronic therapeutic efficacy of ACE inhibitors.

In conclusion, the ACE gene is not a useful predictor of the short-term antihypertensive effects of ACE inhibitors.

REFERENCES


