ACE (I/D) Genotype as a Predictor of the Magnitude and Duration of the Response to an ACE Inhibitor Drug (Enalaprilat) in Humans

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Background—We have investigated the possible effects of contrasting ACE (I/D) genotypes on the responses to the ACE inhibitor enalaprilat in normotensive men.

Methods and Results—Subjects with DD (n = 12) and II (n = 11) ACE genotypes received an intravenous infusion of enalaprilat or placebo. Pressor responses to stepwise, incremental doses of angiotensin I were measured at 1 and 10 hours after dosing. The dose required to raise mean blood pressure by 20 mm Hg (PD20) was calculated individually, and the ratio of PD20 during enalaprilat to that during placebo (dose ratio, DR) was used for assessment of the extent of ACE inhibition. The pressor response was significantly attenuated at 1 hour after enalaprilat in both groups, but significant attenuation was evident at 10 hours after dose only in the II subjects. The DRs at both 1 hour (median, 5.43 versus 2.82, \( P = 0.0035 \)) and 10 hours (2.06 versus 0.84, \( P = 0.0008 \)) after enalaprilat were significantly higher in II subjects than in DD subjects.

Conclusions—The effect of enalaprilat was significantly greater and lasted longer in normotensive men homozygous for the II ACE genotype. By multivariate analysis, ACE (I/D) genotype and plasma angiotensin II levels were predictive of >50% of the variation in response to ACE inhibition. (Circulation. 1998;98:2148-2153.)

Key Words: angiotensin | enzymes | enalaprilat | genes

Angiotensin-converting enzyme inhibitor drugs are established in the treatment of patients with hypertension and heart failure. However, there is a large interindividual variability in the hemodynamic and hormonal responses to this class of drug, and studies investigating the factors that might contribute to intersubject variations in responsiveness (eg, age, ethnic origin, sodium status) have failed to fully explain this heterogeneity. Thus, the pretreatment activity of the renin-angiotensin system (RAS) has been shown to affect the response to ACE inhibition so that activation of the system by dietary sodium restriction enhances the drug-related fall in blood pressure. However, the intersubject variability of plasma renin activity in salt-replete hypertensive patients is not large enough to explain the variation of drug response, and with enalapril, it has been shown that pretreatment plasma renin activity accounts for <10% of the variability. Differences in serum ACE activity have been thought to be less important in regulating the response to ACE inhibition, because the enzyme has not been considered to be rate-limiting for the production of angiotensin II. However, there is large interindividual but small intraindividual variation in ACE activity, recent evidence suggests that >50% of this is familial and can be explained by a single major gene effect. The insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene acts as a convenient marker of this allelic variation, which influences not only serum ACE concentration and activity but also ACE activity in T lymphocytes and in cardiac tissue.

The ACE gene polymorphism has also been extensively investigated as a candidate genetic locus for cardiovascular disease. Although adverse pathological outcomes have been correlated with genetic variability at the ACE locus, the pathophysiological consequences of the concomitant differences in ACE activity and, in turn, the influence of this on the response to ACE inhibitor drug treatment have not been well documented.

In a recent study, we demonstrated an enhanced pressor response to exogenous angiotensin I and increased production of angiotensin II in normotensive men homozygous for the DD allele. These results suggested that genetically determined differences in the level of ACE activity modulated the responsiveness of the RAS via the differential generation of angiotensin II. The principal aim of the present study was to evaluate whether or not there were similar, differential
responses to ACE inhibitor drug treatment. This study therefore evaluated the magnitude and time course of the ACE-inhibitory effects after intravenous enalaprilat in normotensive men with differing levels of ACE activity and contrasting ACE (I/D) genotype.

Methods

Identification of Subjects

Two hundred healthy, normotensive men who had volunteered for research studies in the preceding 12 months gave additional written informed consent for blood sampling for DNA and ACE genotyping. From the subjects found to be homozygous for II and DD genotypes, 12 subjects with DD and 11 with II genotype gave written informed consent to participate in the study, which was approved by the West Ethics Committee of the Greater Glasgow Health Board.

ACE Genotyping

Details of the polymerase chain reaction (PCR) method used to establish ACE genotype have been described elsewhere. In brief, D and I alleles were amplified by PCR using standard primers in a reaction mixture containing 5% DMSO and size-fractionated on agarose gels. Putative DD genotypes were further confirmed by the triple-primer method.20

Enalaprilat Infusion Study

Subjects undertook 2 separate study days at least 2 weeks apart, with intravenous enalaprilat or placebo administered according to a randomized, double-blind, crossover design. All subjects were instructed to maintain a normal-sodium diet (150 mmol/d) for 3 days before each of the 2 study days. After an overnight fast and avoidance of alcohol and caffeine-containing drinks for at least 12 hours, subjects attended the Clinical Investigation and Research Unit, Western Infirmary, Glasgow, at 7:30 AM. An intravenous cannula was inserted into each forearm and, after a period of ≥30 minutes of bed rest, subjects received an infusion of 0.01 mg/kg of enalaprilat (Merck Sharp & Dohme) or placebo (isotonic saline) for 30 minutes. Blood pressure was measured and blood samples were taken at 0, 0.5, 1, 4, 6, 8, and 10 hours after the infusion for measurement of plasma enalaprilat, angiotensin I and II, aldosterone, and active renin concentrations.

Angiotensin I Infusion Studies

Angiotensin I infusion studies were performed at 1 and 10 hours after the end of the enalaprilat or placebo infusion. Each subject received incremental doses, each of 8 minutes' duration, from 1 to 20 ng·kg⁻¹·min⁻¹ of angiotensin I (Clinalpha AG). Blood pressure was measured every minute by semiautomated sphygmomanometer (Critikon). When the rise in blood pressure was <20 mm Hg from baseline at 20 ng·kg⁻¹·min⁻¹, additional doses were infused. Blood samples for measurement of plasma angiotensin II concentrations were obtained from the contralateral arm after each dose of angiotensin I.

Analysis of Pressor Response Data

Details of the methods and analysis for the pressor infusion study have been described elsewhere.21 Changes from baseline in mean blood pressure were plotted against the log-transformed dose of angiotensin I and then adapted to a quadratic function for each individual infusion. The PD20, which is the dose of angiotensin I required to raise mean blood pressure by 20 mm Hg, was calculated from the individual fit in each subject. The dose ratio (DR) for angiotensin I, ie, the ratio of the PD20 after enalaprilat to that after placebo, was then calculated for each infusion time. The dose ratios therefore provide indices of the magnitude of the attenuation by enalaprilat of the angiotensin I pressor response; thus, a high DR represents marked ACE inhibition, whereas a DR of ~1 indicates no significant ACE inhibition. Changes in blood pressure were also plotted against the achieved plasma concentra-
25.2 kg/m²; and mean arterial pressure, 85 and 82 mm Hg, respectively.

**Basal Activity of the RAS**

Table 1 shows baseline activities and concentrations for each component of the RAS. As expected, ACE activity in the subjects with DD genotype was significantly higher than in those with II genotype. There were no significant differences in plasma angiotensin II, angiotensin I, angiotensin II/I ratio, aldosterone, and active renin concentration at baseline.

There was no significant relationship between baseline ACE activity and baseline plasma angiotensin II, angiotensin I, and aldosterone. There was a weak correlation between ACE activity and angiotensin II/I ratio ($r^2=0.152$), but this did not reach statistical significance ($P=0.0656$).

**Plasma Concentrations of Enalaprilat**

Plasma enalaprilat concentrations at 1 hour after intravenous enalaprilat were 17.4±6.8 ng/mL in II genotype and 16.7±5.5 ng/mL in DD genotype subjects (mean±SD), and at 10 hours after dose, 1.8±0.9 and 2.2±1.1 ng/mL, respectively.

**Time Course of Activity of the RAS and Blood Pressure After Enalaprilat**

The time courses for the absolute values and the percentage changes in ACE activity after enalaprilat and placebo are shown in Figure 1. In both groups, enalaprilat inhibited ACE activity by ~90% at 1 hour after dose and by up to 40% at 10 hours after dose. Despite the absolute values for ACE activity being higher in DD subjects than in II subjects at all time points, the percentage inhibition of ACE activity did not differ between the 2 groups.

Changes in mean blood pressure and changes in each component of the RAS are summarized in Table 2. Plasma angiotensin II concentrations in DD subjects returned to baseline levels at 10 hours after enalaprilat, whereas those in II subjects remained suppressed, but there was no statistically significant difference between the 2 groups (ANOVA). Furthermore, there were no significant differences between the 2 groups in the time courses for the changes in the other measured components of the RAS. Although active renin concentrations after enalaprilat tended to be higher in DD subjects than those in II subjects, this did not reach statistical significance. There was no significant correlation between pretreatment ACE activity and changes in blood pressure, plasma angiotensin I, angiotensin II/I ratio, and plasma active renin concentrations.

**Angiotensin II Levels During Angiotensin I Infusion**

The changes in angiotensin II levels during the angiotensin I infusion are shown in Figures 2 and 3. At 1 hour after enalaprilat (Figure 2), plasma angiotensin II levels were significantly decreased in both groups compared with those after placebo ($P<0.0001$ by ANOVA). At 10 hours after enalaprilat (Figure 2), plasma angiotensin II levels were significantly lower than those after placebo in the subjects with II genotype ($P=0.02$ by ANOVA) but not in those with DD genotype.

**Angiotensin I Pressor Response After Enalaprilat**

The individual dose ratios for angiotensin I at 1 and 10 hours after enalaprilat are shown in Figure 3. There were no

<table>
<thead>
<tr>
<th>Component</th>
<th>II</th>
<th>DD</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active renin concentration, μU/mL</td>
<td>15.8 (6.4–39.9)</td>
<td>20.3 (12.5–38.5)</td>
<td>(−0.4, 14.4)</td>
</tr>
<tr>
<td>Angiotensin I, pmol/L</td>
<td>28.3 (17.8–46.3)</td>
<td>23.5 (9.2–55.7)</td>
<td>(−11.5, 13.2)</td>
</tr>
<tr>
<td>Angiotensin II, pmol/L</td>
<td>8.4 (2.3–16.1)</td>
<td>10.2 (2.8–18.6)</td>
<td>(−2.4, 6.4)</td>
</tr>
<tr>
<td>Angiotensin II/I×10²</td>
<td>29.5 (12.6–67.2)</td>
<td>42.4 (13.0–91.4)</td>
<td>(−4.0, 37.4)</td>
</tr>
<tr>
<td>Plasma aldosterone concentration, pmol/L</td>
<td>326 (189–511)</td>
<td>402 (139–589)</td>
<td>(−46, 174)</td>
</tr>
<tr>
<td>ACE, EU/L</td>
<td>15.8 (7.1–21.7)</td>
<td>26.0 (17.7–36.7)</td>
<td>(6.0, 18.0)*</td>
</tr>
</tbody>
</table>

EU indicates enzyme units. Data are median (range).

*P<0.0004.
significant changes in sensitivity to angiotensin II at either 1 or 10 hours: at 1 hour, the CR for angiotensin II was 0.62 (0.51, 1.12) in DD subjects and 0.79 (0.44, 1.06) in II subjects, and at 10 hours, 0.72 (0.58, 1.23) in DD subjects and 0.99 (0.72, 1.59) in II subjects [mean (95% CI)]. There were no significant differences between the 2 groups in sensitivity to angiotensin II at either time point: 95% CI (−0.33, 0.41) at 1 hour and (−0.75, 0.18) at 10 hours after enalaprilat.

At 1 hour after enalaprilat administration, angiotensin I pressor responses were significantly attenuated in both groups. Unadjusted DR was 2.82 (2.03, 3.45) in DD subjects and 5.43 (3.45, 13.36) in II subjects. The DRs adjusted for angiotensin II sensitivity were 4.20 (2.22, 5.77) in DD subjects and 9.39 (3.13, 21.01) in II subjects [median (95% CI)]. The magnitude of attenuation in subjects with II genotype was significantly greater than in subjects with DD genotype [unadjusted DR, P<0.0035, 95% CI (0.77, 12.80); adjusted DR, P=0.0178, 95% CI (0.83, 14.87)].

At 10 hours after enalaprilat, the angiotensin I pressor response was significantly attenuated only in II subjects: unadjusted DR of 2.06 (1.43, 2.86) and adjusted DR of 2.33 (1.41, 2.66) in II subjects compared with unadjusted DR of 0.84 (0.54, 1.42) and adjusted DR of 1.05 (0.76, 1.46) in DD subjects. The DR was also significantly higher in II subjects than DD subjects: unadjusted DR 95% CI (0.61, 1.95), P=0.0008; adjusted DR 95% CI (0.36, 1.57), P=0.0023.

**Table 2. Changes in RAS and Blood Pressure After Enalaprilat**

<table>
<thead>
<tr>
<th>Time after Enalaprilat, h</th>
<th>0.5</th>
<th>1</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active renin concentration, μU/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>21.0 (36.3)</td>
<td>21.4 (34.7)</td>
<td>20.0 (22.9)</td>
<td>20.5 (16.2)</td>
<td>10.4 (11.8)</td>
<td>7.8 (8.0)</td>
</tr>
<tr>
<td>DD</td>
<td>18.4 (21.2)</td>
<td>32.4 (30.5)</td>
<td>20.0 (18.5)</td>
<td>31.5 (36.7)</td>
<td>34.9 (28.1)</td>
<td>13.7 (19.2)</td>
</tr>
<tr>
<td>Angiotensin II, pmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>−1.9 (6.9)</td>
<td>−3.7 (4.3)</td>
<td>−2.6 (7.6)</td>
<td>−3.3 (5.8)</td>
<td>−1.6 (4.1)</td>
<td>−2.7 (5.3)</td>
</tr>
<tr>
<td>DD</td>
<td>−2.7 (3.8)</td>
<td>−2.1 (6.5)</td>
<td>−2.5 (7.9)</td>
<td>−2.2 (6.0)</td>
<td>−0.8 (3.1)</td>
<td>0.6 (6.6)</td>
</tr>
<tr>
<td>Angiotensin II×10^2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>−12.5 (26.0)</td>
<td>−20.8 (31.3)</td>
<td>−15.6 (23.7)</td>
<td>−27.9 (47.2)</td>
<td>−16.6 (29.6)</td>
<td>−10.1 (13.3)</td>
</tr>
<tr>
<td>DD</td>
<td>−23.7 (27.9)</td>
<td>−20.8 (29.9)</td>
<td>−21.8 (27.3)</td>
<td>−26.2 (32.4)</td>
<td>−18.0 (23.2)</td>
<td>−15.3 (38.9)</td>
</tr>
<tr>
<td>Aldosterone, pmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>−46.0 (167)</td>
<td>−66.1 (173)</td>
<td>−88.1 (112)</td>
<td>−35.1 (71.8)</td>
<td>−20.4 (104)</td>
<td>−47.4 (89.0)</td>
</tr>
<tr>
<td>DD</td>
<td>−75.9 (87.0)</td>
<td>−126.2 (214)</td>
<td>−89.3 (119)</td>
<td>−11.7 (144)</td>
<td>32.3 (155)</td>
<td>−4.9 (80.7)</td>
</tr>
<tr>
<td>Mean blood pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>−4.2 (5.4)</td>
<td>−5.3 (6.5)</td>
<td>−4.6 (6.8)</td>
<td>−3.7 (5.8)</td>
<td>−3.1 (3.9)</td>
<td>−3.5 (4.5)</td>
</tr>
<tr>
<td>DD</td>
<td>−3.1 (4.2)</td>
<td>−4.2 (6.5)</td>
<td>−4.1 (5.3)</td>
<td>−1.8 (6.1)</td>
<td>−3.1 (5.5)</td>
<td>0.1 (4.2)</td>
</tr>
</tbody>
</table>

Data are shown as mean (SD).

**Figure 2.** Top, Changes in plasma angiotensin II concentrations during angiotensin I infusion at 1 hour after enalaprilat and placebo in subjects with II (left) and DD genotype (right). Bottom, Changes in plasma angiotensin II concentrations during angiotensin I infusion at 10 hours after enalaprilat and placebo in subjects with II (left) and DD (right) genotype.

**Figure 3.** Dose ratios for angiotensin I at 1 hour and 10 hours post dose in II (○) and DD (●) subjects. *P=0.003 and **P=0.001 by Mann-Whitney U test.
Interrelationships Between Activity of the RAS and Attenuation of the Pressor Response to Angiotensin I During ACE Inhibition

At 10 hours after enalaprilat, there were statistically significant differences between II and DD subjects in the extent of the ACE inhibitory effect as determined by the DRs for the angiotensin I response. By univariate analysis, both ACE genotype and ACE activity were significant predictors of the ACE inhibitory effect (coefficients of determination, 50.5%; $P=0.000$ and 40.9%; $P=0.001$), but there were no other significant relationships for age, renin, angiotensin II, or aldosterone, which all had coefficients of determination of <6.5%. An all-subsets regression (multivariate) analysis showed that 48.1% of the variability could be explained by ACE genotype ($P=0.012$) and that this progressively increased to 61.7% and 70.4% with the combination of ACE genotype with angiotensin II ($P=0.000$) and then with aldosterone concentrations ($P=0.000$). The final regression equation, which incorporated ACE activity ($P=0.005$) to account for 78.1% of the variability in response, was $\text{DR}_{\text{II}}=2.59-0.0433 \text{ ACE} +0.0425 \text{ AII} – 1.27$ genotype to 0.00702 aldosterone.

Discussion

This study has demonstrated that the attenuation by enalaprilat of the angiotensin I pressor response in normotensive healthy men was of significantly greater magnitude and longer duration in the group homozygous for the I allele at the ACE locus. Furthermore, although a significant reduction in plasma angiotensin II levels at 10 hours after enalaprilat was apparent in the II subjects, there was no significant reduction in DD subjects at this time. This, in turn, is wholly consistent with the attenuated angiotensin I pressor response at 10 hours after dose in the II subjects and reflects persisting ACE inhibition in these subjects.

By univariate analysis, both ACE genotype and ACE activity were predictive of 48.1% and 38.1%, respectively, of the variation in the ACE inhibition response to enalaprilat. However, the multivariate analysis revealed that ACE genotype was the most important single factor: in turn, ACE genotype in combination with measurements of angiotensin II and aldosterone concentrations accounted for 70% of the variation in response, and the further incorporation of ACE activity then increased the predictive power to 78.1%. Thus, ACE genotype predicted the response to the ACE inhibitor independently of ACE activity. However, although ACE genotype was the most important single factor, this analysis might also be interpreted as showing that ACE activity has an important regulatory role in the RAS via the gene expression of angiotensin II and aldosterone. This possibility is wholly consistent with the results of our previous study, which suggested that the influence of ACE gene polymorphism on in vivo ACE activity was most obvious in the presence of relatively high angiotensin I concentrations.

Other recent studies investigating the influence of the ACE gene polymorphism on the response to ACE inhibition have tended to produce inconclusive results. For example, a retrospective analysis of a clinical trial with 20 mg enalapril showed that there was no difference in blood pressure reduction for hypertensive patients with DD and II ACE genotypes. However, a 20-mg dose of the ACE inhibitor enalapril, for example, is likely to cause >95% peak ACE inhibition, and as seen in the present study, near-maximum ACE inhibition may mask interindividual differences in responses. For this reason, we chose a dose of enalaprilat expected to produce a submaximal effect at peak, ie, located on the “shoulder” portion of the dose-response curve. This corresponds approximately to the effect of an oral dose of <2.5 mg enalapril. In studies using conventional doses, therefore, the doses are relatively high and likely to evoke close to maximal responses (on the flat portion of the dose-response curve). Consistent with this concept is the demonstration in the present study of equivalent ACE inhibition at 1 hour after dose on both groups.

Although a number of reports focus on the ACE genotype as a genetic cardiovascular risk factor, the relationship between ACE activity itself and cardiovascular disease has been little investigated, although 1 report suggests that ACE activity may be a risk factor for myocardial infarction, independently of the I/D polymorphism. The fact that the inclusion of angiotensin II, aldosterone, and ACE activity as independent variables significantly improved the predictive power lends some support for this concept. Overall, however, the results of the multivariate analysis and the demonstration of a 2-fold greater attenuation in II subjects despite similar angiotensin II concentrations at 1 hour after dose suggest that categorization of the I/D genotype may be the more precise way of predicting the response to an ACE inhibitor drug. In turn, this suggests that genetically determined factors other than circulating ACE activity may influence the response to ACE inhibition. Alternatively, categorization of ACE (I/D) genotype may simply reduce the “noise” and eliminate such extraneous influences as dietary intake, physical activity, or concomitant drug treatment, which render the measurement of ACE activity (relatively) unreliable. In pragmatic terms, therefore, relatively low doses of an ACE inhibitor drug may be sufficient for patients with low ACE activity (which is known to be ≈50% genetically determined) and low levels of angiotensin II (ie, those with II genotype) but insufficient for full and sustained inhibition in patients with high ACE levels and higher levels of angiotensin II, ie, those with DD genotype.

In conclusion, the results of this study indicate that the magnitude and duration of the effect of an ACE inhibitor drug will vary in different subgroups that can be identified by the I/D genotype. Furthermore, these results are also consistent with the concept of a regulatory role for ACE via differing levels of ACE activity and the differential generation of angiotensin II concentrations, particularly in circumstances of an activated RAS. In turn, the differential pathological consequences and therapeutic outcomes that have been attributed directly to the I/D genotype may, alternatively and/or additionally, reflect differences in the relative therapeutic effectiveness of standard ACE inhibitor drug doses.

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References


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