Effects of Hormone Replacement Therapy on Serum Angiotensin-Converting Enzyme Activity and Plasma Bradykinin in Postmenopausal Women According to Angiotensin-Converting Enzyme-Genotype

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An insertion/deletion (I/D) polymorphism in the angiotensin-converting enzyme (ACE) gene determines serum ACE levels. The D allele is associated with increased ACE activity and is linked to cardiovascular disease. Hormone replacement therapy (HRT) in postmenopausal women (PMW) decreases serum ACE activity and concomitantly increases plasma bradykinin. We investigated the effect of HRT on these parameters in PMW according to ACE-genotype. We assessed 68 PMW during 12-month oral HRT (0.625 mg conjugated estrogen + 2.5 mg medroxyprogesterone acetate). ACE genotype was determined at baseline, and serum ACE activity and plasma bradykinin were measured at baseline and after 3-, 6-, and 12-month HRT. We divided the PMW into three groups according to ACE genotype: groups I/I (n = 26), I/D (n = 33), and D/D (n = 9). HRT resulted in a significant reduction in the genotype-associated increase in ACE activity in the ACE I/D and D/D groups after 6-month (p < 0.001 and p < 0.05, respectively) and 12-month HRT (p < 0.001 and p < 0.01, respectively), but not in the I/I group. While the reduction of ACE activity was expected to increase bradykinin in the ACE I/D and D/D groups, HRT significantly increased the bradykinin levels not only in these two groups but also in the ACE I/I group at both 6 months (p < 0.01, p < 0.05, and p < 0.001, respectively) and 12 months after the start of HRT (p < 0.01, p < 0.01, and p < 0.01, respectively). These results suggest that the increased plasma bradykinin of PMW by HRT might not be induced solely by the reduction in serum ACE activity. (Hypertens Res 2003; 26: 53–58)

Key Words: angiotensin, bradykinin, estrogen, polymorphism, women

Introduction

An insertion/deletion (I/D) polymorphism in intron 16 of the angiotensin-converting enzyme (ACE) gene accounts for 50% of the variability in human serum ACE levels (1). The D allele is associated with increased ACE activity and is linked to cardiovascular disease (2). Hormone replacement
therapy (HRT) reduces serum ACE activity in postmenopausal women (PMW), suggesting that lower ACE activity may be one of the factors that protects against cardiovascular disease (3). We also recently found that HRT decreases the serum ACE activity of PMW (4–7) and concomitantly increases their circulating plasma bradykinin concentrations (4–6). Since ACE is the same as the bradykinin-degrading enzyme (kininase II) (8), the plasma bradykinin concentrations would be expected to increase when ACE activity decreases. Thus, it is important to determine whether the decrease in serum ACE activity and increase in plasma bradykinin induced by HRT differ according to ACE genotype.

We investigated whether the ACE genotype affects the decrease in serum ACE activity induced by HRT and whether the increase in plasma bradykinin concentrations is dependent on the ACE-genotype.

Methods

Subjects
We enrolled 72 consecutive Japanese PMW in this study and assessed the 68 PMW (mean age, 57.3 ± 0.7 years; range, 46 to 71 years) for whom complete data for 12-month HRT were available. All women were postmenopausal (>12 months’ amenorrhea) as confirmed by elevated serum concentrations of follicular-stimulating hormone (FSH) and low serum estradiol (E2) concentrations. None had received HRT before enrollment or had any contraindications to such treatment. All subjects were nonsmokers with no evidence of gynecologic disorders or any other disease, and with no history of cardiovascular disease. The study was approved by the Institutional Committee on Human Research of the Cardiovascular Hospital of Central Japan, and written informed consent was obtained from every patient.

Study Protocol
Each subject received a daily dose of HRT (0.625 mg conjugated equine estrogen combined with 2.5 mg oral medroxyprogesterone acetate) orally for 12 months. All subjects attended the HRT clinic of the Cardiovascular Hospital of Central Japan once a month for physical checkups and to provide blood specimens at baseline and after 3, 6, and 12 months of HRT. The blood specimens were collected and blood pressure and heart rate were measured after the subject had rested for at least 30 min in the supine position in the morning after a 12-h fast. After centrifuging, the blood specimens were stored at -80°C until assayed. ACE genotype was determined at baseline, and serum ACE activity, FSH, and E2 and plasma bradykinin concentrations were measured at baseline and after 3, 6, and 12 months of HRT. We divided the consecutive 68 PMW into three groups according to ACE genotype: groups I/I (n = 26), I/D (n = 33), and D/D (n = 9).

ACE Genotyping
Genomic DNA was isolated by phenol-chloroform extraction from whole blood drawn into tubes containing potassium ethylenediamine tetra-acetic acid (EDTA) (9). Polymerase chain reaction was used to detect the two alleles of 490 and 190 bp corresponding, respectively, to the I and D fragments of ACE (10). Genomic DNA (250 ng) was used in a final volume of 50 µl containing 1.5 mmol/l MgCl2, 50 mmol/l KCl, 10 mmol/l Tris hydrochloride (pH 8.4), 50 pmol of each primer (5'-CTGGAGACCACCTCCCATCCTTT-3' and 5'-GATGTCGCCACATATTGTCGAT-3'), 250 µmol/l each of the four dNTPs, and 0.4 U Taq polymerase. DNAs were amplified on a programmable thermal controller (MJ Research, Waterstone, USA) for 30 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 1 min. The genotypes were analyzed by electrophoresis in 4% agarose gel and visualized by ethidium bromide staining.

Biochemical Assays
For the measurement of ACE activity, 3 ml of venous blood was drawn into a polyethylene tube, immediately placed in ice water, and centrifuged at 3,000 rpm for 10 min. Serum ACE activity was assayed by measuring hippuric acid production from the substrate (Hip-His-Leu). The concentration of hippuric acid was measured by a colorimetric method (11) using high-performance liquid chromatography (HPLC) with a Model Tri-Rotor (Japan Spectroscopic Co., Tokyo, Japan). To measure bradykinin, the samples were placed in siliconized vacuum tubes containing aprotinin, soybean trypsin inhibitor, protamine sulfate, and disodium EDTA, and all tubes were placed in a box filled with ice until centrifuged at 3,000 rpm for 10 min. The supernatants were stored in a sealed polypropylene tube at -80°C until analyzed. The plasma bradykinin concentrations were measured by radioimmunoassay (RIA) (12). The intra-assay coefficients of variation for serum ACE activity and plasma bradykinin were 5–7% and 5–10%, respectively, and the inter-assay coefficients of variation for serum ACE activity and plasma bradykinin were 2–8% and 5–10%, respectively.

Serum FSH and E2 concentrations were measured by RIA with a commercially available kit (Boehringer Mannheim, Mannheim, Germany).

Statistical Analysis
Data are reported as the mean ± SEM. Clinical characteristics among the three genotypes were compared by one-way analysis of variance (ANOVA). The changes in body mass index, blood pressure, heart rate, ACE activity, and hormone and bradykinin concentrations after the start of HRT in the
Table 1. Clinical Characteristics of the Subjects and Change in These Factors According to ACE Genotype

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>ACE I/I (n = 26)</th>
<th>ACE I/D (n = 33)</th>
<th>ACE D/D (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55.1 ± 1.6</td>
<td>55.8 ± 1.3</td>
<td>56.0 ± 1.4</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>22.5 ± 1.4</td>
<td>23.0 ± 0.9</td>
<td>22.7 ± 1.3</td>
</tr>
<tr>
<td>3 months</td>
<td>22.5 ± 1.4</td>
<td>23.1 ± 0.9</td>
<td>22.8 ± 1.3</td>
</tr>
<tr>
<td>6 months</td>
<td>22.5 ± 1.4</td>
<td>23.1 ± 0.9</td>
<td>22.6 ± 1.3</td>
</tr>
<tr>
<td>12 months</td>
<td>22.5 ± 1.4</td>
<td>23.1 ± 0.9</td>
<td>22.7 ± 1.3</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>125.8 ± 2.9</td>
<td>124.2 ± 2.1</td>
<td>125.8 ± 6.5</td>
</tr>
<tr>
<td>3 months</td>
<td>125.8 ± 2.3</td>
<td>125.0 ± 2.5</td>
<td>124.4 ± 4.7</td>
</tr>
<tr>
<td>6 months</td>
<td>125.2 ± 2.7</td>
<td>125.2 ± 2.7</td>
<td>124.9 ± 5.1</td>
</tr>
<tr>
<td>12 months</td>
<td>125.8 ± 3.2</td>
<td>124.6 ± 2.6</td>
<td>125.1 ± 4.7</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>79.8 ± 1.9</td>
<td>79.5 ± 1.4</td>
<td>79.6 ± 4.5</td>
</tr>
<tr>
<td>3 months</td>
<td>80.5 ± 1.2</td>
<td>79.0 ± 1.7</td>
<td>79.1 ± 2.5</td>
</tr>
<tr>
<td>6 months</td>
<td>81.0 ± 2.1</td>
<td>79.4 ± 1.6</td>
<td>80.2 ± 4.0</td>
</tr>
<tr>
<td>12 months</td>
<td>80.1 ± 1.3</td>
<td>79.7 ± 1.9</td>
<td>79.8 ± 4.4</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>62.4 ± 1.4</td>
<td>62.5 ± 1.2</td>
<td>62.7 ± 1.3</td>
</tr>
<tr>
<td>3 months</td>
<td>62.8 ± 1.3</td>
<td>62.4 ± 0.9</td>
<td>62.7 ± 2.1</td>
</tr>
<tr>
<td>6 months</td>
<td>62.7 ± 1.2</td>
<td>62.8 ± 1.1</td>
<td>62.7 ± 1.3</td>
</tr>
<tr>
<td>12 months</td>
<td>62.5 ± 1.1</td>
<td>62.8 ± 1.0</td>
<td>62.2 ± 1.7</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM. ACE, angiotensin-converting enzyme; I/D, insertion/deletion; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Three genotypes were compared by two-way ANOVA for repeated measurements, and when the analysis was significant, Scheffe’s F-test was applied. All probability values are 2-tailed. A value of $p < 0.05$ was accepted as statistically significant.

**Results**

Before treatment, there were no significant differences in age among the groups. There were no significant differences in body mass index, systolic and diastolic blood pressures, or heart rate between the groups at baseline and after 3, 6, and 12 months of HRT. HRT also did not change body mass index, systolic and diastolic blood pressures, or heart rate in the three groups during treatment (Table 1).

HRT was associated with increased serum concentrations of E2 and decreased FSH, confirming patients’ compliance with the regimen (Table 2). Before treatment, serum ACE activity was highest in the D/D genotype (18.3 ± 2.0 IU/l; $p < 0.05$ vs. I/I genotype), intermediate in the I/D genotype (16.5 ± 0.6 IU/l; $p < 0.05$ vs. I/I genotype), and lowest in the I/I genotype (11.9 ± 1.0 IU/l) group. After 6 and 12 months of HRT, there were no differences in ACE activity among the three groups. In the ACE I/D and D/D groups, HRT significantly reduced serum ACE activity after 6 ($p < 0.001$ and $p < 0.05$, respectively) and 12 months of HRT ($p < 0.001$ and $p < 0.01$, respectively), but no significant changes in serum ACE activity were observed in the I/I group during treatment (Table 2).

There were no significant differences in plasma bradykinin concentrations between the groups at baseline or after 3, 6, and 12 months of HRT. HRT significantly increased the plasma bradykinin concentrations in the ACE I/I, I/D, and D/D groups 6 months after the start of HRT ($p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively) and 12 months after the start of HRT ($p < 0.01$, $p < 0.01$, and $p < 0.01$, respectively) (Table 2).

**Discussion**

Our study showed that HRT reduced the serum ACE activity of PMW with the ACE I/D and D/D genotypes but not the I/I genotype, whereas it increased the plasma bradykinin concentrations of the PMW in all three ACE-genotype groups.

Proudlerr et al. (3) demonstrated that HRT reduces serum ACE activity in PMW and speculated that an HRT-depen-
dent reduction in ACE activity might affect vascular function through changes in angiotensin II and kinin concentrations. Our previous studies (4–7) reported that HRT reduces serum ACE activity and increases plasma bradykinin concentration in PMW.

ACE is involved in coronary thrombosis (13), vasoconstriction (14), and smooth muscle cell proliferation (15), and the D/D genotype, which is associated with high ACE levels, has been identified as a novel risk factor for myocardial infarction (16), cardiomyopathy (17), and left ventricular hypertrophy (18), while one report demonstrated that there is no association between the ACE gene and left ventricular hypertrophy in essential hypertension occurring in the Chinese population (19). Moreover, systolic blood pressure after hospitalization is higher in normotensive subjects who possesses the D allele of ACE polymorphism (20). After administration of antihypertensive drugs, the % reduction in mean systolic blood pressure in hypertensive patients with a deletion homozygote of the ACE gene is lower than that in patients with an I allele of the ACE gene (21). Thus, elevated serum ACE activity and the deletion polymorphism of the ACE gene may be independently associated with increased risk of cardiovascular disease.

Many studies (3–7, 22) have reported that HRT reduces ACE activity in PMW and that estrogen downregulates ACE mRNA in rat tissues, suggesting that the reduction in the activity of serum and tissue ACE may represent beneficial effects of HRT on cardiovascular disease in women. In rat tissues, estrogen downregulates ACE mRNA concentrations (22). Estrogens regulate cellular processes through specific hormone receptor-mediated mechanisms (23). The estrogen-receptor complex regulates transcription of a variety of genes by binding to estrogen response elements and affecting RNA polymerase activity. Such directly activated genes are referred to as primary response genes. The estrogen-receptor complex also mediates gene transcription independent of its hormone response element. By this mechanism, the estrogen-receptor complex interacts directly with transcription factors, forming a multiprotein complex that binds to specific gene response elements (22). No estrogen response element (5’GGTCANNNTGACC-3’) was reported in the 5 flanking region of the ACE coding sequence; however, the ACE promoter does contain a consensus AP1 site in the 300-base pair region upstream from the start site (24–27). Gallagher et al. (22) discuss that the ACE mRNA regulation by estrogen treatment occurs through a receptor-mediated complex interaction with the Fos-Jun heterodimer at an AP1 site with other possible mechanisms. Estrogen also binds to imperfect estrogen response elements, albeit with lower affinity, by forming heterodimers with nonhormone proteins or through other protein–protein interactions. Thus, additional analyses of the regulatory regions of the ACE gene are needed to elucidate the precise molecular mechanism of the regulation of ACE mRNA by estrogen.

From the current studies in vivo we can only speculate as to the mechanisms by which HRT decreases the serum ACE activity. Estrogen stimulates the release of endothelium-derived nitric oxide, which is known to mediate vascular relaxation in response to various endothelium-dependent vasodilators (28, 29) and increases circulating nitrite/nitrate levels (30, 31). Recently, Sanada et al. (32) reported that the change in the serum level of nitrite/nitrate after 3 months of estrogen therapy shows an inverse correlation with the change in the plasma level of ACE activity in PMW. Although we did not measure circulating nitrite/nitrate levels in this study, the estrogen-stimulated nitric oxide production may inhibit ACE activity.

A recent study (33) reported on the association between HRT in PMW and ACE genotype and ACE activity. A significant decrease in plasma ACE activity during 3 months of conjugated equine estrogen therapy was seen in the I/D and I/I genotypes, but not in the D/D genotype, which is inconsistent with our own findings. In the present study, a 3 month course of conjugated equine estrogen combined with medroxyprogesterone acetate did not decrease serum ACE activity in any of the ACE genotypes, but 6 and 12 months of therapy significantly decreased serum ACE activity of PMW with the ACE I/D and D/D genotype but not the I/I genotype. This discrepancy may be due to the differences in route of administration and/or duration of HRT. The mechanism whereby HRT reduces serum ACE activity in PMW with the ACE I/D and D/D genotype, but not the I/I genotype is uncertain, but one possible scenario is as follows. The ACE activity of the PMW with the ACE I/I genotype may have been lower than that in either of the other two groups, and/or the sensitivity of serum ACE activity measurement by a colorimetric method may have been above 1.0 IU/l in this study. Since 3 of 26 subjects had serum ACE levels of below 1.0 IU/l, the sensitivity of serum ACE activity measurement may have been related to the lack of an HRT-induced reduction in serum ACE activity of the PMW with the ACE I/I genotype.

Bradykinin, on the other hand, is a potent vasodilator with a beneficial effect on cardiovascular disease (34–36), reducing both systemic and coronary resistances (34). In addition, bradykinin has a positive inotropic effect (35) and is a cardioprotective agent that may decrease myocardial oxygen consumption and ischemia (36). In this study, HRT reduced the serum ACE activity of PMW with the ACE I/D and D/D genotypes and increased the plasma bradykinin concentrations in all three ACE-genotype groups. The decreases in ACE activity or increases in bradykinin produced by HRT may represent beneficial effects of estrogens on cardiovascular disease.

While the decrease in ACE activity caused by HRT in PMW with the ACE I/D and D/D genotypes may increase their plasma bradykinin levels, it is interesting that HRT increased plasma bradykinin concentrations in PMW with the ACE I/I genotype without decreasing serum ACE activity. Murphey et al. (37) observed no differences in plasma
bradykinin concentrations in forearm venous blood during intrabrachial bradykinin infusion among healthy subjects with the ACE I/I, I/D, or D/D genotype, suggesting that the D allele of ACE is unassociated with plasma bradykinin concentrations, although degradation of bradykinin through the ACE pathway was greatest in the ACE D homozygotes, least in the ACE I homozygotes, and intermediate in the heterozygotes. Moreover, it is possible that local kinin production is affected in a different manner in individuals with the D genotype. However, since the half-life of bradykinin is short (17), the plasma bradykinin levels may not reflect local production of kinins, which are paracrine factors. Thus, the decrease in ACE activity by HRT alone appears incapable of accounting for the increase in bradykinin.

The limitation of the present study in terms of the small number of subjects, especially those having the D/D genotype, should be taken into consideration in assessing these results.

In conclusion, HRT reduced the serum ACE activity and increased the plasma bradykinin concentrations of PMW with the ACE I/D and D/D genotypes. The observation of a genetic polymorphism has clinical implications, particularly for individualized medicine, such as in pretreatment prediction of the efficacy of HRT.

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References


