Comparative Effects of Estrogen and Angiotensin-Converting Enzyme Inhibition on Plasminogen Activator Inhibitor-1 in Healthy Postmenopausal Women

Nancy J. Brown, MD; Amira Abbas, MD; Daniel Byrne, MS; John A. Schoenhard, BS; Douglas E. Vaughan, MD

Background—This study compares the effect of estrogens and ACE inhibition on plasminogen activator inhibitor-1 (PAI-1) concentrations in healthy postmenopausal women, genotyped for a 4G/5G polymorphism in the PAI-1 promoter, a polymorphism shown to influence PAI-1 concentrations.

Methods and Results—Morning estradiol, PAI-1, tissue plasminogen activator, plasma renin activity, angiotensin II, and aldosterone were measured in 19 postmenopausal women (5G/5G:4G/5G:4G4G = 5:10:4, respectively) at baseline and during randomized, single-blind, crossover treatment with conjugated equine estrogens 0.625 mg per os per day, ramipril 10 mg per os per day, and combination estrogens and ramipril. Estradiol (P < 0.005) and angiotensin II (P < 0.01) were significantly higher during estrogens. Plasma renin activity was significantly increased during ACE inhibition (P < 0.05). Both conjugated estrogens [PAI-1 antigen from 12.5 (7.6, 17.4) [mean (95% CI)] baseline to 6.6 (2.6, 10.7) ng/mL, P < 0.01] and ACE inhibition [8.3 (4.9, 11.7) ng/mL, P < 0.005] decreased PAI-1 without decreasing tissue plasminogen activator. The effect of combined therapy on PAI-1 [5.6 (2.3, 8.8) ng/mL] was significantly greater than that of ramipril alone (P < 0.05). There was a significant effect of PAI-1 4G/5G genotype on baseline PAI-1 concentrations (P = 0.001) and a significant interactive effect of 4G/5G genotype and treatment, such that genotype influenced the change in PAI-1 during ramipril (P = 0.011) or combined therapy (P = 0.006) but not during estrogens (P = 0.715).

Conclusions—ACE inhibition with ramipril and conjugated estrogens similarly decrease PAI-1 antigen concentrations in postmenopausal women. Larger studies that use clinical outcomes are needed to determine whether PAI-1 4G/5G genotype should influence the choice of conjugated estrogens or ACE inhibition for the treatment of healthy postmenopausal women. (Circulation. 2002;105:304-309.)

Key Words: hormones ■ angiotensin ■ plasminogen activators

Observational studies suggest that estrogen replacement reduces the risk of coronary heart disease in postmenopausal women, although data from a randomized clinical trial have been inconclusive. Estrogen exerts a number of effects that could favorably affect cardiovascular mortality rates, including raising HDL cholesterol and lowering LDL cholesterol and improving endothelium-dependent vasodilator function through increased nitric oxide bioavailability. In addition, oral estrogen improves fibrinolytic balance by decreasing plasminogen activator inhibitor-1 (PAI-1), the primary inhibitor of tissue-type plasminogen activator (TPA). PAI-1 concentrations are increased in postmenopausal women compared with premenopausal women and may contribute to the increased risk of atherosclerotic events after menopause. Increased PAI-1 expression has been demonstrated in atherosclerotic lesions, and elevated PAI-1 concentrations have been associated with an increased risk of myocardial infarction.

Despite the favorable effects of oral estrogen treatment on fibrinolytic balance and its potential for reducing cardiovascular mortality rates, there are a number of disadvantages to the use of oral estrogens for reduction of cardiovascular risk. First, although estrogens improve fibrinolytic balance, they activate the coagulation pathway. This effect may contribute to the increased risk of venous thromboembolism associated with estrogen use and may account for the early increase in cardiovascular mortality rates observed in the Heart and Estrogen/progestin Study (HERS), a secondary prevention study. In addition, estrogen therapy may increase the risk of breast and endometrial cancer and causes such adverse

Received August 29, 2001; revision received November 7, 2001; accepted November 8, 2001.

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effects as vaginal bleeding and breast tenderness. Although the newer estrogen receptor modulators lack many of these adverse effects, they also do not appear to improve fibrinolytic balance.17,18

One potential cardioprotective alternative to estrogen replacement in postmenopausal women is ACE inhibition. Recent data from the Heart Outcomes Prevention Evaluation (HOPE) study suggest that ACE inhibitors reduce the incidence of cardiovascular events in high-risk patients with normal left ventricular function.19 Although there are numerous mechanisms whereby interruption of the renin-angiotensin-aldosterone system (RAAS) by ACE inhibition could reduce cardiovascular mortality rates, studies indicate that ACE inhibition, like estrogen therapy, decreases PAI-1 concentrations and the molar ratio of PAI-1 to TPA.20,21 Thus, the purpose of the present study was to compare the effect of estrogen replacement and ACE inhibition on PAI-1 in healthy postmenopausal women. A second aim of the study was to test the hypothesis that a 4G/5G polymorphism in the PAI-1 promoter that has been shown to influence PAI-1 concentrations22 modulates the effect of either estrogen or ACE inhibition on fibrinolytic balance.

Methods

Study Population
Healthy, normotensive women were asked to participate in this single-blind, crossover design study. All subjects underwent a complete medical history and physical examination before the investigation. Subjects with significant cardiovascular, renal, endocrine, or pulmonary disease, or who were taking any medication other than acetaminophen, or who were smokers, were excluded. Women were defined as postmenopausal if they had had no menstrual period for at least 1 year or had had a bilateral oophorectomy and if plasma estradiol levels were <50 pg/mL. Women taking hormone replacement therapy (HRT) were required to discontinue therapy and if plasma estradiol levels were <50 pg/mL. Women taking estrogen or estrogen analogues were excluded. Women taking anticoagulant medications other than acetaminophen, or who were smokers, were excluded. Women were defined as postmenopausal if they had had no menstrual cycle for at least 1 year or had had a bilateral oophorectomy and if plasma estradiol levels were <50 pg/mL. Women taking hormone replacement therapy (HRT) were required to discontinue therapy and if plasma estradiol levels were <50 pg/mL. Women taking estrogen or estrogen analogues were excluded. Women taking anticoagulant medications other than acetaminophen, or who were smokers, were excluded.

Study Protocol
After screening and, if appropriate, washout from HRT (visit 1), subjects underwent the first of 6 study days. They reported to the Vanderbilt General Clinical Research Center between 6:30 and 8:30 AM after an overnight fast. A catheter was placed in a forearm vein for blood drawing. Blood pressure (BP) and heart rate were measured. Blood was then drawn through the indwelling catheter after the subject had been supine for 30 minutes for measurement of PAI-1 antigen, TPA antigen, plasma estradiol, plasma renin activity (PRA), angiotensin II (Ang II), and aldosterone. In 15 subjects, blood was drawn for measurement of serum triglycerides. Baseline measurements were repeated 1 week later.

At the end of the second study day, women were randomized to treatment with either 0.625 mg conjugated equine estrogens (CEE) or 10 mg ramipril per day. Ramipril was initiated at a dose of 1.25 mg and titrated to 10 mg per day over 1 week. At the end of 4 weeks, the study day was repeated. Subjects then underwent a 4-week washout period, and baseline hemodynamic measurements and blood drawing were repeated. After this fourth study day, subjects were crossed over to the opposite drug for 4 weeks and the study day was repeated. Finally, subjects were treated with the combination of both CEE and ramipril for 4 weeks, and the study day was repeated.

Laboratory Analyses
Blood samples were collected on ice and centrifuged immediately at 0°C for 20 minutes. All plasma or serum was separated and stored at −70°C until the time of assay, except for aldosterone, estradiol, and renin, which were stored at −20°C. Blood for measurement of PAI-1 and TPA antigen was collected in Vacutainer tubes containing 0.105 mmol/L acidified sodium citrate, and antigen levels were determined with the use of a 2-site ELISA (Biopool AB). Blood for PRA and aldosterone was drawn into chilled tubes containing EDTA. PRA was measured by radioimmunoassay for Ang I formation at pH 7.4 and 37°C. Blood for Ang II was collected in chilled tubes containing a cocktail of protease inhibitors.23 Ang II measurements were made by radioimmunoassay, as previously described.24,25 Aldosterone and estradiol concentrations were measured with the use of a commercially available radioimmunoassay (Diagnostic Product Corp). Serum triglycerides were determined by means of standard enzymatic methods on an automated system (ACE, Schiapparelli Bio Systems).

PAI-1 4G/5G Genotyping
A commercially available process (Qiagen Inc) was used for the extraction of DNA from EDTA-anticoagulated blood. The PAI-1 4G/5G polymorphism was genotyped with a 25-μL mixture containing standard PCR buffer, 10 mmol/L deoxynucleotide triphosphates, 4 μmol/L primers, 0.4U Tag polymerase, and 25 ng genomic DNA and previously published primers, 5’-CACAGAGAGACTCTGGTCTCAGCT-3’ and 5’-CACAGAGGACTCTTGGTCAGT-3’. The upstream primer was modified by substituting a cytosine for adenine at position −681, thereby introducing a BsiRI restriction site in the presence of the 5G allele, as detailed elsewhere.26

Statistical Analysis
Data are expressed as means and 95% confidence intervals in the text and means and SEM in the figures. Because there were no differences in any of the study variables among baseline study days, mean baseline values were used for all analyses. Based on previous studies,20,21 the sample size of 20 was chosen to give 80% power to detect a difference in PAI-1 antigen of 5.3 ng/mL between treatment arms with α=0.05. The effect of therapy on endocrine and fibrinolytic parameters was assessed with the use of a general linear model repeated-measures ANOVA in which the within-subject variable was treatment and the between-subjects variable was PAI-1 4G/5G genotype. Post hoc comparisons between treatment arms were made with a paired t test. Where there was evidence for a treatment by 4G/5G genotype interaction, the effect of increasing number of 4G alleles on the response to a specific treatment was determined with 1-way ANOVA, followed by Bonferroni test for multiple comparisons. A value of P<0.05 was considered statistically significant. All tests were 2-tailed. All statistical analyses were performed with the statistical package SPSS for Windows (Version 10.0, SPSS).

Results
Twenty women participated in the study. One woman’s data were excluded from analysis because she had an intercurrent infection during the study. The mean age of the remaining 19 women was 52.4 (50.2, 54.6) years. The mean body mass index was 27.2 (24.0, 30.3) kg/m². Thirteen women were white, 5 black, and 1 Asian (Indian).
of CEE, ACE inhibition, or the combination of fasting serum triglycerides.

ACE inhibition significantly reduced diastolic blood pressure [from 79.4 (74.7, 84.3) to 75.7 (71.3, 80.1) mm Hg, \( P=0.048 \)] but not systolic blood pressure (\( P=0.531 \)). The effect of ACE inhibition on diastolic blood pressure was similar in the presence and absence of CEE.

**Renin-Angiotensin-Aldosterone Measurements**

ACE inhibition, either alone or in combination with CEE, significantly increased PRA (Table 1). There was no effect of CEE on PRA; CEE did not modify the effect of ACE inhibition on PRA. In contrast, CEE significantly increased plasma Ang II concentrations (Table 1), whereas there was no significant effect of ACE inhibition on Ang II concentrations. Neither CEE nor ACE inhibition significantly altered serum aldosterone (\( P=0.175 \) for treatment effect).

**Fibrinolytic Measurements**

CEE, ACE inhibition, and combination CEE/ACE inhibition all significantly decreased PAI-1 antigen concentrations compared with baseline (Table 1 and Figure 1). The effects of CEE and ACE inhibition on PAI-1 antigen were similar. The effect of combined CEE and ACE inhibition on PAI-1 was greater than that of ACE inhibition alone (\( P=0.039 \)) and tended to be greater than that of CEE alone (\( P=0.064 \)). There was no effect of treatment order on PAI-1 antigen (\( P=0.054 \)). The effect of CEE on the molar ratio of PAI-1 to TPA tended to be greater than that of ACE inhibition (\( P=0.017 \)).

**Effect of PAI-1 4G/5G Polymorphism on Response to Therapy**

The frequency of the 4G/5G genotypes (5G/5G:4G/5G:4G/4G = 5:10:4, respectively) was similar to that previously reported.\(^{22}\) There was no effect of PAI-1 4G/5G genotype on estradiol (\( P=0.455 \)), PRA (\( P=0.073 \)), Ang II (\( P=0.463 \)), aldosterone (\( P=0.779 \)), or TPA antigen (\( P=0.382 \)). There was a significant interactive effect of PAI-1 4G/5G genotype and treatment on both PRA (\( P=0.037 \)) and aldosterone (\( P=0.019 \)) but not estradiol (\( P=0.481 \)), Ang II (\( P=0.910 \)), or TPA antigen (0.086). Thus, during combined therapy there was a significant effect of genotype on PRA [1.5 (−0.1, 3.1), 1.8 (0.5, 3.1), and 9.7 (−1.5, 20.9) ng Ang I/mL per hour in the 5G/5G, 4G/5G, and 4G/4G subjects, respectively, \( P=0.003 \)], although there was no effect of genotype on PRA during CEE (\( P=0.183 \)) or ACE inhibition alone (\( P=0.220 \)). In addition, the change in aldosterone in response to ramipril varied significantly with genotype [95 (−5, 196), −10 (−43,

<table>
<thead>
<tr>
<th>TABLE 1. Effect of Treatment on Renin-Angiotensin-Aldosterone and Fibrinolytic Systems</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
</tr>
<tr>
<td>Estradiol, pg/mL</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
</tr>
<tr>
<td>PRA, ng Ang I/mL per h</td>
</tr>
<tr>
<td>Ang II, pg/mL</td>
</tr>
<tr>
<td>Aldosterone, pmol/L</td>
</tr>
<tr>
<td>PAI-1 antigen, ng/mL</td>
</tr>
<tr>
<td>TPA antigen, ng/mL</td>
</tr>
<tr>
<td>Molar ratio, PAI-1/TPA</td>
</tr>
</tbody>
</table>

\[ *P<0.05, \dagger P<0.01, \ddagger P<0.005 \text{ vs baseline.} \]

\[ \ddagger P<0.05 \text{ vs ramipril.} \]

\[ \ddagger P<0.005 \text{ vs baseline.} \]

\[ \ddagger P<0.005 \text{ vs ramipril.} \]
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Figure 2. Effect of treatment with CEE, ramipril, or combination CEE and ramipril on PAI-1 antigen concentrations as a function of PAI-1 4G/5G genotype. Data are presented as mean±SEM. **P<0.05, ***P<0.005 vs baseline; †P<0.05 vs ramipril; ‡P<0.05 vs combination therapy.

Table 2. Effect of PAI-1 4G/5G Genotype on Fibrinolytic Response to Therapy

<table>
<thead>
<tr>
<th>PAI-1 Genotype</th>
<th>5G/5G</th>
<th>4G/5G</th>
<th>4G/4G</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline PAI-1 antigen, ng/mL</td>
<td>6.1 (0.9, 11.4)</td>
<td>10.4 (4.6, 16.2)</td>
<td>25.7 (11.1, 40.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>Change in PAI-1 antigen, ng/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEE</td>
<td>−4.2 (−7.3, −1.2)</td>
<td>−6.6 (−12.3, −1.0)</td>
<td>−6.0 (−18.9, 6.9)</td>
<td>0.715</td>
</tr>
<tr>
<td>Ramipril</td>
<td>−1.6 (−6.1, 2.9)</td>
<td>−3.7 (−6.8, −0.6)</td>
<td>−8.8 (−9.4, −8.1)*</td>
<td>0.011</td>
</tr>
<tr>
<td>Combination</td>
<td>−1.1 (−4.0, 1.7)</td>
<td>−6.1 (−12.1, 0.0)</td>
<td>−16.4 (−28.1, −4.7)*</td>
<td>0.006</td>
</tr>
<tr>
<td>Baseline TPA antigen, ng/mL</td>
<td>4.5 (3.0, 6.0)</td>
<td>7.7 (5.0, 10.5)</td>
<td>6.4 (4.6, 8.3)</td>
<td>0.360</td>
</tr>
<tr>
<td>Change in TPA antigen, ng/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEE</td>
<td>−0.4 (−2.2, 1.4)</td>
<td>−1.5 (−2.8, −0.1)</td>
<td>1.0 (−0.6, 2.6)</td>
<td>0.235</td>
</tr>
<tr>
<td>Ramipril</td>
<td>0.9 (0.2, 1.5)</td>
<td>−0.7 (−1.9, 0.6)</td>
<td>−0.5 (−1.6, 0.6)</td>
<td>0.155</td>
</tr>
<tr>
<td>Combination</td>
<td>−0.1 (−1.6, 1.3)</td>
<td>−2.5 (−4.8, −0.2)</td>
<td>−0.3 (−2.0, 1.4)</td>
<td>0.938</td>
</tr>
<tr>
<td>Baseline molar ratio, PAI-1/TPA</td>
<td>2.0 (0.4, 3.6)</td>
<td>2.6 (0.9, 4.2)</td>
<td>5.8 (2.5, 9.2)*</td>
<td>0.013</td>
</tr>
<tr>
<td>Change in molar ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEE</td>
<td>−1.4 (−2.9, 0.0)</td>
<td>−1.6 (−3.2, 0.1)</td>
<td>−2.0 (−4.5, 0.6)</td>
<td>0.692</td>
</tr>
<tr>
<td>Ramipril</td>
<td>−0.7 (−2.5, 1.0)</td>
<td>−1.0 (−2.8, 0.8)</td>
<td>−1.6 (−1.9, −1.3)</td>
<td>0.536</td>
</tr>
<tr>
<td>Combination</td>
<td>−0.4 (−2.0, 1.2)</td>
<td>−1.4 (−3.0, 0.2)</td>
<td>−3.9 (−6.7, −1.1)*</td>
<td>0.015</td>
</tr>
</tbody>
</table>

*P<0.05, †P<0.005 vs 5G/5G; ‡P<0.05 vs 4G/5G.

Discussion

Previous studies indicate that treatment of postmenopausal women with oral estrogen therapy favorably affects fibrinolytic balance by lowering PAI-1 antigen without altering TPA antigen.6,7 Similarly, previous studies indicate that ACE inhibition reduces PAI-1 antigen in salt-depleted normotensive subjects,20 in postmenopausal women,27 in patients with hypertension,28 and in patients after myocardial infarction.21 The present study is the first study to compare the effects of oral estrogen therapy and ACE inhibition on fibrinolytic balance in a group of healthy postmenopausal women. The data suggest that CEE and ACE inhibition reduce PAI-1 antigen to a comparable degree.

In the present study, CEE and ACE inhibition exerted opposing effects on the RAAS. As observed in a prior study of hypertensive women,29 CEE significantly increased circulating Ang II concentrations consistent with a stimulatory effect of estrogen on angiotensigen synthesis.30 As reported previously, ACE inhibition significantly increased PRA without altering either Ang II or aldosterone concentrations, consistent with decreased conversion of tissue Ang I to Ang II, a loss of feedback inhibition of renin synthesis by Ang II, and subsequent “escape” of circulating Ang II and aldosterone concentrations.31,32
The finding that both CEE and ACE inhibition decrease PAI-1 even though the drugs exert different effects on the RAAS may indicate that they lower PAI-1 through different mechanisms. Clinical studies indicating that oral but not transdermal HRT reduces PAI-1 suggest that estrogen lowers circulating PAI-1 concentrations primarily by suppressing hepatic PAI-1 production or enhancing hepatic clearance. In vitro, estrogen suppresses PAI-1 expression through an ER β-receptor dependent mechanism (Vaughan DE, unpublished data, 1999).

Whether ACE inhibitors also affect the hepatic clearance of PAI-1 antigen is not known. ACE inhibitors may decrease PAI-1 production by decreasing the formation of Ang II and/or aldosterone. Ang II or its hexapeptide metabolite, Ang IV, and aldosterone increase PAI-1 expression in a variety of cell types.34–38 Ang II stimulates PAI-1 through a serum inducible element localized to a sequence between −47 and −38 bp in the PAI-1 promoter (Vaughan DE, unpublished data, 1998). In the present study, ACE inhibition may have decreased PAI-1 through effects on tissue Ang II, even though circulating Ang II concentrations were not decreased. In addition, it is possible that ACE inhibition decreases PAI-1 through effects of endogenous bradykinin on nitric oxide,39 a potent inhibitor of PAI-1 expression.40 In this regard, estrogen may also lower PAI-1 in part through its effects on nitric oxide production.5

A unique finding of the present study was the observation that PAI-1 4G/5G genotype affected the PAI-1 response to ACE inhibition but not to CEE. As reported in previous studies,22 PAI-1 4G/5G genotype influenced baseline PAI-1 antigen concentrations, such that PAI-1 was highest in those individuals homozygous for the PAI-1 4G allele, lowest in those homozygous for the 5G allele, and intermediate in heterozygotes. The mechanism underlying allelic differences in PAI-1 production has been explained by the observation that both the 4G and 5G alleles bind a transcriptional activator, whereas the 5G allele also binds a transcriptional repressor.41 In the absence of the bound repressor, basal and stimulated PAI-1 transcription is increased. We hypothesize that ACE inhibition influences the transactivation of the PAI-1 promoter through transcriptional mechanisms that are influenced by this polymorphism.

The small number of subjects in the present study make it impossible to exclude an effect of 4G/5G genotype on the PAI-1 response to CEE with certainty. The lack of effect of the 4G/5G genotype contrasts a previously published study by Grancha et al.42 In that study, the authors reported a significantly greater decrease in PAI-1 antigen in response to transdermal estrogen in women with coronary artery disease (CAD) who were either homozygous or heterozygous for the PAI-1 4G allele compared with those who were homozygous for the 5G allele. Estrogen significantly reduced PAI-1 in this study, even though it was administered transdermally, in contrast to several other studies.7,33 Also, the authors observed that PAI-1 4G/5G genotype affected baseline PAI-1 antigen concentrations in women with CAD but not in healthy postmenopausal women; the effect of PAI-1 4G/5G genotype on the fibrinolytic response to transdermal estrogen in healthy women was not reported in this study. On the other hand, the finding that PAI-1 4G/5G genotype influenced the PAI-1 response to ACE inhibition is compatible with a prior study demonstrating that the PAI-1 4G/5G locus modulates the effect of activation of the RAAS by salt depletion on PAI-1 antigen concentrations.26

Observational studies suggest that estrogen use decreases the risk of CAD in postmenopausal women.1,2 The present study confirms that estrogen improves fibrinolytic balance and indicates that ACE inhibition decreases PAI-1 to a similar degree in postmenopausal women. Given that a randomized, prospective study indicates that HRT does not provide the anticipated cardioprotective benefit in women with known CAD,3 we suggest that large clinical trials are needed to assess the effect of ACE inhibition on cardiovascular morbidity and mortality rates in postmenopausal women. Furthermore, these studies should assess the possibility that pharmacogenomic profiling at the PAI-1 4G/5G locus could enhance the therapeutic impact of ACE inhibition.

Acknowledgments

This work was funded by a research grant from Monarch Pharmaceuticals and by National Institutes of Health grants HL-65193, HL-69060, RR-00095, and HL-07411.

References

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Circulation. 2002;105:304-309
doi: 10.1161/hc0302.102570
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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