Insertion-Deletion Polymorphism of the ACE Gene Modulates Reversibility of Endothelial Dysfunction With ACE Inhibition

Abhiram Prasad, MBBS, MRCP; Suresh Narayanan, MBBS; Syed Husain, MBBS; Feroz Padder, MBBS; Myron Waclawiw, PhD; Neil Epstein, MD; Arshed A. Quyyumi, MD, FRCP

Background—The aim of this study was to examine whether angiotensin-converting enzyme (ACE) inhibition improves coronary endothelial dysfunction in patients with atherosclerosis and its risk factors and whether this related to the ACE insertion-deletion (I/D) polymorphism.

Methods and Results—In 56 patients with atherosclerosis or its risk factors, we studied endothelium-dependent responses with acetylcholine and endothelium-independent function with sodium nitroprusside, before and after ACE inhibition with enalaprilat. Enalaprilat did not alter either resting coronary tone or vasodilation with sodium nitroprusside. However, it potentiated the coronary microvascular and epicardial responses with acetylcholine; coronary blood flow increased from 82±7 to 90±8 mL/min (P=0.05) after enalaprilat. Patients with depressed endothelial function (P<0.001) and those with ACE DD or ID genotypes (P=0.002) but not those homozygous for the I allele had the greatest improvement by multivariate analysis. Similarly, acetylcholine-mediated epicardial vasomotion improved in segments that initially constricted (endothelial dysfunction): from −10.1±1% to −1.4±2% (P<0.001) after enalaprilat. No augmentation was observed in segments that dilated (normal endothelial dysfunction) with acetylcholine. Patients with the D allele, hypercholesterolemia, and smokers (all P<0.05) had greater improvement.

Conclusions—Acute ACE inhibition improves coronary epicardial and microvascular endothelium-dependent vasomotion in patients with atherosclerosis or its risk factors who have endothelial dysfunction and presence of the D allele.

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Key Words: atherosclerosis ● genes ● angiotensin ● acetylcholine ● coronary disease

Endothelial cell dysfunction contributes to atherogenesis,1 which appears in part to be due to diminished nitric oxide (NO) bioavailability.2 Thus, strategies that reverse endothelial dysfunction are likely to be of therapeutic value in patients with atherosclerosis. Studies indicate that angiotensin-converting enzyme (ACE) inhibition improves endothelial function.3,4 As an integral component of the circulating and vascular renin-angiotensin and kallikrein-kinin systems, ACE promotes synthesis of angiotensin II and degrades bradykinin; therefore, its inhibition may increase NO activity by reducing angiotensin II–mediated superoxide anion generation and bradykinin-mediated release of NO.

The contribution of genotype to the development of atherosclerosis is evident in the predisposing role of family history, independent of conventional risk factors. An increase in frequency of myocardial infarction in patients with the D allele of the insertion-deletion (I/D) polymorphism in the ACE gene provides 1 genetic predisposing factor.5 The ACE gene is located on chromosome 17, and the polymorphism is characterized by the presence (I) or absence (D) of a 287–base-pair alu repeat within intron 16.6 Because the polymorphism is located in an intron, it is believed to be a neutral marker in strong linkage disequilibrium with 1 or more unknown functional variants located in or close to the ACE gene.7,8 Many studies, including a recent meta-analysis, have reported an association between the DD genotype and increased risks of developing coronary artery disease,9–11 though some have failed to confirm these findings.12,13 Since circulating14 and tissue ACE15 levels are higher in patients with the D allele, we hypothesized that ACE inhibition may result in a greater improvement in endothelial dysfunction in patients with the DD or ID genotypes compared with those with II genotype.

Thus, the aim of this study was to investigate whether in patients with atherosclerosis or its risk factors, acute ACE inhibition (1) improves coronary epicardial and microvascular endothelial function and (2) whether this improvement is related to the ACE I/D polymorphism or to serum ACE levels.

Methods

Study Population
We studied patients undergoing cardiac catheterization for investigation of chest pain or abnormal noninvasive tests. Patients with recent myocardial infarction, valvular heart disease, or those treated with ACE inhibitors in the previous 2 weeks were excluded. All
cardiac medications were withdrawn ≥48 hours before the study, and aspirin or other cyclo-oxygenase inhibitors were discontinued 7 days before. The study was approved by the National Heart, Lung, and Blood Institute Investigational Review Board, and informed consent was obtained from all patients.

Fifty-six patients with atherosclerosis or its risk factors were enrolled into the study; 33 patients had coronary atherosclerosis and 23 had angiographically normal coronary arteries and ≥1 risk factors for atherosclerosis that included presence of hypertension (blood pressure >140/90), hypercholesterolemia (total cholesterol >220 mg/dL), non–insulin-dependent diabetes, current smoking or smoking in the previous year, or age ≥60 years (Table 1).

### Protocol

After diagnostic coronary angiography, a 6F guiding catheter was introduced into the proximal segment of either the left main or the proximal segment of a major epicardial artery, and blood flow velocity was measured with the use of a 0.018-inch wire equipped with a Doppler crystal at its tip (Cardiometrics Flowire, Cardiometrics, Inc). Flow measurements were made in a coronary artery with 0.25- to 0.5-cm segments of mid and distal regions of the epicardial coronary arteries were also measured by quantitative coronary angiography.

### Effect of Enalapril on Responses to Acetylcholine and Sodium Nitroprusside

After a 5-minute infusion of 5% dextrose at 1 mL/min, measurements of coronary blood flow velocity and coronary angiography were performed and repeated after each intervention. Endothelium-dependent vasodilation was estimated by measuring coronary flow and epicardial responses to incremental 2-minute infusions of intracoronary acetylcholine (ACh) starting at 10⁻⁷ mol/L (intracoronary concentration) in patients with atherosclerosis and at 10⁻⁶ mol/L in patients with normal coronary arteries. This was followed by infusions of 10⁻⁶ mol/L ACh in patients with atherosclerosis, so that all patients received the higher dose of ACh.

Ten minutes after ACh measurements were performed, endothelium-independent function was estimated with intracoronary sodium nitroprusside given at 40 μg/min for 3 minutes, and flow reserve was measured with intracoronary adenosine administered at 2.2 mg/min for 2 minutes.

This was followed by a 10-minute intracoronary infusion of the ACE inhibitor enalaprilat (Merck) at 20 μg/min. We have previously demonstrated that this dose of enalaprilat effectively inhibits coronary vascular ACE because bradykinin responses were significantly enhanced. While continuing the infusion of enalaprilat at 20 μg/min, ACh was coinfused at 10⁻⁷ mol/L. Twenty-eight patients had repeat infusions of 40 μg/min sodium nitroprusside for 3 minutes.

### Measurement of Coronary Blood Flow and Diameter

As previously described, coronary blood flow was derived from the coronary blood flow velocity and diameter measurements by use of the formula \( \pi \times \text{average peak velocity} \times 0.125 \times \text{diameter} \). Coronary vascular resistance was calculated as mean arterial pressure divided by blood flow.

In addition to the measurement of the diameter at the level of the Doppler flow wire, 0.25- to 0.5-cm segments of mid and distal regions of the epicardial coronary arteries were also measured by quantitative coronary angiography.

### Determination of ACE Genotype

ACE genotyping was performed by laboratory personnel who had no knowledge of the endothelial function data. ACE genotypes were determined by use of polymerase chain reaction, according to previously published protocols. In brief, a set of primers was designed to encompass the polymorphic region in intron 16 of the ACE gene (sense primer 5' CTGGAGACCACTCCATCCITTTCT 3' and antisense primer 5' GATGTGGCCATACATCTGCAGAT 3'). DNA was amplified for 35 cycles, each cycle composed of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 30 seconds. The products were separated by electrophoresis on 2% agarose gel and identified by ethidium bromide staining. To verify the DD allele, each sample found to be DD was reamplified with the primers hace5a and hace5c, which recognize the inserted sequence, as previously described. This amplification detected the presence of the I allele and produced no product from the DNA with DD genotype.

### Statistical Analysis

Data are expressed as mean±SEM. Differences between means were compared by paired or unpaired Student’s t test, as appropriate. All probability values were 2-tailed, and a value <0.05 was considered statistically significant. Univariate correlations were performed by use of Pearson’s correlation coefficient. Multiple stepwise regression analysis was performed to test whether the magnitude of change with enalaprilat of ACh-mediated vasodilation, measured as the decrease in coronary vascular resistance with ACh, was related to the age, sex, presence of atherosclerosis, hypertension, diabetes, cigarette use, total cholesterol, HDL, serum ACE levels, plasma renin activity, to the initial vasodilator response with ACh, or the ACE ID genotype. This was also performed for the changes in the epicardial diameter with ACh after ACE inhibition.

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### TABLE 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>All</th>
<th>DD</th>
<th>ID</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>56</td>
<td>11</td>
<td>32</td>
<td>13</td>
</tr>
<tr>
<td>ACE level, U/L</td>
<td>9.9±0.6</td>
<td>12.8±1.2</td>
<td>9.8±0.8</td>
<td>7.5±0.8*</td>
</tr>
<tr>
<td>Age, y</td>
<td>52±1</td>
<td>55±3</td>
<td>51±2</td>
<td>54±3</td>
</tr>
<tr>
<td>Whites</td>
<td>45 (80.3)</td>
<td>9 (82%)</td>
<td>26 (81%)</td>
<td>10 (77%)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>14 (25%)</td>
<td>1 (9%)</td>
<td>9 (28%)</td>
<td>4 (31%)</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>227±6</td>
<td>210±11</td>
<td>240±9</td>
<td>210±7</td>
</tr>
<tr>
<td>HDL, mg/dL</td>
<td>40±2</td>
<td>35±4</td>
<td>43±3</td>
<td>38±3</td>
</tr>
<tr>
<td>Hypertension</td>
<td>30 (54%)</td>
<td>7 (64%)</td>
<td>18 (56%)</td>
<td>5 (38%)</td>
</tr>
<tr>
<td>Type II diabetes</td>
<td>7 (13%)</td>
<td>2 (18%)</td>
<td>5 (16%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Plasma renin activity, ng·mL⁻¹·h⁻¹</td>
<td>1.2±0.2</td>
<td>0.9±0.1</td>
<td>1.6±0.3</td>
<td>0.8±0.2</td>
</tr>
</tbody>
</table>

Data shown as mean±SEM. *P<0.001 vs DD.
Results
Coronary Vascular Response to Enalaprilat
After 10 minutes of enalaprilat infusion, there was no change in coronary epicardial diameter, blood flow, or vascular resistance, suggesting that acute ACE inhibition does not influence resting epicardial or microvascular tone (Table 2). Heart rate and mean arterial blood pressure also remained unchanged.

Effect of Enalaprilat on Response to ACh
After enalaprilat, ACh-induced vasodilation was enhanced. Thus, compared with baseline, enalaprilat increased flow from $102 \pm 6\%$ to $127 \pm 6\%$ ($P=0.008$) and reduced coronary vascular resistance, compared with baseline, from $42 \pm 3\%$ to $49 \pm 3\%$ ($P=0.002$, Figure 1).

The potentiation of ACh-mediated vasodilation with enalaprilat correlated with the baseline vasodilator response to ACh ($r=0.44$, $P=0.006$), indicating that patients with a depressed response to ACh had greater improvement with enalaprilat and vice versa. ACE inhibition also improved ACh-mediated coronary epicardial vasomotion; the baseline $2.0 \pm 0.8\%$ change was improved to a $1.6 \pm 1.1\%$ ($P=0.034$) dilation after enalaprilat. As previously reported, coronary epicardial changes with ACh were heterogeneous.17 Because of this variability, we divided epicardial segments into those that constricted with ACh (n=75, endothelial dysfunction) and the remaining that dilated (n=71, normal endothelial function) for studying the response of enalaprilat. Improvement in epicardial coronary artery vasomotion was only observed in segments with endothelial dysfunction ($-9.1 \pm 1\%$ to $-2.5 \pm 1.4\%$, $P<0.001$), whereas segments that dilated with ACh had no change with enalaprilat ($7.9 \pm 0.9\%$ to $6.5 \pm 1.5\%$, $P=0.3$, Figure 1).

Effect of Enalaprilat on Response to Sodium Nitroprusside
Microvascular ($139 \pm 14\%$ increase in coronary blood flow) and epicardial ($19 \pm 2\%$) dilation with sodium nitroprusside remained unchanged after enalaprilat ($158 \pm 15\%$, $P=NS$, and $19 \pm 1\%$, $P=NS$, respectively, Figure 2).

ACE Genotype and Coronary Vascular Responses to Enalaprilat
The frequencies of the DD, ID, and II genotypes (0.20, 0.57, and 0.23, respectively) did not deviate significantly from those predicted by the Hardy-Weinberg equilibrium (0.23, 0.5, 0.27; $\chi^2=1.49$, $P=0.1$). The distribution of risk factors for atherosclerosis, plasma renin activity, and baseline hemo-

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**TABLE 2. Coronary and Systemic Hemodynamic Responses to Enalaprilat**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline</th>
<th>Enalaprilat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean blood pressure, mm Hg</td>
<td>112±2</td>
<td>111±2</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>77±2</td>
<td>78±2</td>
</tr>
<tr>
<td>Epicardial diameter, mm</td>
<td>2.0±0.05</td>
<td>2.0±0.05</td>
</tr>
<tr>
<td>Coronary blood flow, mL/min</td>
<td>42±3</td>
<td>42±3</td>
</tr>
<tr>
<td>Coronary vascular resistance, mm Hg · mL⁻¹ · min</td>
<td>3.6±0.3</td>
<td>3.5±0.4</td>
</tr>
</tbody>
</table>

There were no significant differences between baseline and postenalaprilat values.
Dynamic measurements were similar between the 3 groups of patients. As expected, plasma ACE levels were higher in the DD group compared with the II group (Table 1). Microvascular dilation with ACh was not significantly different among the genotype subgroups (blood flow increased by 68±15%, 107±16%, and 117±23% in DD, ID, and II genotypes, respectively, P = 0.18, ANOVA, Figure 3). Similarly, the mean diameter change with ACh did not vary in patients with different genotypes (DD −0.7±1.8%, ID −1±1.3%, and II −0.5±2.2%; P = 0.97, ANOVA).

The magnitude of enalaprilat-induced improvement in ACh-mediated microvascular dilation, however, correlated with the ACE I/D genotype (r = 0.43, P = 0.001). Significant improvement was only observed in patients with the DD and ID genotypes, who had a 20±10% (P = 0.01) and 18±8% (P < 0.01) further increase in coronary blood flow with enalaprilat, respectively, compared with a nonsignificant 2±8% (P = 0.8) decrease in flow in those homozygous for the I allele (Figure 3).

Because enalaprilat primarily improved epicardial reactivity only in segments that constricted with ACh, the effect of the ACE I/D genotype was examined in these segments. As in the microcirculation, enalaprilat produced significant improvement only in patients with the D allele in whom ACh-induced constriction decreased from −9.4±1.4% to −2.1±1.7% (P < 0.001, Figure 4). In contrast, the change from −8.5±2.0% to −3.5±2.3% (P = 0.1) in patients with the II genotype was insignificant (Figure 4).

Serum ACE Levels and Coronary Vascular Responses to Enalaprilat

Because serum ACE levels correlated with the ACE I/D genotype (r = 0.35, P = 0.005, Table 1), we investigated the relation between plasma ACE levels and the effect of enalaprilat on ACh-mediated vasodilation. No significant correlation was observed between serum ACE levels and either the microvascular or epicardial coronary arterial potentiation of the ACE response with enalaprilat. We divided patients into 2 groups, based on the median value of 9.9 ± 0.6 U/L (Figure 5). Mean ACE levels in the 2 groups were 14.3 ± 0.6 U/L (n = 23) and 6.8 ± 0.3 U/L (n = 33), respectively. Baseline vasodilator responses to ACh were similar between the groups (P = 0.25). After enalaprilat, ACh-mediated microvascular dilation was enhanced in patients with high ACE levels (P = 0.004) but not in those with low ACE levels (P = 0.1, Figure 5). In contrast to the microcirculation, enalaprilat improved ACh-mediated epicardial vessel constriction in patients with high (4±2% net gain, P = 0.004) and low ACE levels (4±2% gain, P = 0.005).

There was no association between plasma renin activity and either the baseline endothelial function or its improvement with enalaprilat.

Multivariate Analysis

The only statistically significant independent predictors of improvement in ACh-mediated microvascular dilation with
enalaprilat were the baseline response to ACh ($P<0.001$) and the presence of the $D$ allele ($P=0.002$). Thus, patients with $DD$ and $ID$ genotypes and those with an initially depressed response to ACh had greater improvement in microvascular dilation with enalaprilat. The interaction between the $ACE$ I/D polymorphism and baseline microvascular endothelial function is illustrated in Figure 6.

Similar multivariate analysis for epicardial segments revealed that the only significant independent predictors of improvement in ACh-mediated epicardial coronary constriction with enalaprilat were the presence of $D$ allele ($P=0.009$), serum cholesterol level ($P=0.002$), and cigarette smoking ($P=0.015$). Thus, constricting segments from patients with $DD$ or $ID$ genotypes, those with elevated cholesterol levels, and those with history of smoking were likely to improve most with enalaprilat.

**Discussion**

Our hypothesis that ACE inhibitors reverse endothelial dysfunction in atherosclerotic human coronary arteries was supported by the findings of this study. Acute ACE inhibition selectively improved endothelium-dependent vasomotion in patients with coronary atherosclerosis and its risk factors, demonstrated by the potentiation of ACh-mediated responses of coronary epicardial arteries and microvessels. We also showed that the $D$ allele in the $ACE$ gene predicted, independent of conventional risk factors and plasma ACE levels, the improvement in conductance and microvessel endothelial dysfunction with ACE inhibition.

We have previously reported that the depression in epicardial and microvascular dilation in response to ACh in patients with risk factors for atherosclerosis is associated with reduced basal and stimulated NO activity. We revealed that acute ACE inhibition reverses coronary endothelial dysfunction, an effect that we have previously demonstrated to be due to improvement in vascular NO bioavailability. This beneficial action was greatest in patients with endothelial dysfunction; a depressed response to ACh in the microcirculation independently correlated with the magnitude of improvement of the ACh response with ACE inhibition. Similarly, segments with ACh-mediated constriction, considered to be a hallmark of endothelial dysfunction in the human epicardial circulation, improved with enalaprilat, and this improvement was greater in smokers and patients with hypercholesterolemia. In contrast, epicardial segments without endothelial dysfunction remained unchanged, indicating, as in the coronary microvasculature, that the action of ACE inhibitors is greatest in areas of endothelial dysfunction. Moreover, there was no change in resting coronary vascular tone or enhancement of endothelium-independent function with sodium nitroprusside after ACE inhibition, indicating that the benefit of enalaprilat was specifically due to its action on the vascular endothelium.

Our data are consistent with previous reports in patients with heart failure and diabetes in whom acute ACE inhibition also improved the forearm vascular responses to ACh and flow-mediated brachial artery vasodilation. Furthermore, our findings indicate that long-term therapy, as in the trial on reversing endothelial dysfunction (TREND) study, is not essential to improve impaired endothelial function and that the effects of ACE inhibitors are at least partly due to local effects on the coronary arteries and not due to other neurohormonal changes that may occur with long-term therapy. Our investigation extends findings obtained in the epicardial circulation in previous studies to the coronary microcirculation and illustrates that the magnitude of improvement relates to both the genotype and baseline endothelial function.
Mechanism of Action of ACE Inhibitors

Experimental studies suggest that ACE inhibitors improve endothelial function by promoting release of NO, prostaglandins, or endothelium-derived hyperpolarizing factor. We have recently confirmed that in humans the improvement in endothelial function with ACE inhibition is also at least partly mediated through increased NO activity.

ACE inhibition may improve vascular NO activity by a reduction in angiotensin II–dependent vascular superoxide anion generation through the vascular NADH/NADPH oxidase system. Angiotensin II infusions produce endothelial dysfunction that can be restored toward normal with either superoxide dismutase or the receptor antagonist losartan. In a recent clinical study, we also demonstrated improvement in peripheral vascular endothelial dysfunction in patients with atherosclerosis after angiotensin AT-1 receptor blockade with losartan.

Another potential mechanism whereby ACE inhibitors improve endothelial NO bioactivity is through an interaction with bradykinin. ACE, also known as kininase II, promotes degradation of bradykinin. Endogenous bradykinin appears to regulate resting coronary dilator tone and contributes to flow-mediated vasodilation of coronary and radial arteries in humans, suggesting that resting and stimulated increase in NO bioavailability may be due to local activity of bradykinin. Kinins vasodilate by releasing NO, prostanoids, and possibly endothelium-derived hyperpolarizing factor from the endothelium. Enhancement of bradykinin activity with ACE inhibition appears to be partially due to increased local levels but also due to augmentation of bradykinin binding to the receptor, reduction in B2 receptor desensitization, and internalization. Finally, we have demonstrated that enalaprilat, in the doses used in this study, improves bradykinin responses in the human coronary vasculature. Moreover, this is due to increased bioavailability of NO and leads to improved flow-mediated coronary vasomotion. Thus, it is likely that the improvement in endothelial dysfunction observed in patients with atherosclerosis is due in part to promotion of local bradykinin activity by ACE inhibition.

Mechanisms Underlying Improvement of Endothelial Function in Patients With ACE D Allele

Baseline vascular responses to ACh were similar in the 3 genotype groups, indicating that the presence of the D allele does not determine muscarinic receptor–stimulated endothelial function, a finding that we confirmed in a larger cohort and that has been previously demonstrated in normal subjects with the use of flow-mediated brachial artery dilation.

Plasma ACE levels lack association with environmental factors and are in part genetically determined, such that the ACE I/D polymorphism accounts for ~50% of interindividual variability. Like plasma levels, tissue ACE concentrations appear to be under genetic control. Left ventricular ACE activity is higher in individuals with the D allele, and conversion of angiotensin II from angiotensin I is greater in patients with the DD genotype, indicating higher vascular tissue ACE activity in the presence of the D allele. Furthermore, upregulation of vascular ACE activity occurs in atherosclerosis. Thus, over the long term, this increased tissue ACE activity may augment the deleterious effects of converting enzyme–induced angiotensin II production and bradykinin breakdown, and its inhibition would be expected to result in proportionately greater benefit in individuals with the D allele. This hypothesis is supported by our findings in which patients with the D allele and higher ACE levels had greater improvement in endothelial dysfunction after treatment with enalaprilat. If these observations are confirmed in prospective trials, it would serve to illustrate the importance of pharmacoepidemiology in cardiovascular diseases.

Implications

In experimental models of atherosclerosis, ACE inhibitors appear to have a protective effect. These findings, together with our observations, suggest that the improvement in NO activity by ACE inhibitors may be antatherogenic in the long term. The impact of the ACE I/D polymorphism on the response to ACE inhibitors warrants further investigation that may be derived from ongoing trials currently in progress to evaluate the long-term effects of converting enzyme inhibition on progression of atherosclerosis and on clinical outcome.

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References


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