Perindopril Alters Vascular Angiotensin-Converting Enzyme, AT₁ Receptor, and Nitric Oxide Synthase Expression in Patients With Coronary Heart Disease

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Abstract—Angiotensin-converting enzyme inhibitors (ACEi) reduce cardiovascular morbidity and mortality by improving coronary perfusion, reducing ventricular hypertrophy and remodeling, and preventing progression of coronary atherosclerosis. However, the cellular mechanisms underlying the beneficial effects of ACEi are not fully understood. We studied the in vivo effects of ACE inhibition with perindopril on cellular expression of ACE, AT₁ receptors and 2 nitric oxide synthase (NOS) isoforms, endothelial (eNOS) and inducible NOS (iNOS), in human blood vessels using quantitative in vitro autoradiography and immunocytochemistry. Seven patients with ischemic heart disease were treated with perindopril (4 mg/d) for up to 5 weeks before elective coronary bypass surgery, whereas controls did not receive the ACEi (n = 7). Perindopril decreased plasma ACE by 70% and the plasma angiotensin II to angiotensin I ratio by 57% and reduced vascular ACE to approximately 65% of control levels in both endothelium and adventitia. By contrast, AT₁ receptor binding in vascular smooth muscle cells was increased by 80% in patients treated with perindopril as confirmed by immunocytochemistry. eNOS was expressed primarily in endothelial cells, whereas little iNOS expression occurred in vascular smooth muscle cells of untreated patients. Both eNOS and iNOS expression seemed to increase during perindopril treatment. These results suggest that suppression of angiotensin II formation in the vascular wall and increased expression of eNOS and iNOS during ACE inhibition may be beneficial in reversing endothelial dysfunction in patients with cardiovascular disease. Because vascular AT₁ receptor expression is increased during chronic ACE inhibition, more clinical studies are required to determine whether it is necessary to combine ACE inhibitors and AT₁ receptor antagonists in clinical management of heart failure, coronary heart disease, and hypertension (Hypertension. 2002;39[part 2]:634-638.)

Key Words: angiotensin-converting enzyme ■ angiotensin II ■ atherosclerosis ■ coronary artery disease ■ ACE inhibitor ■ nitric oxide synthase

Numerous human studies and clinical trials have shown that angiotensin-converting enzyme inhibitors (ACEi) have multiple beneficial effects in treating hypertension and chronic heart failure.¹⁻³ Long-term ACEi treatment significantly reduces cardiovascular morbidity and mortality by improving coronary perfusion and reversing cardiac hypertrophy and ventricular remodeling. Interest in using ACEi to treat or prevent coronary atherosclerotic disease has grown recently after the publication of the Heart Outcomes Prevention Evaluation (HOPE) studies.⁴ This landmark study showed that an ACEi, ramipril, significantly lowered the risk of cardiovascular death, myocardial infarction, or stroke in a broad spectrum of high-risk patients by reducing the need for revascularization and the occurrence of heart failure or diabetic complications.⁴ Thus, tissue ACE, a key enzyme in the formation of the vasoactive peptide angiotensin II (Ang II), may be involved in the pathogenesis of ischemic heart disease and myocardial infarction, making it an important therapeutic target in preventing and treating hypertension, coronary atherosclerosis, and ischemic heart disease.

Although ACE inhibition markedly improves both primary and secondary clinical outcomes in high-risk patients with ischemic heart disease,¹⁻² the cellular mechanisms involved are not fully understood. One of the most plausible mechanisms is inhibition of Ang II formation in the circulation and target tissues. A deletion polymorphism in the ACE gene (DD) has been linked to increased circulating and tissue levels of ACE,⁵ and indeed ACE activity is markedly increased in coronary arteries of patients with acute coronary syndrome.⁶ High levels of circulating and tissue ACE may lead to increased tissue Ang II formation in the vascular wall, which induces vascular injury and subsequent hypertrophy and stenosis. Beneficial effects of ACEi may also be associated with increased bioavailability of nitric oxide (NO) in
high-risk patients with ischemic heart disease. However, there is evidence that the benefits of ACEis are attenuated during prolonged ACEi treatment. The present study was designed to test the hypothesis that chronic ACEi treatment blocks vascular endothelial and adventitial ACE, which is associated with increased expression of endothelial NO synthase (eNOS) and inducible NOS (iNOS) and upregulation of \( \text{AT}_1 \) receptors in the vascular wall in patients with ischemic heart disease. The effects of perindopril on vascular expression of ACE, \( \text{AT}_1 \) receptors, eNOS, and iNOS were evaluated using complementary quantitative in vitro autoradiography and immunohistochemistry.

**Methods**

**Patients**

Fourteen patients with documented ischemic heart disease (single- or triple-vessel disease) were scheduled for elective coronary bypass surgery as described previously. Informed consent was obtained from each patient, and the protocol was approved by the Human Ethics Committee of the Austin and Repatriation Medical Center. There were 10 men and 4 women from 40 to 78 years of age. The patients were randomly divided into 2 groups; half of patients received perindopril (Coversyl) 4 mg/d for up to 35 days (18±5 days) \((n=7)\), and the other half served as controls \((n=7)\). At this dose, perindopril has been shown effectively to inhibit circulating and tissue ACE in humans. ECG, blood pressure, biochemical, and hematological evaluations were carried out, and blood was collected 24 hours before surgery to measure plasma renin activity, plasma ACE, Ang I, and Ang II as described previously. Patients with severe heart failure, unstable myocardial infarction or ECG features, abnormal serum potassium levels \((<3.5 \text{ mmol/L or } >5 \text{ mmol/L})\), or moderate to severe renal failure \((\text{serum creatinine levels } >0.18 \text{ mmol/L})\) were excluded from the study.

**Tissue Preparation**

A segment of the internal mammary artery was removed from each patient and used to bypass the occluded coronary arteries. Both ends of each vessel trimmed out during the surgical procedures were collected for studies of vascular ACE, \( \text{AT}_1 \) receptors, and NOS expression. The vessels were snap-frozen in isopentane on dry ice at −40°C and stored at −80°C. Frozen sections, 10- to 20-μm thick, were cut on a cryostat at −80°C in sealed containers with silica gel for autoradiography and immunohistochemistry as described previously.

**Autoradiographic Measurement of Vascular ACE and \( \text{AT}_1 \) Receptors**

ACE was mapped by \( ^{125}\text{I} \)-labeled 351A, a tyrosyl derivative of the ACEi lisinopril (Merck). The binding properties of this radioligand and its validity for quantitative in vitro autoradiography of tissue ACE have been established. Nonspecific binding was determined in parallel incubations in the presence of 1 mmol/L EDTA. Vascular Ang II receptors were labeled by \( ^{125}\text{I} \)-[Sar\(^{\text{III}}, \text{Ile}^8\] Ang II, and \( \text{AT}_2 \) receptors were discriminated in the presence of 10 μmol/L of an \( \text{AT}_2 \) (PD 123319, a gift of Park-Davis) or an \( \text{AT}_1 \) receptor (losartan, a gift of Dupont) antagonist. Nonspecific binding was determined in the presence of 1 mmol/L unlabeled Ang II (Hypertensin, Ciba). ACE in the vascular endothelium and adventitia and \( \text{AT}_2 \) or \( \text{AT}_1 \) receptors in the medial smooth muscle layer of each section were quantified by a microcomputerized imaging device run on an IBM AT computer with a high-resolution charge-coupled device camera (Imaging Research Institute).

**Immunohistochemistry for ACE, \( \text{AT}_1 \) Receptors, eNOS, and iNOS**

Vascular ACE was localized by immunocytochemistry using a polyclonal antibody generated against a 25-amino acid peptide located near the COOH terminus (a gift from Professor Kunio Hiwada, Ehime University School of Medicine, Ehime, Japan), whose potency and specificity for human renal ACE have been established. AT\(_1\) receptors were localized using a polyclonal antibody generated against the amino acid sequence corresponding to residues 15 to 24 of the human AT\(_1\) receptor, Ac-QDPCPKARGH-\( \text{NH}_2 \), a hydrophilic portion from the amino-terminal extracellular domain coupled to an additional COOH-terminal cysteine (a gift from Professor Tosio Ogihara, Osaka University School of Medicine, Suita, Japan), which is also specific for the human AT\(_1\) receptor. Two isoforms of NOS were labeled by a polyclonal anti-iNOS antibody and an anti-eNOS antibody, respectively (Transduction Laboratory Ltd.). Immunohistochemical localization of ACE, \( \text{AT}_1 \) receptors, eNOS, and iNOS protein was performed using an avidin-biotin complex method. For negative controls, non-immune rabbit serum (DAKO) was used on adjacent sections instead of the primary antibodies.

**Statistical Analysis**

All data are expressed as mean±SEM. Parameters were compared between perindopril-treated and untreated patients using an unpaired Student’s \( t \)-test. A \( P \) value of less than 0.05 was considered significant.

**Results**

**Effects of Chronic Perindopril Treatment on Circulating Angiotensins**

The effects of chronic ACE inhibition with perindopril on the components of the circulating renin-angiotensin system were similar to our previous data. Perindopril reduced plasma ACE activity by approximately 70% \((P<0.01)\) and the plasma Ang II to Ang I ratio, an indicator of ACE activity, by 57% \((P<0.01; \text{Figure } 1)\).
Effects of Chronic Perindopril Treatment on Vascular Endothelial and Adventitial ACE

The effects of chronic perindopril treatment on endothelial and adventitial ACE are illustrated in Figures 2A and 2B. Endothelial ACE was reduced to 65% of untreated patients, whereas adventitial ACE was decreased by perindopril to a similar extent (65%) (Figures 2C and 2D). As expected, ACE immunostaining was primarily localized to the endothelium and adventitia, but it was more intense in untreated patients (Figures 2E and 2F).

Effects of Chronic Perindopril Treatment on Vascular AT\textsubscript{1} and AT\textsubscript{2} Receptors

Autoradiography shows that AT\textsubscript{1} receptor binding is located predominantly in the medial smooth muscle layer (Figures 3A and 3B). In contrast to reduced ACE binding, chronic perindopril treatment was associated with an 80% increase in vascular AT\textsubscript{1} receptor binding (Figure 3C), whereas AT\textsubscript{2} receptor binding remained unaltered (not shown). AT\textsubscript{1} receptor immunostaining was stronger in vascular smooth muscle cells of perindopril-treated patients (Figures 3D and 3E).

Effects of Chronic Perindopril Treatment on Vascular eNOS and iNOS Expression

In untreated patients, low levels of eNOS expression occurred mainly in the endothelium, whereas iNOS expression was negligible in the vascular wall, including vascular smooth muscle cells (Figure 4). In perindopril-treated patients, eNOS expression was increased in the endothelium and iNOS expression in vascular smooth muscle cells (Figure 4).

Discussion

The present study demonstrated that perindopril treatment for more than 2 weeks effectively inhibits both circulating and tissue ACE to a similar extent in the vascular endothelium and adventitia in patients with ischemic heart disease. ACE inhibition with perindopril maintained or increased eNOS expression in the endothelium and increased iNOS expression while at the same time upregulating AT\textsubscript{1} receptor expression in vascular smooth muscle cells. This suggests that inhibition of circulating and tissue Ang II formation in the vascular endothelium and adventitia and increased bioavailability of NO through enhanced eNOS and iNOS expression are two
major mechanisms of action of ACEi in treating essential hypertension, chronic heart failure, and ischemic heart disease. Furthermore, because chronic ACE inhibition also markedly increases AT1 receptor expression in blood vessels, our results indicate that more clinical trials are necessary to determine any additional effects of combined therapy with ACE inhibitors and AT1 receptor antagonists in treating chronic heart failure, coronary heart disease, and hypertension.

It should be emphasized that the present study was not designed to assess the effects of long-term ACE inhibition with perindopril on primary and secondary clinical outcomes in patients with ischemic heart disease but rather (1) whether perindopril administration for more than 2 weeks effectively inhibits ACE in different vascular sites, specifically the endothelium, medial smooth muscle cells, and adventitia; and (2) whether ACE inhibition increases eNOS and iNOS expression in human blood vessels. Effectiveness of ACE inhibition was demonstrated by the 70% inhibition of circulating ACE activity and 57% decrease in the plasma Ang II:Ang I ratio in patients with perindopril treatment. These results show that perindopril has a very high affinity for tissue ACE and effectively inhibits not only endothelial but also adventitial ACE. Although there is a general consensus that inhibition of endothelial ACE is important for the benefits of any ACEi, inhibition of adventitial ACE may be just as important especially in clinical management of hypertension, heart failure, and/or ischemic heart disease or coronary atherosclerosis, because these disorders are associated with vascular hypertrophy, left ventricular remodeling, and atherosclerotic plaque formation. A recent report indicated that coronary artery ACE activity was approximately 5-fold higher in patients with acute coronary syndrome even though serum ACE activity was not different, suggesting that inhibition of vascular and/or cardiac ACE is very important for the clinical benefits of ACEi.

In addition to ACE inhibition and therefore blockade of systemic and tissue Ang II formation, clinical benefits of chronic ACE inhibition may be in part due to increased vascular or tissue NO synthesis either directly or indirectly as a result of elevated bradykinin levels, which in turn increases bioavailability of NO. Although we were unable to measure NO production or expression of eNOS and iNOS mRNA in perindopril-treated and untreated vessels because of the small number of samples, we were able to detect and/or compare eNOS and iNOS between the 2 groups of patients because both enzymes exhibited stronger immunostaining in patients treated with perindopril. eNOS was primarily increased in luminal endothelial cells, whereas iNOS was increased mainly in medial vascular smooth muscle cells. These results may serve as indirect evidence that perindopril treatment increases bioavailability of NO, which then plays an important role in restoring endothelial dysfunction in patients with hypertensive and cardiovascular disorders. However, it has been suggested that iNOS induced locally in macrophages and smooth muscle cells by cytokines or during the inflammatory processes including Ang II may produce a large quantity of NO at sites of its expression; this NO can have an adverse effect on production of free-radical superoxide, which is cytotoxic and proatherosclerotic.

It has been suggested that prolonged ACEi treatment may induce partial escape of ACE inhibition, which affects its beneficial antihypertensive and cardiovascular effects. One mechanism for this partial escape may be upregulation of vascular AT1 receptors. Chronic ACE inhibition has been shown to increase AT1 receptors in vascular smooth muscle cells or renal cells in animal studies, but there are few reports showing similar upregulation of AT1 receptors during chronic ACE inhibition in humans. In the present study, ACE inhibition with perindopril was associated with an 80% increase in AT1 receptors in medial vascular smooth muscle cells, although this may be partly due to the feedback response to suppression of circulating and tissue Ang II formation, as low levels of Ang II may stimulate AT1 receptor expression. These observations are highly relevant to current debates on whether ACE inhibitors, AT1 receptor antagonists, or combined therapy may provide greater clinical benefits in treating hypertension, chronic heart failure, or ischemic heart disease.

Indeed, both ACEi and AT1 receptor antagonists have been shown to be more effective than the other in treating hypertension and chronic heart failure or in reducing left ventricular hypertrophy and remodeling. By contrast, other studies report that ACE inhibition and AT1 receptor antagonist provide similar improvement of endothelial dysfunction. Our results suggest that ACE inhibition may offer greater benefits, because ACEi lower tissue Ang II levels and increase tissue bradykinin levels, which causes NO to increase, whereas AT1 receptor antagonists may only block the AT1 receptor. However, whether it is more beneficial to use both ACEi and AT1 receptor antagonists than either inhibitor alone still awaits the outcome of several ongoing clinical trials.
In summary, we have demonstrated that ACE inhibition with perindopril for more than 2 weeks suppressed both circulating and tissue ACE in the endothelium and adventitia of blood vessels and increased vascular eNOS and iNOS expression in patients with ischemic heart disease. These effects of ACE inhibition may play an important role in restoring endothelial dysfunction in patients with ischemic heart disease. However, as ACE inhibition is also associated with increased vascular AT1 receptor expression, our results suggest that more animal studies and clinical trials are therefore required to address further this important issue.

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References


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