Modulating Atherosclerosis through Inhibition or Blockade of Angiotensin

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Summary: Angiotensin-converting enzyme (ACE) inhibitors are well recognized for their benefits in treating hypertension and congestive heart failure and preventing postmyocardial infarction heart failure or left ventricular (LV) dysfunction. Recently, blockade of the angiotensin II type 1 (AT1) receptor was shown to reduce cardiovascular events in hypertensive subjects with LV hypertrophy. Several lines of evidence are now converging to show that ACE inhibitors may affect the atherosclerotic process itself. Emerging clinical data indicate that angiotensin-receptor blockers (ARBs) may possibly modulate atherosclerosis as well. The antiatherogenic properties of ACE inhibitors and ARBs may derive from inhibition or blockade of angiotensin II, now recognized as an agent that increases oxidative stress. Angiotensin-converting enzyme inhibition and angiotensin-receptor blockade also increase endothelial nitric oxide formation, which improves endothelial function. In contrast to the effects of ARBs, the vascular effects of ACE inhibitors may, in part, be mediated by an increase in bradykinin. This article reviews some of the biologic mechanisms whereby ACE inhibitors and ARBs may modulate atherosclerosis.

Key words: atherosclerosis, angiotensin-converting enzyme inhibitors, angiotensin-receptor blockers, oxidative stress, endothelial dysfunction, angiotensin II, bradykinin, nitric oxide

Introduction

Angiotensin-converting enzyme (ACE) inhibitors have gained widespread acceptance for use in hypertension, congestive heart failure (CHF), and the postmyocardial infarction (MI) period when there is evidence of CHF or left ventricular dysfunction (LVD).1–6 Angiotensin II type 1 (AT1) receptor blockers (ARBs) reduce cardiovascular events in hypertensive subjects with left ventricular hypertrophy.7 New data show that ACE inhibitors and ARBs may modulate the atherosclerotic process itself.

Perhaps the most compelling new findings involve the role of oxidative stress in cardiovascular disease and the recognition of angiotensin as an agent of oxidative stress.8, 9 Angiotensin II is a central component of oxidative signaling that causes vascular inflammation and endothelial dysfunction. Agents that inhibit angiotensin formation—such as ACE inhibitors—may be particularly well suited to modulate atherosclerosis.

Recent evidence from clinical trials, in particular the Heart Outcomes Prevention Evaluation (HOPE) trial and its substudies, as well as the Losartan Intervention for Endpoint Reduction in Hypertension study (LIFE), will be reviewed to support an expanding role for ACE inhibitors and a possible role for ARBs. In HOPE and Microalbuminurias, Cardiovascular, and Renal Outcomes in HOPE (MICRO-HOPE), long-term treatment with the ACE inhibitor ramipril significantly lowered rates of cardiovascular death, MI, and stroke among patients at high risk for cardiovascular events.10, 11 In the Study to Evaluate Carotid Ultrasound changes in patients treated with Ramipril and vitamin E (SECURE), a HOPE substudy, ACE inhibition, but not treatment with the antioxidant vitamin E, prevented the progression of atherosclerosis, as measured by the intimal-to-medial ratio of the carotid artery.12 This is an important contrast to previous findings that suggested that use of an antioxidant vitamin might mitigate atherosclerosis by reducing excessive low-density lipoprotein (LDL) cholesterol oxidation. A brief overview of the role of ACE inhibitors and ARBs in lessening oxidative stress in the vascular system follows.

New Insights into the Initiation and Progression of Atherosclerosis

Healthy endothelium, important in modulating smooth muscle cell function and growth, produces nitric oxide (NO) and maintains a homeostatic balance between NO and reactive oxygen species (ROS). Nitric oxide plays a critical role in maintaining endothelial function via vasodilatory and vascular hypertrophic-inhibiting effects, particularly as an inhibitor of monocyte adhesion. Although the endothelium may be physi-
cally intact in atherosclerosis, it functions differently; with endothelial dysfunction, NO activity is decreased, whereas angiotensin—with its vasoconstrictive, pressor, and mitogenic effects—is increased.8

Oxidative stress

Many of the major risk factors for cardiovascular diseases, including hypertension, smoking, hypercholesterolemia, and diabetes mellitus, stimulate oxidative stress and may generate ROS in vascular cells.13 When there is increased activity of superoxide anion and other ROS, oxidative stress can, in turn, set off increased catabolism of NO. When NO activity is impaired, endothelial dysfunction can follow.5,9,14,15

The results of oxidative stress and excess production of intracellular ROS are oxidative damage and cellular cytotoxicity. Increased levels of oxidative stress induce vascular inflammatory genes, whereas lower levels sustain a noninflammatory or vascular-protective effect.8,9,14,15

Angiotensin II

There is also increasing evidence that angiotensin II, a powerful vasoconstrictor and likely promoter of plaque rupture,11 contributes to the development of atherosclerosis. Angiotensin II mediates recruitment of inflammatory cells into the atherosclerotic lesion and generates ROS. In response to angiotensin II, enzymes in the endothelium, smooth muscle, and fibroblasts that use nicotinamide adenine dinucleotide (NADH), nicotinamide adenine dinucleotide phosphate (NADPH) substrates, or both, are activated and yield superoxide anion.16–20 Therefore, we would expect superoxide production to increase when the local or systemic renin-angiotensin system (RAS) is activated—and increased vascular NADH/NADPH oxidase activity has been demonstrated in animal models of early atherosclerosis along with activation of the RAS.21 When NADH/NADPH oxidase is activated, the amount of superoxide within the vascular wall increases, and atherosclerosis can progress. Superoxide can combine with NO in a diffusion-limited reaction to produce peroxynitrite, which has limited NO-like properties.22,23 The result is that NO is directed away from its usual targets that maintain vasodilation and inhibit platelet activation. In short, increased vascular superoxide both decreases NO bioactivity and allows vascular oxidative stress to continue.24,25

Other atherogenic activities have been noted for angiotensin. Increases in plasminogen activator inhibitor-1 (PAI-1), the primary inhibitor of tissue-type plasminogen activator and a critical regulator of fibrinolysis, have been demonstrated in human coronary arteries. Angiotensin has also been shown to regulate PAI-1 expression in cultured endothelial cells.26,27

New Insights into the Role of the Renin-Angiotensin System in Atherosclerosis

Since its discovery more than a century ago, the RAS has been implicated in several cardiovascular diseases, including hypertension, CHF, post-MI LVD and CHF, and diabetic nephropathy.28,29 It is now being increasingly implicated in atherosclerosis. Renin, an enzyme that acts on the renin substrate angiotensinogen to catalyze formation of angiotensin I, is a major determinant of the rate of angiotensin II production.29,30 Angiotensin II is cleaved from angiotensin I by the action of the ACE.30

Another important action of the ACE is to catalyze the degradation of bradykinin. Bradykinin permits vasodilation by stimulating production of endothelium-derived NO.30

Several atherogenic activities have been described for angiotensin II. It stimulates hypertrophic growth of vascular smooth muscle cells;31 it induces synthesis of basic fibroblast growth factor, a potent mitogen for vascular smooth muscle cells;32 and it has been shown to recruit monocytes into the vessel wall (a critical early step in the development of atherosclerosis) in a rabbit model of early accelerated atherosclerosis.33

Tissue ACE has been shown to accumulate in atherosclerotic human coronary arteries as well as in luminal endothelial cells and inflammatory cells, especially in regions of clustered macrophages and T lymphocytes, possibly contributing to increased production of local angiotensin.34

The Role of Angiotensin-Converting Enzyme Inhibition and Angiotensin II Type 1 Receptor Blockers in Modulating Atherosclerosis

Angiotensin-converting enzyme regulates the balance between the vasodilatory/natriuretic properties of bradykinin and the vasoconstrictive/salt-retaining properties of angiotensin II.30 Angiotensin-converting enzyme inhibitors lessen angiotensin II formation and bradykinin degradation,30 interrupting the cycle of inflammation, lesion formation, and disease progression in vascular lesions.8 They may differ from ARBs because the latter drugs act solely on the angiotensin II receptor, whereas ACE inhibitors, as noted, also have bradykinin-mediating properties.35

The vascular activities of ACE inhibitors have conventionally been related to their ability to inhibit angiotensin II production. New findings show that when kininase II (recently found to be the same as ACE) is inhibited, bradykinin production is elevated, which, in turn, stimulates a bradykinin receptor, B2, to release NO, prostacyclin, and endothelium-derived hyperpolarizing factor (Fig. 1).36,37 Angiotensin-converting enzyme inhibition has also been shown to prevent the rapid tachyphylaxis of the B2 receptor.37

Angiotensin II affects oxidative signaling and vascular inflammatory gene expression through its effects on NADPH oxidase and lipoxygenase and subsequent generation of ROS. Specifically, several proinflammatory cytokines and growth factors (tumor necrosis factor-alpha [TNF-α], interleukin-1β [IL-1β], and interferon-gamma [IFN-γ]) as well as angiotensin II activate membrane-bound NADPH-like oxidase activity in endothelial and vascular smooth muscle cells, resulting in superoxide anion production.16,17,19,21 Superoxide and other free radicals promote LDL oxidation and degradation of NO. The ROS that are responsible for LDL oxidation
also stimulate the synthesis of other vascular inflammatory products and adhesion molecules that include monocyte chemoattractant protein-1 (MCP-1), vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1).

Angiotensin II upregulates the lectin-like oxidized LDL receptor-1 (LOX-1), which is an endothelial localized receptor that binds oxidized LDL. Oxidized LDL binding to LOX-1 upregulates a nuclear transcription factor NF-κB, a redox-sensitive transcription factor for proinflammatory genes.

Evidence from Experimental Animal Models

The vast array of effects of angiotensin II in vascular pathology may be mediated through immune response elements. The impact of AT1 receptor blockade has been evaluated in two animal models. These studies report an antiatherogenic effect of AT1 receptor blockade that may be mediated by reduced LDL oxidative susceptibility, reduced MCP-1 levels, and depressed expression of CD11b on circulating monocytes.

Evidence from Clinical Trials

In a recent large-scale clinical trial, long-term treatment with an ACE inhibitor significantly lowered rates of death, MI, and stroke among patients at high risk for cardiovascular (CV) events. The HOPE study assessed the effect of the ACE inhibitor ramipril in high-risk patients who did not have hypertension, heart failure, or low ejection fraction. Three substudies of patients in the HOPE trial provide intriguing connections between ACE-inhibitor activity and atherosclerosis.

HOPE: This trial randomized more than 9,000 patients to receive either ramipril 10 mg/day, vitamin E, or their respective placebo controls for a planned follow-up of 5 years. All participants were 55 years or older, were normotensive or had controlled hypertension, and had evidence of vascular disease or diabetes. All patients with diabetes had at least one other risk factor for CV disease (total cholesterol level > 5.2 mmol/l, high-density lipoprotein [HDL] cholesterol level ≤ 0.9 mmol/l, controlled hypertension, known microalbuminuria, or current smoking). In the overall population, 80% had known coronary artery disease (CAD), 43% had peripheral vascular disease, 11% had a previous stroke/transient ischemic attack, and 38% were diabetic.

The study was stopped after 4.5 years because the ramipril-treated patients had a 22% reduction in the composite primary endpoint of MI, stroke, or death from CV disease (p < 0.001). Ramipril showed a consistent benefit across all subgroups—patients with and without diabetes, with and without hypertension, older and younger than 65 years, and with and without microalbuminuria (Table I). This risk reduction occurred independently of an effect on blood pressure: mean systolic and diastolic blood pressures at entry

| Table I Reduced incidence of outcomes with ramipril in three HOPE trials |
|------------------|------------------|------------------|------------------|
| HOPE10           | Ramipril Placebo |
| Primary outcome  | n = 4,645       | n = 4,652        | p Value          |
| MI, stroke, or death from CV causes (%) | 651 (14) | 826 (17.8) | <0.001 |
| Secondary outcomes | Revascularization (%) | 742 (16) | 852 (18.3) | 0.002 |
| Death from CV causes (%) | 282 (6.1) | 377 (8.1) | <0.001 |
| MI (%) | 459 (9.9) | 579 (12.3) | <0.001 |
| Stroke (%) | 156 (3.4) | 226 (4.9) | <0.001 |

MICRO-HOPE11

<table>
<thead>
<tr>
<th>MICRO-HOPE11</th>
<th>Ramipril Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary outcome</td>
<td>n = 1,808</td>
</tr>
<tr>
<td>Combined (%)</td>
<td>277 (15.3)</td>
</tr>
<tr>
<td>MI (%)</td>
<td>185 (10.2)</td>
</tr>
<tr>
<td>Stroke (%)</td>
<td>76 (4.2)</td>
</tr>
<tr>
<td>CV death (%)</td>
<td>112 (6.2)</td>
</tr>
</tbody>
</table>

Secondary outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Total stroke (%)</th>
<th>n = 4,645</th>
<th>n = 4,652</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOPE Stroke Substudy12</td>
<td>Ramipril Placebo</td>
<td>156 (3.4)</td>
<td>226 (4.9)</td>
</tr>
</tbody>
</table>

Abbreviations: HOPE = Heart Outcomes Prevention Evaluation, MICRO-HOPE = Microalbuminuria, Cardiovascular, and Renal Outcomes in HOPE, MI = myocardial infarction, CV = cardiovascular.
were 139/79 mmHg in the ramipril treatment group and 136/76 mmHg at the study’s end.10

Use of ramipril decreased rates of death, MI, stroke, revascularization, cardiac arrest, heart failure, diabetes-related complications, and new cases of diabetes mellitus in a wide spectrum of high-risk patients. Vitamin E had no beneficial effect. This finding from the SECURE substudy is detailed below.10, 12

MICRO-HOPE Substudy: This substudy sought to determine whether ACE inhibition would lower risk factors for CV and renal disease in persons with diabetes. Criteria matched the HOPE trial, and MICRO-HOPE participants (n = 3,577) could also not have clinical proteinuria. Over 4.5 years, ramipril significantly lowered the risk of the composite primary outcome (MI, stroke, or CV death) by 25%; it lowered the risk of MI by 22%, stroke by 33%, and CV death by 37% in a broad range of patients with diabetes mellitus. Ramipril also lowered the risk of overt nephropathy by 24%, dialysis by 20%, and laser therapy for retinopathy by 22%. Again, the benefits were greater than those attributable to a decrease in blood pressure alone.11

HOPE Stroke Substudy: In the most recent HOPE substudy, the effects of ramipril treatment on the risk of stroke and stroke sequelae were analyzed separately. The relative risk of any stroke was reduced by 32% in the ramipril treatment group. The relative risk of fatal stroke was reduced by 61% (95% confidence interval [CI], 0.22–0.67). It is also important to note that among the ramipril-treated patients who did experience stroke, significantly fewer patients had functional impairment (cognition, motor weakness, speech, and difficulty swallowing).42

SECURE: As noted previously, the activation of oxidative modification of LDL cholesterol is likely to be significant in atherogenesis. In the SECURE trial, the benefits of long-term ACE-inhibitor therapy, given with or without the antioxidant vitamin E, on the intimal-to-medial thickness of the carotid artery were investigated. Participants (n = 732) were randomly assigned to receive either ramipril 2.5 or 10 mg per day and vitamin E or their matching placebos over an average follow-up of 4.5 years.12

Atherosclerosis progression, measured using carotid ultrasound/carotid intimal-to-medial thickness, was significantly less in the ramipril 10 mg versus the placebo-treated patients (p = 0.03). Although there was a trend toward slower progression in the ramipril 2.5 mg group, the difference did not achieve statistical significance. There was no difference in atherosclerosis progression rates, as measured between the vitamin E and the placebo-treated groups.12

This finding is an important contrast to previous evidence suggesting that use of an antioxidant agent might be an effective means of reducing excessive LDL cholesterol oxidation. It is likely that vitamin E therapy is limited to scavenging lipid-soluble oxidants, whereas ACE inhibition blocks vascular superoxide production at its source. These very different therapeutic targets may explain why use of antioxidant vitamins has not been effective in influencing CV disease progression. Vitamin E is not effective against all the oxidants related to atherosclerosis. It may also not completely inhibit oxidative stress and may be ineffective in other atherosclerotic processes such as smooth muscle cell proliferation.24

As noted, participants in HOPE and its substudies were either normotensive or had well-controlled hypertension at entry, and the effect of ramipril on further lowering blood pressure was modest. Although there is discussion that even a modest blood pressure reduction could have contributed to the benefits shown, these trials provide strong evidence that long-term ACE inhibition with ramipril slows the progression of atherosclerosis and can block an array of ischemic events via nonhemodynamic mechanisms.43

Randomized trials using other ACE inhibitors have not shown a benefit in retarding atherosclerosis progression. In the Quinapril Ischemic Event Trial (QUIET), there was no overall benefit with quinapril on coronary angiography-measured progression of atherosclerosis.44 Over 3 years of follow-up in 1,750 patients (mean age 58 years) with normal LV function who were also normotensive and normocholesterolemic, QUIET showed no significant differences between quinapril and placebo in any of the coronary angiography measurements.44 The QUIET investigators have posited that the quinapril dosage (20 mg/day) may have been too low to produce the desired effects and that 16% of patients were also taking lipid-lowering therapy, which can slow progression of coronary atherosclerosis (Table II).44

Similarly, the Simvastatin/Enalapril Coronary Atherosclerosis Trial (SCAT) showed no overall benefit with simvastatin/enalapril, either alone or in combination, on atherosclerosis progression, measured using coronary angiography in 460 patients with CAD and normal cholesterol levels followed for 3 to 5 years.45

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**Table II**  Placebo-controlled clinical trials evaluating effects of angiotensin-converting enzyme inhibitors on outcomes measured by ultrasound or angiography

<table>
<thead>
<tr>
<th>Study</th>
<th>Drug</th>
<th>No. of patients</th>
<th>Patient characteristics</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>SECURE12</td>
<td>Ramipril</td>
<td>818</td>
<td>Vascular disease or diabetes</td>
<td>↓AS progression a</td>
</tr>
<tr>
<td>QUIET44</td>
<td>Quinapril</td>
<td>377</td>
<td>CAD</td>
<td>Neutral effect on AS progression b</td>
</tr>
<tr>
<td>SCAT45</td>
<td>Enalapril</td>
<td>460</td>
<td>CAD, normocholesterolemic</td>
<td>Neutral effect on lumen diameter or % stenosis b</td>
</tr>
</tbody>
</table>

a Measured using carotid ultrasound.
b Measured using coronary angiography.

Abbreviations: SECURE = Study to Evaluate Carotid Ultrasound changes in patients treated with Ramipril and vitamin E, QUIET = Quinapril Ischemic Event Trial, SCAT = Simvastatin/Enalapril Coronary Atherosclerosis Trial, AS = atherosclerosis, CAD = coronary artery disease.
The SCAT investigators suggest a number of possible explanations for the neutral effect of enalapril therapy on CAD progression, most prominently the difficulty in detecting normalization of endothelial dysfunction and plaque formation/stabilization with quantitative coronary angiography. Changes in wall thickness measurements can be detected by intravascular ultrasound before changes in lumen diameter can be shown by quantitative coronary angiography. Because the study lacked intravascular ultrasound data, the investigators could not conclude that enalapril lacked efficacy.45

The investigators also noted that 90% of the participants in SCAT were taking concomitant aspirin. There are data suggesting that use of aspirin attenuates ACE inhibition. In brief, the biologic basis for such attenuation is that aspirin inhibits prostaglandin synthesis, while one of the effects of ACE inhibition is to potentiate bradykinin, which in turn increases synthesis of vasodilatory prostaglandin.46, 47

Clinical data, however, are not consistent. While the SCAT investigators suggest that concomitant aspirin use might have negated the effects of enalapril, other large-scale or more recent smaller studies using an ACE inhibitor and aspirin have reported clinical benefits in similar patient groups.45, 48, 49 Notably, in HOPE, 75% of the patients treated with ramipril were also taking aspirin, and significantly reduced rates of death, MI, and stroke were demonstrated.10 Given such contradictory results, prospective randomized, controlled trials are needed to determine the clinical relevance of a theoretically possible interaction. Furthermore, the use of ACE inhibitors in the prevention of atherosclerosis-mediated clinical events should not be considered a “class effect.”50

Recently, the LIFE study demonstrated significant decreases in the composite endpoint of cardiovascular death, MI, or stroke with use of the ARB losartan compared with the beta blocker atenolol.7 This multinational, randomized, prospective, parallel-group trial enrolled 9,193 patients (55 to 80 years) with hypertension and LV hypertrophy documented by electrocardiogram. Therapy lasted for at least 4 years and until 1,040 patients had a primary cardiovascular endpoint (death, MI, or stroke). Blood pressure fell by 30.2/16.6 and 29.1/16.8 mmHg in the losartan and atenolol groups, respectively. There was a significant adjusted risk reduction of 13% favoring losartan in the primary composite endpoint (p = 0.021 vs. atenolol) and of 24.9% in fatal or nonfatal stroke (losartan vs. atenolol, p = 0.001). There were also fewer occurrences of new-onset diabetes with losartan.7

Because both the ARB and the beta blocker lowered blood pressure similarly, the LIFE investigators suggested that losartan conferred benefits beyond blood pressure lowering, and they attributed significant benefit to losartan’s more potent blockade of angiotensin II.51 However, it is important to note that the reduction in the primary cardiovascular composite endpoint in LIFE was driven by a reduction in stroke. The myocardial infarction endpoint was better with atenolol than with losartan.7

Investigators for the multicenter, double-blind, randomized Optimal Trial in Myocardial Infarction with the Angiotensin II Antagonist Losartan (OPTIMAAL) recently reported a non-significant difference in total mortality in favor of captopril in patients with evidence of heart failure or LVD after acute MI. Patients (n = 5,477) were ≥50 years of age and were followed for just less than 4 years. Relative risk for all-cause mortality was 1.13 (95% CI: 0.99–1.28) for losartan. The OPTIMAAL investigators recommended the continued use of ACE inhibitors in this patient group.53

Conclusion

The efficacy and safety of ACE inhibitors in treating patients with hypertension, CHF, and post-MI LVD or CHF have made them mainstays in the treatment of these diseases. The beneficial effects of ACE inhibition are now being extended to other settings. Several recent lines of evidence are converging to show that long-term ACE inhibition, by inhibiting angiotensin II formation and potentiating bradykinin, may lessen oxidative stress and increase NO formation in the endothelium, lessening the endothelial dysfunction that is key to atherosclerosis development and progression.

In the HOPE trial and its substudies, long-term ACE inhibition with ramipril significantly lowered rates of death, MI, and stroke among patients at high risk for CV events. These benefits likely derive from the protective effects of ACE inhibition on the arterial wall.11, 54 Angiotensin-converting enzyme inhibitors lower levels of angiotensin II, a powerful vasoconstrictor and likely promoter of plaque rupture.11 ACE inhibition may also stabilize atherosclerotic lesions, as shown in many animal models.11, 54 The other major effect of ACE inhibition is to potentiate bradykinin, a direct vasodilator that also promotes release of NO and prostacyclin. In the SECURE substudy, ramipril limited the progression of carotid intimal thickening, whereas vitamin E did not. A likely reason for this is that ACE inhibition blocks vascular superoxide production at its source, whereas antioxidant vitamin therapy targets only scavenging lipid-soluble oxidants.

Clinicians may want to consider a broader use of ACE inhibition in appropriate patients to slow progression of atherosclerotic disease or to prevent its development. Most patients who are over 55 years of age, with atherosclerosis or diabetes but without hypertension, CHF, or post-MI LVD or CHF, similar to those in the HOPE trial, should be considered for ACE inhibitor therapy with ramipril. The role of ARBs in preventing atherosclerosis continues to be investigated, as does the role of combination ACE inhibitor/ARB therapy.

Acknowledgment

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