Acute and Chronic Angiotensin-1 Receptor Antagonism Reverses Endothelial Dysfunction in Atherosclerosis

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Background—The renin-angiotensin system may contribute to atherogenesis through the promotion of endothelial dysfunction. The present study was performed to determine whether angiotensin-1 (AT\(_1\)) receptor inhibition improves endothelial dysfunction.

Methods and Results—In the femoral circulation of 19 patients with atherosclerosis and of 9 control subjects, we studied microvascular responses to reactive hyperemia, angiotensin II, acetylcholine, and sodium nitroprusside before and after the administration of intra-arterial losartan (10 mg). Femoral artery flow velocity was measured with a Doppler flow wire, and the femoral vascular resistance index (FVRI) was calculated as mean arterial pressure divided by flow velocity. Losartan induced a minor (5.9±2%, \(P=0.02\)) reduction in FVRI and inhibited angiotensin II–mediated vasoconstriction in both patient groups (\(P<0.01\)). After the administration of losartan, acetylcholine-mediated vasodilation was augmented in patients (44±5% to 58±4% reduction in FVRI with infusion at a rate of 150 \(\mu\)g/min, \(P<0.001\)) but not control subjects. Vasodilation during reactive hyperemia was also greater after AT\(_1\) receptor inhibition (\(P=0.03\)) in patients, but the response to sodium nitroprusside remained unchanged. In a separate group of 31 patients with atherosclerosis, we investigated the effect of 8 weeks of oral losartan therapy on brachial artery flow-mediated vasodilation with the use of high-resolution ultrasound. Oral losartan therapy improved flow-mediated brachial artery dilation (1.4±0.9% to 3.2±0.8%, \(P=0.03\)) but had no effect on the nitroglycerin response. Serum nitrogen oxide levels increased from 21.6±1.7 to 26.7±2.4 \(\mu\)mol/L (\(P=0.008\)).

Conclusions—The results of the present study indicate that inhibition of the AT\(_1\) receptor in patients with atherosclerosis reverses endothelial dysfunction by improving NO availability and therefore may have long-term therapeutic benefits. (Circulation. 2000;101:2349-2354.)

Key Words: atherosclerosis ■ angiotensin ■ nitric oxide ■ endothelium ■ receptors ■ losartan

The renin-angiotensin system (RAS) is an important regulator of blood pressure and body fluid homeostasis in healthy individuals. The results of epidemiological,\(^1\) genetic,\(^2\) and clinical trials with ACE inhibitors\(^3\) strongly suggest that the RAS contributes to atherogenesis, potentially through angiotensin-induced endothelial dysfunction. The endothelium is central to the regulation of a variety of vascular functions, including smooth muscle tone, hemostasis, reduction-oxidation (redox) state, and the inflammatory response to injury.\(^4\) Endothelial dysfunction, an early feature of atherosclerosis, contributes to atherogenesis through the promotion of abnormal vasomotion, a procoagulant state, and excessive infiltration of inflammatory cells into the vessel.\(^5\)

Although the precise mechanisms remain unclear, alterations in the redox state, inactivation of NO, and increase in activity of the transcription factor nuclear factor-\(\kappa\)B appear to be important.\(^6,7\) Moreover, angiotensin II promotes endothelial dysfunction through angiotensin-1 (AT\(_1\)) receptor–mediated generation of superoxide anions from smooth muscle and endothelial cell membrane–bound reduced nicotinamide adenine dinucleotide–dependent oxidase.\(^8-11\)

We hypothesized that the RAS contributes to endothelial injury and that angiotensin II inhibition with AT\(_1\) receptor blockade will reverse endothelial dysfunction in patients with atherosclerosis. Thus, we determined whether (1) short-term treatment with losartan, an AT\(_1\) receptor antagonist, reverses endothelium-dependent vasomotor dysfunction, (2) any observed benefit persists during long-term therapy, and (3) long-term oral therapy with losartan increases NO generation.
Short-Term Study

Patients
We studied 19 patients with atherosclerosis of the coronary circulation and 9 healthy volunteers (control subjects). Patients with recent myocardial infarction, valvular heart disease, or peripheral vascular disease or those treated with ACE inhibitors or angiotensin receptor antagonists in the previous 2 weeks were excluded. The mean patient age was 61±2 years, 15 (79%) were male, 8 were hypertensive, 4 were either current smokers or had smoked in the previous year, and 2 had diabetes. For the patients, the mean total cholesterol level was 202±8 mg/dL, the HDL cholesterol level was 38±2 mg/dL, the mean LDL cholesterol level was 131±7 mg/dL, and the mean triglyceride level was 174±23 mg/dL. The mean ejection fraction was 0.53±0.03. The mean age of the control subjects was 46±2 years (P<0.01 compared with patients), 7 were male, and none were hypertensive, hypercholesterolemic (total cholesterol level >200 mg/dL), diabetic, or smokers. For the control subjects, the mean total cholesterol level was 172±12 mg/dL, the mean HDL cholesterol level was 54±11 mg/dL, the mean LDL cholesterol level was 104±11 mg/dL (all P<0.05 compared with patients), and the mean triglyceride level was 116±21 mg/dL.

Protocol
All cardiac medications were withdrawn at least 48 hours before the study and aspirin was discontinued 7 days before the study. The study was approved by the institute review board, and informed consent was obtained from the patients and control subjects. A 6F multipurpose A2 (Cordis, Inc) catheter was introduced 1 cm beyond the antecubital crease. As previously described, blood was flowed through the catheter at 5 mL/min, the end of a 7F femoral artery sheath. Sodium nitroprusside (Novartis) at 100, 500, and 1000 pmol/min. After a 20-minute rest period in the supine position in a quiet room, 2-dimensional and pulsed-Doppler blood flow images were obtained at 2 to 10 cm above the antecubital crease. The arterial diameter was measured at a fixed distance from an anatomic landmark such as a fascial plane or a vein. After baseline measurements, hyperemia was induced through the inflation of a blood pressure cuff on the proximal portion of the forearm to occlude arterial flow (220 mm Hg). After 5 minutes, the cuff was deflated rapidly, and blood flow velocity was recorded for the first 15 seconds and the 2-dimensional image for the next 75 seconds. A 10-minute recovery period was followed by measurements before and 3 minutes after the administration of sublingual nitroglycerin (0.4 mg).

Images were obtained with a Hewlett-Packard Sonos 1500 high-resolution 7.5-MHz linear array transducer as described previously. Analysis was blindly performed by 1 observer. Arterial diameter was calculated as the mean value of 2 measurements over a 1-cm segment from the anterior to the posterior “m” line at the end of diastole, concurrent with the onset of the QRS complex. The average of diameters over 3 cardiac cycles was calculated. Arterial flow was calculated as the product of the vessel cross-sectional area (m2/4), the velocity-time integral of the Doppler flow signal, and the heart rate.

The 8-week reproducibility of flow-mediated and nitroglycerin-induced brachial artery dilation was studied in 7 patients. Brachial artery diameter measurements at rest (3.77 and 3.72, r=0.99), flow-mediated dilation (4.02 and 4.0, r=0.97), and with nitroglycerin (4.23 and 4.09, r=0.88) were reproducible. Flow-mediated vasodilation (6.8±0.7% and 7.4±0.5%, P=0.5) and nitroglycerin-mediated dilation were similar at baseline and at 8 weeks (12.7±0.8% and 11.9±0.8%, P=0.7).

Serum Nitrogen Oxide Level
Patients were on a low nitrate diet for 24 hours before the measurement of fasting serum nitrogen oxides (NOx). Blood samples were centrifuged at 3000 rpm for 10 minutes at 10°C. The supernatant was stored at −70°C until analysis with use of the Sievers Nitric Oxide Analyzer (model 280).

Statistical Analysis
Data are expressed as mean±SEM. Mean values were compared with the use of paired or unpaired Student’s t test, as appropriate. The differences in the dose-response curves were compared with the use of ANOVA for repeated measures. The effect of losartan on reactive hyperemia was studied with the repeated measures ANOVA from 5-second to 5-minute time points that included patients, medications
(losartan/control), and time as main effects and incorporated the 2-factor interactions between them. All probability values are 2 tailed. Correlation analysis was performed with Pearson’s correlation coefficient.

**Results**

**Acute Study**

**Femoral Vascular Response to Losartan**

After 10 minutes of intra-arterial losartan infusion, there was mild vasodilation in both control subjects and patients with a 5.9±2% reduction in FVRI from (6.6±0.3 to 6.2±0.4 mm Hg \(\cdot\) cm \(\cdot\) s, \(P=0.02\)) and a reduction in mean arterial blood pressure from 105±2 to 102±2 mm Hg (\(P=0.005\)).

**Effect of Losartan on the Response to Angiotensin II**

Angiotensin II infusions produced similar vasoconstriction in patients and control subjects. The 3 doses of angiotensin II increased FVRI by 22±3%, 43±5%, and 60±8% in patients and by 24±8%, 44±9%, and 80±17% in control subjects (\(P=0.5\), ANOVA between groups). There was significant inhibition of angiotensin II–induced microvascular constriction by losartan in both groups (Figure 1, \(P<0.01\), ANOVA). Thus, after losartan, the highest dose of angiotensin II produced a 31±5% and 32±8% increase in FVRI in patients and control subjects, respectively, indicating the inhibition of femoral vascular AT1 receptors in both groups (Figure 1).

**Effect of Losartan on ACh Responses**

ACh infusions produced graded vasodilation in both patients and control subjects. The trend toward a reduced response in patients compared with control subjects did not reach statistical significance; at the peak dose of ACh, flow velocity was 172±28% higher in patients compared with a 231±47% increase in control subjects (Figure 2).

Intra-arterial losartan infusion improved ACh responses in patients (\(P<0.001\)) but not in control subjects (\(P=0.9\), Figure 2), indicating that losartan selectively enhances ACh-mediated vasodilation in patients with atherosclerosis. Improvement in vasodilation with ACh in patients was confirmed with a simultaneous reduction in arteriovenous oxygen difference (from 10.5±1.3% before to 7.8±1% after losartan, at the peak dose of ACh; \(P=0.04\), Figure 2).

There was an inverse correlation between the decrease in FVRI with ACh (300 \(\mu\)g/min), representing endothelial function at baseline, and the magnitude of improvement in ACh response with losartan (\(r=-0.46\), \(P=0.06\)), indicating that patients with a de-
pressed dilator response with ACh had greater improvement with AT1 receptor inhibition, and vice versa.

**Effect of Losartan on Response to Sodium Nitroprusside**

Patients and control subjects had a similar vasodilator response to sodium nitroprusside ($P=0.4$). Thus, at the peak dose of sodium nitroprusside, FVRI decreased by 64±2% in patients and by 62±2% in control subjects. After losartan, there was no change in the magnitude of vasodilation with sodium nitroprusside in either the control subjects or the patients ($P>0.5$, ANOVA, Figure 3).

**Effect of Losartan on Reactive Hyperemia**

In patients, vasodilation during reactive hyperemia was greater after losartan ($P=0.03$, ANOVA, Figure 4). This difference was not, however, present at the peak response (FVRI 2.4±0.2 mm Hg cm$^{-1}$ s$^{-1}$ before and 2.4±0.2 mm Hg cm$^{-1}$ s$^{-1}$ after losartan, $P=NS$).

**Measurements During Oral Losartan Therapy**

<table>
<thead>
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<th></th>
<th>Baseline</th>
<th>After Losartan</th>
<th>$P$</th>
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<tbody>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>141±3</td>
<td>135±4</td>
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<td>Diastolic blood pressure, mm Hg</td>
<td>80±2</td>
<td>77±2</td>
<td>0.3</td>
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<td>Heart rate, bpm</td>
<td>66±2</td>
<td>67±2</td>
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<tr>
<td>Baseline vessel size, mm</td>
<td>5.1±0.2</td>
<td>5.1±0.2</td>
<td>0.8</td>
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<tr>
<td>Hyperemia, % increase in flow</td>
<td>385±34</td>
<td>427±31</td>
<td>0.2</td>
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<tr>
<td>Serum nitrate, μmol/L</td>
<td>21.6±8.8</td>
<td>26.0±8.7</td>
<td>0.008</td>
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<tr>
<td>ACE level, U/L</td>
<td>9.3±0.6</td>
<td>8.9±0.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Plasma renin activity, ng · mL$^{-1}$ · h$^{-1}$</td>
<td>1.2±0.4</td>
<td>2.7±0.5</td>
<td>0.001</td>
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</table>

In contrast, reactive hyperemia was unchanged in control subjects after losartan (Figure 4). Control subjects also did not enhance their peak hyperemic response (FVRI 2.1±0.3 mm Hg cm$^{-1}$ s$^{-1}$ before and 1.9±0.2 mm Hg cm$^{-1}$ s$^{-1}$ after losartan $P=NS$).

**Chronic Study**

There was a mild reduction in systolic, but not diastolic, blood pressure 8 weeks after the administration of oral losartan (Table). Resting heart rate was similar and plasma renin activity was higher after losartan therapy. Baseline brachial artery diameter remained unaltered (Table). Before treatment with losartan, mean endothelium-dependent, flow-mediated brachial artery dilation was 1.4±0.9%. After 8 weeks of oral losartan, flow-mediated dilation was enhanced to 3.2±0.8% ($P=0.03$ compared with before losartan (Figure 5). As in the acute study, there was no difference in peak increase in hyperemic blood flow before compared with 8 weeks after losartan (Table). Endothelium-independent brachial artery dilation assessed with the use of sublingual nitroglycerin remained unchanged 8 weeks after treatment with losartan (8.0±1.7% and 8.8±1.1%, $P=0.69$, Figure 5).

There was a significant increase in serum NOx levels 8 weeks after treatment with losartan (Table).

**Discussion**

We hypothesized that inhibition of the RAS might reverse endothelial dysfunction in patients with atherosclerosis. The results of the present study support this hypothesis by demonstrating that AT1 receptor blockade with losartan selectively improves endothelium-dependent peripheral vaso-motion in patients with coronary atherosclerosis as indicated by the potentiation of ACh-mediated dilation of femoral microvessels and of flow-mediated dilation of the brachial artery. Furthermore, the reversal of endothelial dysfunction can be achieved acutely and persists during long-term oral therapy and is associated with increased NO bioavailability.

**AT1 Receptor Blockade and Basal Vascular Tone**

Short-term AT1 receptor blockade with intra-arterial losartan produced minor but statistically significant femoral microvascular dilation. This was confirmed during oral therapy that also resulted in a small reduction in arterial blood pressure. However, conductance vessel tone remained constant, illustrated by the unchanged brachial arterial size. Our findings...
indicate that endogenous angiotensin II is a determinant of resting peripheral microvascular but not conductance vessel tone and is consistent with its anti hypertensive action, which is at least in part due to blockade of microvascular AT1 receptors. These observations are at variance with the results of previous studies in young healthy volunteers.16,20 and may be due to the higher dose of losartan used and the older age of our study population.

Hypercholesterolemia-induced AT1 receptor expression in the media and intima of atherosclerotic vessels of cholesterol-fed rabbits is associated with marked augmentation of the angiotensin II response.21 However, constriction with angiotensin II in the femoral microcirculation was similar in control subjects and patients in our study, suggesting that atherosclerosis of conductance vessels is not associated with the upregulation of AT1 receptors in human microvasculature. This observation does not exclude the possibility that AT1 receptors and other components of RAS, such as ACE and angiotensin II, are upregulated in atherosclerotic conductance arteries.22

Intra-arterial losartan inhibited constriction in response to exogenous angiotensin II, indicating adequate femoral vascular AT1 receptor blockade. As previously shown, this blockade persists for at least 60 minutes,20 the period during which we investigated the effects of losartan on ACh, hyperemia, and sodium nitroprusside responses.

AT1 Receptor Blockade and Endothelial Dysfunction
Losartan improved ACh-mediated microvascular dilation in patients but not in control subjects. This was confirmed by measurement of arteriovenous oxygen differences, which also narrowed after losartan administration in patients. In contrast, responses to sodium nitroprusside were not altered, suggesting that AT1 receptor antagonism selectively improves endothelium-dependent but not endothelium-independent vasodilation in patients with atherosclerosis. Furthermore, the magnitude of improvement in ACh responses with parenteral losartan correlated inversely with the initial response with ACh, suggesting that the improvement was greatest in those with the most depressed endothelial function.

During oral losartan therapy, we observed significant improvement in flow-mediated brachial artery dilation, confirming that reversal of endothelial dysfunction also occurs in conduit arteries of patients with atherosclerosis and persists during long-term AT1 receptor blockade. As in the acute study, endothelium-independent responses, measured with sublingual nitroglycerin, remained unaltered with oral losartan. Finally, the increase in serum NOx levels further illustrates that there was an increase in NO availability with losartan therapy.

Previous studies have demonstrated that ACE inhibition can reverse endothelial dysfunction in atherosclerosis, and this effect was at least in part attributed to the increased bioavailability of bradykinin.23,24 Whether reduced generation of angiotensin II was also instrumental in this action was unknown. Results from the present study indicate that the prevention of angiotensin II synthesis with ACE inhibitors may also contribute to their beneficial vascular effects.

AT1 Receptor Blockade and Reactive Hyperemia
Local factors that contribute to the reactive hyperemic response include changes in interstitial potassium and hydrogen ions, osmolality, carbon dioxide, catecholamines, prostaglandins, adenosine, and the ATP-sensitive potassium channels.25 The crucial role of the endothelium in modulation of arteriolar reactive hyperemia, and particularly of NO, in the late phase of the hyperemic response was recently demonstrated.26,27 Patients with atherosclerosis had reduced inhibition of the hyperemic response with Nω-monomethyl-L-arginine, indicating diminished contribution of NO to reactive hyperemia in patients with endothelial dysfunction.14 Our observation in the present study that the late phase of the hyperemic response could be improved with losartan in patients with endothelial dysfunction but not in control subjects suggests that improved NO bioavailability with AT1 receptor blockade in this population translated into the improved vasodilation during physiological testing.

Potential Mechanisms That Underlie the Improvement in Endothelial Function With AT1 Receptor Antagonism
Experimental evidence illustrates the presence of increased angiotensin II in atherosclerotic lesions28 and that angiotensin II is a powerful stimulus for NADH/NADPH oxidase-dependent vascular superoxide anion generation.9–11 Increased oxidant levels inactivate NO, and this is instrumental in the precipitation of endothelial dysfunction. Infusions of angiotensin II and norepinephrine into rats produced similar increases in blood pressure, but only angiotensin II stimulated superoxide anion production and NADPH-dependent oxidase activity. Furthermore, endothelium-dependent relaxation was abnormal in vessels from rats treated with angiotensin II but not in norepinephrine-treated rats. The endothelial responses were restored with treatment with superoxide dismutase or the AT1 receptor antagonist losartan. Indeed, losartan lowered the production of superoxide to below the levels seen in normal animals, suggesting that endogenous angiotensin II may modulate basal superoxide anion production.

In canine coronary arteries, angiotensins I, II, III, IV, and (1-7) promote NO release, which can be inhibited by NO synthase inhibition, bradykinin B2 receptor blockade, and protease inhibitors. AT1 and AT2 receptor antagonists abolish the response to all fragments except angiotensin IV, which is inhibited only by AT2 receptor blockade. These observations suggest that angiotensin peptides promote NO activity due to the activation of local kinin production. Furthermore, the effect is mediated through both AT1 and AT2 receptors.29 AT2 receptor-mediated NO release has also been observed in rat kidneys during sodium depletion.30 AT1 receptor blockade interferes with the negative feedback of angiotensin II on the release and synthesis of renin from the kidneys, leading to an increase in renin, angiotensin I, and angiotensin II levels.31 Plasma renin activity was also increased with oral losartan in our study. Consequently, it has been suggested that increased stimulation of AT2 receptors during AT1 receptor blockade may promote NO release, possibly through enhanced kinin activity. This hypothesis is supported by studies that demonstrate that the cardioprotective action of losartan in the rat...
heart failure model\(^{12}\) and of short-term administration of candesartan in the porcine myocardial infarction model can be blocked by either AT\(_2\) receptor or bradykinin B\(_2\) receptor antagonism.\(^{35}\) In the latter investigation, cyclooxygenase inhibition also abolished the effects of candesartan, suggesting that prostaglandins may also contribute to the cardioprotection of AT\(_1\) receptor blockade.

Thus, AT\(_1\) receptor antagonism may reverse endothelial dysfunction in humans through improvement in NO bioavailability by either AT\(_1\) receptor–mediated reduction in oxidant stress or AT\(_2\) receptor–mediated increase in NO synthesis. The present study supports these concepts by demonstrating an increased response to ACh, improvement in flow-mediated dilation that is considered to be primarily due to NO release with shear, and an increase in serum NO\(_x\) levels after the administration of losartan.

**Implications**

In experimental atherosclerosis, AT\(_1\) receptor blockade appears to have a protective effect.\(^{34}\) Potential mechanisms include the prevention of endothelial injury,\(^{35,36}\) the augmentation of NO activity,\(^37\) the inhibition of lipid peroxidation,\(^{34}\) and an antiproliferative effect. These findings, together with our observations that losartan improved endothelial function and NO activity, suggest that AT\(_1\) receptor antagonism may also be antiatherogenic in patients with atherosclerosis.

**References**


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