Pleiotropic Effects of Angiotensin II Receptor Blocker in Hypertensive Patients

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OBJECTIVES
We investigated the vascular effects of candesartan in hypertensive patients.

BACKGROUND
The renin-angiotensin system may contribute to atherogenesis through the promotion of endothelial dysfunction. The plausible mechanisms are that angiotensin II promotes superoxide anion generation, endothelial dysfunction, inflammation, and impaired fibrinolysis. The effects of candesartan on these conditions have not been clearly observed.

METHODS
We administered placebo or candesartan 16 mg daily during two months to 45 patients with mild-to-moderate hypertension. This was a randomized, double-blind, placebo-controlled, crossover study in design.

RESULTS
Candesartan did not significantly change lipoprotein levels. However, compared with placebo, candesartan significantly reduced plasma levels of malondialdehyde from 1.50 ± 0.07 to 1.29 ± 0.09 μM (p = 0.009); improved the percent flow-mediated dilator response to hyperemia from 5.17 ± 0.24 to 6.22 ± 0.26% (p < 0.001); and, furthermore, reduced plasma levels of monocyte chemoattractant protein (MCP-1) from 213 ± 8 to 190 ± 7 pg/ml (p = 0.003), tumor necrosis factor-alpha from 2.93 to 2.22 pg/ml (p = 0.026), and plasminogen activator inhibitor type 1 from 74 ± 4 to 53 ± 4 ng/ml (p < 0.001) but not C-reactive protein (CRP), matrix metalloproteinase protein, and fibrinogen. There were no significant correlations between these changes and reduction of systolic blood pressure (BP) (−0.247 ≤ r ≤ 0.195) and between these changes and reduction of diastolic BP (−0.262 ≤ r ≤ 0.197). There were no significant correlations between markers of inflammation and flow-mediated dilation percent or reduction of oxidant stress (−0.119 ≤ r ≤ 0.127). Furthermore, we observed no significant correlations between CRP and MCP-1 levels (r = −0.162).

CONCLUSIONS
Inhibition of the angiotensin II type 1 (AT1) receptor in hypertensive patients reverses endothelial dysfunction, measured as an improvement in flow-mediated dilation and fibrinolysis and reduction of oxidant stress and inflammatory cytokines, suggesting that AT1 receptor blocker therapy has antatherogenic effects. (J Am Coll Cardiol 2003;42:905–10)

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The Losartan Intervention For Endpoint Reduction in Hypertension study (LIFE) demonstrated that losartan, angiotensin II type 1 (AT1) receptor blocker, therapy prevented or retarded the progression of coronary heart disease. This study suggests benefits beyond reduction in blood pressure (BP) (1). Endothelial dysfunction of epicardial coronary arteries precedes development of atherosclerotic disease that is either angiographically apparent or of sufficient obstructive severity to cause myocardial ischemia and angina pectoris (2). Hypertensive patients have impaired functions of the endothelium, which is a marker of future cardiovascular events (3). The vessel wall in endothelial dysfunction may promote inflammation, plaque rupture, and thrombus formation, which contribute to development and clinical expression of atherosclerosis (4).

Recent experimental studies have confirmed that angiotensin II (AII) accelerates the development of atherosclerosis (5,6). The plausible mechanisms are that AII promotes superoxide anion generation and endothelial dysfunction (7,8). Angiotensin II activates nuclear transcription factor (NFκB) induced by oxidative stress (9). This effect is mediated by AT1 receptor (10). Nuclear transcription factor activates proinflammatory transcription factors and, thus, stimulates the synthesis of protein products such as cell adhesion molecules and chemokines (9,11,12). In hypertensive patients AII infusion leads to a rapid increase in plasma intercellular adhesion molecule-1 via an AT1 receptor-dependent mechanism (13). Angiotensin II type 1 receptor blocker could diminish intracellular production of superoxide anions via reduced activity of AII-dependent oxidases in the endothelium and vascular smooth muscle (14,15), thus protecting nitric oxide (NO) from oxidant degradation to biologically inert or toxic molecules (4,16). Inhibition of the production of superoxide anions could also limit the oxidation of low-density lipoprotein (LDL), thus contributing to increased NO bioactivity by enhancing NO syn-
thirty and limiting oxidative degradation of NO (4,16). In this regard, AT1 receptor blocker inhibited LDL oxidation and attenuated atherosclerosis independent of lowering BP (17). In addition to improving vasomotor tone, increased NO bioactivity within the vasculature may prevent activation of proinflammatory transcription factors and, thus, reduce synthesis of protein products such as chemokines (18). On the other hand, plasminogen activator inhibitor type-1 (PAI-1) expression is affected by AT1 receptor, and interestingly, PAI-1 expression was suppressed by candesartan (19,20), in contrast with losartan (21), showing no effects.

However, the effects of AT1 receptor blocker on endothelium-dependent vasomotor responsiveness are conflicting in patients (22–25). Furthermore, the effects of AT1 receptor blockers on inflammation (26–31) and fibrinolysis (31–34) are conflicting in patients. In this study, we investigated the potential benefit of candesartan, an AT1 receptor blocker, on endothelium-dependent vasomotor responsiveness, oxidant stress, and markers of inflammation, hemostasis, and plaque stability in hypertensive patients.

**METHODS**

**Study population and design.** Forty-seven patients with mild-to-moderate hypertension participated in this study. We used World Health Organization/International Society of Hypertension definitions (35) for hypertension defined as systolic and diastolic BP ≥140 or ≥90 mm Hg, respectively. In order to minimize acute side effects to candesartan, study medication was titrated from 8 to 16 mg upwards over the second treatment period initiated upon completion of the first treatment period (without washout phase). In order to avoid effects from other drugs, no other medications were allowed during the study period. The study was approved by the Gil Hospital Institute Review Board, and all participants gave written, informed consent.

**Laboratory assays.** Blood samples for laboratory assays were obtained at approximately 8:00 AM after overnight fasting, at baseline, and at the end of each treatment period and immediately coded so that investigators performing laboratory assays were blinded to subject identity or study sequence. Assays for lipids, plasma malondialdehyde (MDA), monocyte chemoattractant protein (MCP)-1, tumor necrosis factor (TNF)-alpha, matrix metalloproteinase protein (MMP)-9, PAI-1, and serum C-reactive protein (CRP) were performed in duplicate by ELISA (R & D Systems, Inc., Minneapolis, Minnesota; BIOXYTECH LPO-586, OxisResearch, Portland, Oregon; BioPool, Ventura, California; Rate nephelometry; IMMAGEO, Beckman Coulter, Brea, California), as previously described (36–40). C-reactive protein levels were determined with an immunonephelometry system according to methods described by the manufacturer. The measurement range is 0.1 to 98 mg/dl. All samples from the same patient (batch samples) were measured in blinded pairs on the same ELISA kit to minimize run-to-run variability. The inter-assay and intra-assay coefficients of variation were <6%.

**Vascular studies.** Imaging studies of the right brachial artery were performed using a ATL HDI 3000 ultrasound machine equipped with a 10-MHz linear-array transducer, based on a previously published technique (36,37,39,40). All images were transmitted to a personal computer via Ethernet with DICOM format (Digital Imaging and Communication in Medicine) and then saved on the hard disk of a personal computer as a bitmap format. Arterial diameters were measured with Image Tool for Windows version 2.0 (University of Texas Health Science Center, San Antonio, Texas). Measurements were performed by two independent investigators (D.K.J. and H.S.K.) blinded to the subject’s identity and medication status. Measurements of maximum diameter and percent flow-mediated dilation were made in 10 studies selected at random. The interobserver and intraobserver variability for repeated measurement of maximum diameter were 0.004 ± 0.039 mm and 0.005 ± 0.089 mm, respectively. The interobserver and intraobserver variability for repeated measurement of percent flow-mediated dilation were 0.07 ± 1.27% and 0.15 ± 1.24%, respectively.

**Statistical analysis.** Data are expressed as mean ± SEM or median (range: 25% to 75%). After testing data for normal-
Table 1. Effects of Placebo or Oral Candesartan on Lipids and Endothelial Function in Hypertensive Patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo</th>
<th>Candesartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipids (mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>208 ± 7</td>
<td>202 ± 6</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>50 ± 2</td>
<td>51 ± 3</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>116 ± 5</td>
<td>113 ± 5</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>203 ± 22</td>
<td>193 ± 19</td>
</tr>
<tr>
<td>Vasomotor function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flow-mediated dilation (%)</td>
<td>5.17 ± 0.24</td>
<td>6.22 ± 0.26‡</td>
</tr>
<tr>
<td>Nitroglycerin dilation (%)</td>
<td>13.73 ± 0.58</td>
<td>14.18 ± 0.57</td>
</tr>
<tr>
<td>MDA (µM)</td>
<td>1.50 ± 0.07</td>
<td>1.29 ± 0.09‡</td>
</tr>
<tr>
<td>Inflammation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP-1 (pg/ml)</td>
<td>213 ± 8</td>
<td>190 ± 7†</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>2.93 (1.87–4.31)</td>
<td>2.22 (1.85–3.75)*</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>0.16 (0.11–0.27)</td>
<td>0.17 (0.11–0.26)</td>
</tr>
<tr>
<td>Hemostasis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antithrombin III (mg/dl)</td>
<td>28.8 ± 0.5</td>
<td>29.0 ± 0.5</td>
</tr>
<tr>
<td>PAI-1 (ng/ml)</td>
<td>74 ± 4</td>
<td>53 ± 4‡</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>285 ± 10</td>
<td>285 ± 11</td>
</tr>
<tr>
<td>MMP-9 (ng/ml)</td>
<td>24.4 ± 1.7</td>
<td>27.2 ± 2.5</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM or median (25% to 75%). *p < 0.05; †p < 0.01; ‡p < 0.001 vs. placebo.

CRP = C-reactive protein; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MCP = monocyte chemoattractant protein; MDA = malondialdehyde; MMP = matrix metalloproteinase; PAI = plasminogen activator inhibitor; TNF = tumor necrosis factor.

ity, we used the Student paired t or Wilcoxon signed rank test to compare values after placebo and candesartan therapies, as reported in Table 1. We decided to use the end of a two-month treatment period without washout and the second baseline. Indeed, we found no carryover effect in this study (see Results section). Pearson correlation coefficient analysis was used to assess associations between measured parameters. We calculated that 30 subjects would provide 80% power for detecting an absolute increase of ≥2.1% (α = 0.05, based on our previous studies) (39) flow-mediated dilation of the brachial artery between baseline and candesartan. A value of p < 0.05 was considered statistically significant.

RESULTS

To assess the possibility of a carryover effect from the initial treatment periods to the next treatment period, we compared the percent changes of: 1) the first treatment placebo, and the second treatment placebo 2) the first treatment candesartan and the second treatment candesartan, relative to baseline values. There were no significant differences in age and baseline values—vascular function (diameter and flow) and markers of inflammation—between each group. No significant differences were found in the above two comparisons (data not shown).

Effects of therapies on BP and lipids. Compared with placebo, candesartan therapy reduced systolic (160 ± 2 vs. 140 ± 2 mm Hg, p < 0.001) and diastolic (99 ± 1 vs. 87 ± 1 mm Hg, p < 0.001) BP after two months’ administration of oral candesartan. Resting heart rate (83 ± 1 vs. 84 ± 1, p = 0.575) was similar after candesartan therapy. Candesartan therapy did not change lipoprotein levels (Table 1).

Effects of therapies on malondialdehyde and vasomotor function. Basal brachial artery diameter and forearm blood flows were similar to those during placebo or candesartan treatment periods, as were the peak brachial artery diameters and forearm blood flows during reactive hyperemia and the percent increase in flow during hyperemia (data not shown). Compared with placebo, candesartan therapy significantly reduced plasma levels of MDA and improved the percent flow-mediated dilator response to hyperemia by 14 ± 5% and 29 ± 7%, respectively (p = 0.009 and p < 0.001, respectively). The brachial artery dilator response to nitroglycerin between each therapy was not significantly different (p = 0.296).

Effects of therapies on markers of inflammation. Compared with placebo, candesartan therapy significantly lowered plasma levels of MCP-1 by 9 ± 3% and TNF-alpha by 10 ± 4% from the respective baseline levels (p = 0.003 and p = 0.026, respectively, Fig. 1). We observed that patients with the highest baseline MCP-1 levels showed the greatest extent of reductions with candesartan (r = −0.363, p = 0.013). However, candesartan therapy did not significantly lower serum levels of CRP (p = 0.799).

Effects of therapies on markers of hemostasis and plaque stability. Compared with placebo, candesartan therapy significantly lowered plasma levels of PAI-1 by 21 ± 5% from the respective baseline levels (p < 0.001; Fig. 2). However, candesartan therapy did not significantly lower plasma levels of antithrombin III, fibrinogen, and MMP-9 (p = 0.284, p = 0.768, and p = 0.282, respectively).

We investigated whether candesartan-induced changes in the percent flow-mediated dilator response to hyperemia
CRP and MCP-1 levels (r/H11005/H11002)ing a mechanism for the regulation of CRP and MCP-1 (0.127). Furthermore, despite an experimental study showing improvement in NO bioactivity or reduction of oxidant stress, we observed no significant correlations (fi/H11002/0.119). There were no significant correlations between these changes and reduction of systolic BP (r/H11349/0.262) and between these changes and reduction of diastolic BP (r/H11349/0.197). We also investigated whether the candesartan-induced reduction in serological markers of inflammation was mediated by improvement in NO bioactivity or reduction of oxidant stress. There were no significant correlations (r/H11349/0.119 ≤ r ≤ 0.127). Furthermore, despite an experimental study showing a mechanism for the regulation of CRP and MCP-1 levels (41), we observed no significant correlations between CRP and MCP-1 levels (r = −0.162, p = 0.307).

DISCUSSION

We observed that two months of candesartan therapy significantly improved the percent flow-mediated dilator response to hyperemia and reduced levels of oxidant stress and inflammatory and impaired fibrinolysis markers in hypertensive patients, independent of lipoprotein and BP changes.

Our finding of improved endothelium-dependent vaso- motor responsiveness is consistent with other groups’ results (23–25). Thus, Schiffrin et al. (24) demonstrated that, in contrast with atenolol, losartan corrected endothelial dysfunction in patients with essential hypertension, and Ghia- doni et al. (25) also demonstrated similar improvement in endothelial function with candesartan. We reasoned that candesartan might improve endothelium-dependent vaso- motor responsiveness by reducing oxidant stress and augmenting NO bioactivity, considering that AII is a potent vasoconstrictor that also promotes oxidant stress. We observed that two months of candesartan therapy significantly reduced plasma levels of MDA, compared with placebo. Although we did not measure prostaglandin F2α, studies have reported an excellent correlation between prostaglan- din F2α and MDA levels (42,43).

In order to gain insight into mechanisms of potential vasculoprotective effects of candesartan, we measured surrogate markers for vascular inflammation. Inflammatory cytokines secreted by macrophages and T lymphocytes can modify endothelial function, smooth muscle cell proliferation, collagen degradation, and thrombosis (4,44). We observed that candesartan therapy significantly reduced plasma levels of MCP-1 and TNF-alpha but not CRP and fibrinogen levels.

Tomita et al. (45) observed that inhibition of NO synthesis with Nω-nitro-L-arginine methyl ester induced inflammatory changes and MCP-1 expression in rat hearts and vessels. Activated platelets stimulated MCP-1 production in endothelial cells via an NFκB-dependent mechanism (46). On the other hand, experimental studies demonstrated that AII activates NFκB through AT1 and AT2 receptor stimulation (10) and induces MCP-1 expression in cell culture (47); furthermore, candesartan appears to inhibit the enhancement of MCP-1 expression in rats (48). However, the effects of AT1 receptor blockade on MCP-1 are controversial in patients (29–31). For example, losartan at a mean dose of 77 mg daily did not significantly alter the levels of soluble cell adhesion molecules (consistent with our previous findings [28]) or MCP-1 (29). However, candesartan administered at 16 mg daily for six weeks significantly reduced levels of soluble intercellular adhesion molecule-1 and MCP-1 in hypercholesterolemic patients (30,31), a finding that is consistent with our current study. The difference between losartan and candesartan may partly be attributed to a tenfold stronger AT1 blocking effect of the latter agent. We hypothesize that candesartan may reduce plasma MCP-1 levels via inactivation of NF-κB, due to an improved bioactivity of NO, as shown by improvement in flow-mediated dilation. However, we did not observe a significant correlation between the changes in flow-mediated dilation and plasma levels of MCP-1, a finding that may be explained by the complex pathogenesis of atherosclerosis involving multiple interactions and the relatively small sample size (β error).

We also observed that candesartan reduced plasma levels of TNF-alpha. Other studies have observed this effect in patients with heart failure (26) and coronary artery disease (27). Experimental studies demonstrated that CRP stimulates the synthesis of MCP-1 (41). In the current study, candesartan did not alter serum CRP levels, consistent with other studies (27,28). We observed no correlations between CRP levels and MCP-1 levels in humans.

Previous studies have shown that the AT1 receptor...
blocker losartan partially blocks AII-induced PAI-1 expression in cell culture studies (21). In contrast, other reports have suggested that the AT1 receptor does not mediate AII-stimulated PAI-1 expression (49). However, recent studies demonstrated the role of the AT1 receptor in mediating the effects of AII on PAI-1 expression (19,20,50). Furthermore, unlike losartan, candesartan abolished the stimulatory action of AII on PAI-1 expression (19,50). In human studies the effect of losartan on PAI-1 antigen remains controversial. For example, Erdem et al. (33) reported that losartan and perindopril significantly reduced PAI-1 antigen levels in patients with hypertension. Goodfield et al. (34) reported that, in patients with heart failure, losartan 50 mg, but not enalapril, reduced PAI-1 levels. In contrast, other studies demonstrated that losartan did not change in patients (32,51–53). The effect of candesartan on PAI-1 antigen is controversial. In postmenopausal women, candesartan 8 mg daily for 12 weeks significantly increased plasma PAI-1 antigen (32). However, in hypercholesterolemic patients, candesartan 16 mg daily for six weeks significantly decreased plasma PAI-1 antigen (30,31). This discrepancy may result from the duration of treatment, the patient population, and the drug dosage. On the other hand, the complete blockade of AII-induced PAI-1 expression by candesartan may be attributed to its higher affinity and slower dissociation kinetics than losartan (54,55). Thus, candesartan reduces maximal AII binding to the AT1 receptor, whereas losartan causes a rightward shift in the AII dose response without reducing maximal AII binding (55). It is therefore likely that the partial reduction of AII-induced PAI-1 expression by losartan (21) was due to incomplete antagonism of the AT1 receptor. In this regard, we observed that candesartan 16 mg daily for eight weeks significantly reduced plasma levels of PAI-1 antigen. Further, of the six postmenopausal women in our study, five showed candesartan-induced reduction in PAI-1 antigen levels relative to baseline values. The mean reduction of PAI-1 antigen levels in all six were similar to the effect of candesartan in all 45 patients (27% vs. 21%). Several studies hypothesized that the effects of AT1 receptor blockers on endothelial function go beyond the reduction of BP (1,24,30,56). We observed that candesartan changed the percent flow-mediated dilator response to hyperemia and serological markers of oxidant stress, inflammation, and fibrinolysis independent of lowering BP. In summary, we observed that candesartan therapy in hypertensive patients reversed endothelial dysfunction, measured as an improvement in flow-mediated dilatation and fibrinolysis and reduction of oxidant stress and inflammatory cytokines independent of BP changes, suggesting that AT1 receptor blocker therapy has antiatherogenic effects.

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**REFERENCES**


