Vascular Effects of ACE Inhibition Independent of the Renin-Angiotensin System in Hypertensive Renovascular Disease

A Randomized, Double-Blind, Crossover Trial

Jacobine M.A. van Ampting, Michel L. Hijmering, Jaap J. Beutler, Ronald E. van Etten, Hein A. Koomans, Ton J. Rabelink, Erik S.G. Stroes

Abstract—To evaluate whether ACE inhibition and angiotensin II type 1 blockade exert beneficial effects on NO availability independent of their blood pressure–lowering effect, we used a double-blind crossover design to study vascular function in 18 patients with hypertensive renovascular disease during 6 weeks of therapy with enalapril (Ena) and valsartan (Val) compared with non–renin-angiotensin system–mediated treatment with the α₁-blocker doxazosin (Dox). Control measurements were performed in 13 age-matched volunteers. Forearm blood flow was assessed with venous occlusion plethysmography, and serotonin and nitroprusside were used as endothelium-dependent and -independent vasodilators, respectively. Blood pressure was similar during all treatment periods. Serotonin-induced vasodilation was decreased in patients during Dox treatment (n = 12) compared with control subjects (n = 13) (increase 42 ± 20% versus 107 ± 65%, P < 0.05). Crossover from Dox to Val (n = 6) had no effect on serotonin response (increase 50 ± 14%), but crossover to Ena (n = 6) caused a significant improvement (increase 79 ± 39%, P < 0.05 versus Dox). In an assessment of all patients, serotonin-induced vasodilation during Ena (n = 12, increase 75 ± 31%) was increased compared with both Val and Dox (43 ± 14% and 42 ± 20%, respectively; both P < 0.05 versus Ena). The nitroprusside response remained unaltered during all treatment periods. In conclusion, ACE inhibition improves the impaired endothelium-dependent vascular function in patients with hypertensive renovascular disease. This effect is unrelated to blood pressure–lowering or angiotensin II–mediated effects. (Hypertension. 2001;37:40-45.)

Key Words: endothelium • angiotensin-converting enzyme inhibitors • angiotensin II receptors, angiotensin • nitric oxide • hypertension, renovascular

During the past decade, the assessment of endothelial function has been incorporated in cardiovascular prevention trials as a potential intermediate end point for cardiovascular disease. 1,2 Currently, data are accumulating to show that endothelial dysfunction has prognostic value for the occurrence of future cardiovascular disease. 3–5 The latter observation, taken together with the large burden of evidence showing that NO is a pivotal player in the antiatherogenic effects of the endothelium, 6,7 underscores the relevance of improving endothelial function as a potential therapeutic target.

The renin-angiotensin system interacts with endothelial function and, in particular, with NO availability in several ways. First, angiotensin II (Ang II) not only induces a potent vasoconstrictor response but also stimulates NAD(P)H:oxidase–dependent oxygen radical release. 8–10 Both of these actions are mediated through the Ang II type 1 receptor. 5,11 Oxygen radicals react with NO at a diffusion limited rate and thus contribute to impaired NO availability. 12,13 Second, the Ang II type 2 receptor has been suggested to induce NO release on activation by Ang II. 14 Third, ACE not only mediates conversion from Ang I to Ang II but is also responsible for the degradation of bradykinin. 15,16 Bradykinin has direct stimulatory effects on NO synthase, resulting in increased NO release. Thus, theoretically, both Ang II type 1 receptor antagonists and ACE inhibitors may have beneficial effects on endothelial function. Thus far, several studies have shown an improvement in endothelial function by ACE inhibition in atherosclerotic, 17,18 diabetic, 19,20 and hypertensive 21 subjects. In contrast, the effect of Ang II type 1 receptor antagonists on endothelial function remains to be established.

To evaluate the extent to which the various pathways (ie, Ang II, bradykinin, and/or blood pressure lowering per se)
The procedures followed were in accordance with institutional guidelines. All subjects refrained from tobacco, alcohol, and caffeine-containing drinks for ≥12 hours before measurements were performed. All subjects gave written informed consent. The study was approved by the University Medical Center Utrecht Ethical Committee for Studies in Humans. The procedures followed were in accordance with institutional guidelines.

Study Protocol

All studies were performed in a quiet room maintained at a controlled temperature between 22°C and 24.5°C. The subjects were supine with both forearms resting slightly above heart level. A 20-gauge needle was inserted into the brachial artery after local anesthesia. Forearm blood flow (FBF) was measured simultaneously in both arms with venous occlusion plethysmography as described previously. Baseline measurements were started ≥30 minutes after cannulation of the brachial artery, when FBF had stabilized.

For an assessment of endothelium-dependent vasodilatation, serotonin (Sigma Chemical Co) was infused into the brachial artery in increasing doses of 0, 0.2, 0.6, 1.8, and 6.0 ng/100 mL forearm volume (FAV)/min for 5 min/dose. These dosages have previously been shown to cause specific NO-mediated vasodilation. For an assessment of endothelium-independent vasodilation, sodium nitroprusside (Merck) was administered intra-arterially at incremental doses of 0, 6, 60, 180, and 600 ng ⋅ 100 mL FAV−1 ⋅ min−1 for 5 min/dose. The order of these 2 infusion blocks was randomized. Blood samples taken before FBF measurements were tested for creatinine, glucose, total cholesterol, HDL cholesterol, and triglyceride levels.

Analysis

FBF was expressed as mL ⋅ 100 mL forearm tissue−1 ⋅ min−1. Results are expressed as mean±SD. Baseline characteristics of patients and control subjects were compared with use of an unpaired t test. Average values of FBF in both arms were obtained for the last 5 or 6 consecutive recordings of each measurement period. Changes in percent increase/decrease in FBF during serotonin and nitroprusside infusion were evaluated with repeated measures ANOVA for the 3 crossover groups (3×n=6). For a comparison of all enalapril therapy measurements (n=12), all valsartan measurements (n=12) and all doxazosin measurements (n=12), as well as for a comparison of the responses in patients versus control subjects, nonrepeated measures ANOVA was used. If variance ratios reached statistical significance, differences between the means were analyzed with the Student-Newman-Keuls test for P<0.05.

Results

The characteristics of patients and control subjects are shown in Table 1. In control subjects, serotonin induced a 107±65%
increase in FBF (absolute FBF in the infused arm from 2.5±0.8 to 4.8±2.0; Figure 1). On sodium nitroprusside infusion, FBF increased from 100% to 412±97% (2.6±0.8 to 10.4±3.8; Figure 1). During non–renin-angiotensin system (RAS)–mediated antihypertensive treatment (ie, doxazosin) serotonin-induced, endothelium-dependent vasodilation was significantly impaired in patients versus control subjects (P<0.05; Figure 1), whereas endothelium-independent vasodilation was not significantly different (Figure 1). In both control subjects and patients, FBF in the control arm did not change significantly during the infusion blocks.

**Doxazosin-Enalapril Crossover**
Serotonin-induced vasodilation increased significantly in patients during enalapril compared with during doxazosin. During doxazosin treatment, serotonin induced a 38±19% increase in FBF (absolute FBF in the infused arm increased from 2.8±0.6 to 3.9±0.9), whereas during enalapril, the increase was 79±39% (2.6±0.6 to 4.6±1.5) (P<0.05, doxazosin versus enalapril; Figure 2).

On sodium nitroprusside infusion, the FBF increased from 100% to 419±112% (3.1±1.1 to 12.3±2) during doxazosin and from 100% to 436±145% (2.9±0.6 to 12.5±3.4) during enalapril (NS, Figure 3). The mean arterial pressure was 104±16 mm Hg during doxazosin treatment and 97±7 mm Hg during enalapril treatment.

**Doxazosin-Valsartan Crossover**
Serotonin-induced vasodilation in patients during doxazosin treatment was not significantly different from serotonin-induced vasodilation during valsartan. Doxazosin treatment, serotonin induced a 45±23% increase in FBF (absolute FBF in the infused arm from 2.5±1.2 to 3.9±2), whereas during valsartan, the increase was 50±14% (2.9±0.6 to 4.1±0.7) (NS, Figure 2). On sodium nitroprusside infusion, the FBF increased from 100% to 415±120% (2.9±1.3 to 11.7±3.5) during doxazosin and from 100% to 434±123% (2.7±0.9 to 11.4±4.1) during valsartan (NS, Figure 3). The mean arterial pressure was 101±10 mm Hg during doxazosin treatment and 103±15 mm Hg during valsartan treatment.

**Enalapril-Valsartan Crossover**
Serotonin-induced vasodilation in patients during enalapril treatment was not significantly different from serotonin-induced vasodilation with valsartan. FBF increased from 100% to 172±25% (absolute FBF in the infused arm from 3.2±1.1 to 5.6±2.2) during enalapril and from 100% to 136±10% (3.3±1 to 4.4±1.4) during valsartan (NS, Figure 2). During sodium nitroprusside infusion, the FBF changed from 100% to 432±121% (2.9±0.8 to 11.9±3.7) with enalapril and from 100% to 422±131% (3.3±1.1 to 12.3±6) with valsartan (Figure 3). The mean arterial pressure was 96±21 mm Hg during enalapril treatment and 97±16 mm Hg during valsartan treatment.

On analysis of all treatment periods with serotonin in the patient group, serotonin-induced vasodilation during enalapril (n=12) was significantly increased compared with the response during both doxazosin (n=12) and valsartan (n=12). FBF increased from 100% to 175±31% (2.7±1.0 to 4.7±1.9) with enalapril, from 100% to 142±20% with doxazosin (2.7±0.9 to 3.9±1.5), and from 100% to 143±14% with valsartan (3.1±0.8 to 4.3±1.1) (P<0.05 enalapril versus doxazosin and enalapril versus valsartan; Figure 4). Serum creatinine levels did not change significantly during medication switches.
The absence of an improvement in vascular function by Ang II type I receptor inhibition is in accordance with the BANFF trial, in which the Ang II type I receptor losartan had no effect on endothelium-dependent vasomotion. Accordingly, 2 months of therapy with the Ang II type I receptor blocker candesartan had no effect on acetylcholine-induced vasodilation in patients with essential hypertension. In contrast, recent data have suggested a positive effect of Ang II type 1 receptor blockade. However, in 1 study, a short-term, local intra-arterial infusion of an Ang II type 1 receptor was administered whereas in another study, the effects were observed in diabetic patients without overt macrovascular disease. Hence, these data cannot be compared with our results.

Of note, in a previous study, insufficient dosing of the Ang II receptor antagonist has been suggested to potentially explain the lack of vascular effects. In the present study, however, we have used a highly potent, noncompetitive Ang II type 1 receptor antagonist, at a dose near the top of the dose-response curve, making insufficient dosing of the receptor blocker less likely.

**ACE Inhibition**

In the present study, RAS inhibition with an ACE inhibitor resulted in a significant improvement in serotonin-stimulated endothelium-dependent vasomotion compared with blood pressure lowering with doxazosin therapy. This finding is in agreement with earlier studies in diabetic patients, in patients with coronary artery disease, in hypertensive patients, and in healthy volunteers. This selective improvement in endothelium-dependent vasomotion cannot be related to inhibition of Ang II formation, because Ang II receptor antagonism had no effect on endothelium-dependent vasomotion. Also, it cannot be explained by the blood pressure–lowering effect of ACE inhibition, because again both α-blockade and angiotensin receptor antagonism had no effect on serotonin-induced vasodilation.

Attention has focused on the dual action of ACE inhibition, which, in addition to mediating the conversion of Ang I to Ang II, is responsible for the inactivation of bradykinin, the latter being a potent activator of endothelial NO synthase. In this respect, it has been demonstrated in vivo that both the hypotensive and the vascular effects of ACE inhibition are at least in part mediated by bradykinin. However, the improved endothelium-dependent vasomotion during ACE inhibition in our study cannot be attributed to a bradykinin effect for 2 reasons: First, we infused serotonin as NO agonist. Serotonin acts directly through the 5-hydroxytryptamine receptor and thus acts independent of bradykinin. Moreover, degradation of serotonin is independent of kinase activity. Second, serotonin-induced vasodilation is completely blocked by NO inhibition whereas microvascular vasodilation in the forearm by bradykinin cannot be inhibited by NO inhibition.

What mechanisms could then be held responsible for the enhanced endothelium dependent vasodilation to acetylcholine and serotonin during ACE inhibition? In view of the former discussion, it most likely involves increased NO release, which is supported by recent findings in patients with
essential hypertension, where ACE inhibition caused a significant increase in plasma NO metabolites. In line, recent data have shown that ACE inhibition results in a sustained, ~2-fold increase in endothelial NO synthase expression in endothelial cells, which is accompanied by an enhanced production of NO after agonist-induced stimulation. Further studies are needed to elucidate whether changes in NO production in vivo are responsible for the different vascular effects during ACE inhibition and/or Ang II type I receptor inhibition in these patients.

Study Limitations
In the present study, we show a beneficial effect of ACE inhibition on NO availability. Besides the interaction of the RAS with NO-mediated vasodilatation, Ang II also has clear interactions with several “proatherogenic” pathways, including stimulation of transforming growth factor-β, a key factor in the transition from initial tissue injury to tissue fibrosis, and stimulation of endothelin-1 release, which exerts strong vasoconstrictor and proliferative effects. In this respect, the “selective” improvement in vascular function during ACE inhibition has to be interpreted with caution as a surrogate end point for future cardiovascular disease, because the effects of changes in other proatherogenic, vessel-damaging pathways by, for example, Ang II type 1 receptor antagonists may also be of importance for cardiovascular “damage control.”

Clinical Implications
In the present study, we show that ACE inhibition has selective beneficial effects on endothelial dysfunction in a high-risk population with generalized atherosclerosis, hypertension, and older age. It is interesting to note that recent data from the HOPE trial have emphasized that the blood pressure–lowering effect of ACE inhibitors can be held responsible for only ~30% of the total cardiovascular benefit, seen after relatively short-term ACE inhibitor therapy in patients at an increased cardiovascular risk. As such, it is a challenge to determine the extent to which improvement in endothelial function by ACE inhibition contributes to improved cardiovascular outcome for cardiovascularly compromised patients and the extent to which endothelial function testing can contribute to further optimization of cardiovascular preventive strategies for the individual patient.

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References


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