Effects of Angiotensin Converting Enzyme Inhibitor and Calcium Antagonist on Endothelial Function in Patients with Essential Hypertension

Young-Keun ON, Cheol-Ho KIM*, Byung-Hee OH*, Myoung-Mook LEE*, and Young-Bae PARK*

The endothelium plays an important role in maintaining vascular tone and function. Essential hypertension is associated with alterations in endothelial function. The effects of antihypertensive agents on endothelial function have not been fully evaluated in human hypertension and data on the forearm circulation of humans are controversial. The aim of this study was to determine whether treatment with an angiotensin converting enzyme (ACE) inhibitor or a calcium antagonist improves endothelial dysfunction in hypertensive patients and whether the mechanism involved could be related to antioxidant activity. Endothelial function was estimated using venous occlusion plethysmography in 18 hypertensive patients and 11 healthy volunteers. The patients in the hypertension group were treated with enalapril or amlodipine. The change of forearm blood flow (FBF) was measured during acetylcholine infusion through the brachial artery and also during intra-arterial vitamin C infusion to explore the effects of vitamin C on responses to acetylcholine. FBF response to acetylcholine was significantly enhanced by intra-arterial infusion of vitamin C in the hypertensive group before antihypertensive treatment. Co-infusion of L-NMMA (N\textsuperscript{G}-monomethyl-L-arginine), an inhibitor of nitric oxide synthase, blunted forearm blood flow response to acetylcholine. After antihypertensive treatment with enalapril or amlodipine for 2 months in the hypertensive group, endothelium-dependent vasorelaxation (vasodilatory response to acetylcholine) was significantly improved. Even though the mechanisms leading to depressed endothelial function in essential hypertension remain to be elucidated, our study shows that treatment with an ACE inhibitor or a calcium antagonist resulted in demonstrable improvement by a mechanism that is probably related to antioxidant activity. (Hypertens Res 2002; 25: 365–371)

Key Words: plethysmography, endothelium, ascorbic acid, angiotensin converting enzyme inhibitor, calcium antagonist

Introduction

The endothelium plays an important role in maintaining vascular tone and function. The main endothelium derived factor is nitric oxide (NO), which is not only a potent vasodilator but also an inhibitor of platelet aggregation, smooth muscle cell migration and proliferation, monocyte adhesion and adhesion molecule expression, thereby protecting the vessel wall against the development of atherosclerosis and thrombosis.

Essential hypertension is associated with alterations in endothelial function. Endothelium-dependent vasodilation has been shown to be reduced in the brachial (1–3), coronary (4), renal (5, 6) and femoral (7) arteries in patients with essential hypertension. Impairment of endothelial function has been
shown to play an important role in the development and maintenance of hypertension (8). Therefore, an important aim of antihypertensive therapy would be to not only normalize blood pressure values but also reverse endothelial dysfunction by restoring NO availability.

Several studies have demonstrated that restoration of endothelial function through the administration of antihypertensive agents is essential in the treatment of hypertensive patients (8–10), while others have shown that effective antihypertensive therapy did not restore impaired endothelium-dependent vasodilation in the forearm circulation of hypertensive patients (11, 12).

The effects of antihypertensive agents on endothelial function have not been fully evaluated in human hypertension, and data on the forearm circulation of humans are controversial. It may be clinically important to select an appropriate antihypertensive agent that is effective in improving endothelial dysfunction in patients with established essential hypertension.

Recently, the role of superoxide anion and its interaction with NO have been investigated (13). Under physiological conditions, these oxygen free radicals are potent chemical inactivators of NO (14, 15). And the balance between NO and superoxide is more important than the absolute levels of either alone (16).

Vitamin C is an important antioxidant in human plasma, capable of scavenging oxygen free radicals and sparing other endogenous antioxidants from consumption (17).

Therefore, the aim of this study was to determine whether treatment with an angiotensin converting enzyme (ACE) inhibitor or a calcium antagonist would improve endothelial dysfunction in hypertensive patients and, if so, whether the mechanism involved could be related to antioxidant activity.

Methods

Subjects

Eighteen hypertensive patients (7 men and 11 women; age range, 35 to 73 years) were recruited. They had a clinical blood pressure reading (the average of 3 different sphygmomanometric measurements, each performed on 3 separate days) of >140/90 mmHg. The possibility of secondary causes of hypertension was excluded by standard clinical and laboratory tests. Exclusion criteria were 1) evidence of overt atherosclerotic disease, i.e., coronary artery disease, peripheral vascular disease, stroke, etc.; 2) the presence of other risk factors of atherosclerosis, i.e., current smoking and smoking within 1 year, severe hypercholesterolemia (>240 mg/dl), and diabetes mellitus; 3) advanced organ failure; and 4) malignancy. In all patients, a noninvasive 24-h blood pressure monitoring was performed at baseline and after treatment. Eleven normal volunteers with normal blood pressure were enrolled. Exclusion criteria were the same as for the hypertensive group.

Study Design

Subjects were randomly assigned to one of two groups, a group receiving enalapril 10–20 mg/day and a group receiving amlodipine 5–10 mg/day for at least 2 months. Only hypertensive subjects underwent the treatments. Clinical profiles of the subjects are shown in Table 1. Forearm vascular function was studied before and after antihypertensive treatment. Subjects were required to refrain from drinking alcohol or caffeinated beverages for 12 h before the study.

The protocol of the study was approved by the ethics committee of our institution, and informed consent was obtained from each participant. The procedures followed were in accordance with institutional guidelines.

Venous Occlusion Plethysmography

The examination was done with subjects in a supine position. The brachial artery of the nondominant arm was cannulated with a 20 gauge cannula. A mercury-filled silastic strain gauge was placed around the thickest part of the forearm. The size of the strain gauge was selected to be about 2 cm less than the maximal forearm circumference. The strain gauge was connected to a plethysmograph (EC-R5; Hokanson, Issaquah, USA) to record the forearm volume change. A rapid cuff inflator (E-10; Hokanson) was used to inflate the arm cuff to 40 mmHg instantaneously, thus occluding venous return from the forearm. A wrist cuff was inflated to 20 mmHg above the systolic pressure to cut off the arterial flow to the hand. Intra-arterial pressure was measured continuously (Space Lab., Redmond, USA) throughout the study. Drug infusions were administered using a constant rate infusion pump. The measurement of forearm volume change was repeated 7 times for each stage. Between infusions, the cuffs were deflated, allowing at least 15 min for forearm blood flow to recover from the preceding infusion and before further baseline measures were recorded. All solutions were prepared aseptically from sterile stock solutions or ampoules immediately before infusion into the brachial artery. Acetylcholine and N\textsuperscript{G} monomethyl-L-arginine (L-NMMA) had been diluted in distilled water and filtered through 0.22 \textmu m

Table 1. Clinical Characteristics of the Hypertensive Patients (HT) and Normal Controls (NC)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NC</th>
<th>HT</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>11</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>50</td>
<td>57</td>
<td>0.13</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>4:7</td>
<td>7:11</td>
<td>0.90</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>182</td>
<td>199</td>
<td>0.43</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>115</td>
<td>166</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>65</td>
<td>94</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.
Table 2. Absolute FBF Values before and after L-NMMA Infusion

<table>
<thead>
<tr>
<th></th>
<th>NC</th>
<th>HT</th>
<th>Enalapril Tx</th>
<th>Amlodipine Tx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>4.2 ± 0.6</td>
<td>5.7 ± 0.5</td>
<td>4.3 ± 0.7</td>
<td>4.9 ± 0.3</td>
</tr>
<tr>
<td>L-NMMA</td>
<td>4.6 ± 1.8</td>
<td>6.5 ± 0.4</td>
<td>5.2 ± 0.7</td>
<td>5.4 ± 0.4</td>
</tr>
<tr>
<td>Vit C basal</td>
<td>6.1 ± 1.1</td>
<td>7.8 ± 0.9</td>
<td>6.2 ± 1.2</td>
<td>6.9 ± 0.9</td>
</tr>
<tr>
<td>Vit C + L-NMMA</td>
<td>8.6 ± 0.9*</td>
<td>7.8 ± 0.9</td>
<td>7.8 ± 1.0*</td>
<td>12.4 ± 2.2*</td>
</tr>
</tbody>
</table>

Values are means ± SEM in ml/100 ml forearm/min. NC, normal controls; HT, hypertensive patients; Tx, treated; L-NMMA, Nω-monomethyl-L-arginine; Vit C, vitamin C. *p < 0.05 L-NMMA vs. Vit C + L-NMMA.

**Evaluation of Endothelium-Dependent Vasodilation and the Effect of Vitamin C**

An intra-arterial infusion of 5% dextrose solution was begun at 1 ml/min and continued throughout the drug infusion. Basal measurement was obtained during the infusion of dextrose solution. Acetylcholine (Sigma Chemicals, St. Louis, USA) was infused intra-arterially at 7.5, 15 and 30 µg/min. Acetylcholine was infused for 5 min for each dose level, and forearm blood flow was measured during the last 2 min of each dose. After a 15-min wash-out period, vitamin C was intra-arterially infused at 24 mg/min for 10 min. During continued infusion of vitamin C at the same rate, acetylcholine was infused at incremental doses as in the previous stage, and forearm blood flow (FBF) was measured during the last 2 min of each dose. After an additional 15 min of rest, intra-arterial infusion of vitamin C at the same rate plus infusion of L-NMMA(Sigma Chemicals), an inhibitor of nitric oxide synthesis, at 100 µg/min was begun and continued for 5 min. Then acetylcholine was infused in incremental doses and forearm blood flow was measured as in the previous stages. After a 15-min wash-out period, sodium nitroprusside was infused intra-arterially at 1, 2 and 4 µg/min. Sodium nitroprusside was infused for 5 min for each dose level, and forearm blood flow was measured during the last 2 min of each dose.

**Statistical Analysis**

Forearm blood flow was measured and FBF changes during acetylcholine infusion were expressed as percentage changes from the baseline immediately preceding each drug administration. Forearm blood flow changes were compared between the normal and hypertensive groups with and without vitamin C infusion. Comparison was also made between before and after antihypertensive treatment in the hypertensive group. Finally, FBF was compared in the hypertensive group with and that without vitamin C infusion. Repeated measures ANOVA were used by means of SAS® ver. 6.12. All values were expressed as the mean ± SE and values of p < 0.05 were considered to indicate statistical significance.

**Results**

**Clinical Characteristics**

Eighteen hypertensive patients (HT) and eleven normotensive controls (NC) (11 men and 18 women; age range, 35 to 73 years) were recruited. There were no significant differences in cholesterol level between normotensive controls and hypertensive patients. Only systolic and diastolic blood pressure differed between the two groups (Table 1). After 2-month antihypertensive treatment, there was a significant decrease in systolic and diastolic blood pressure (enalapril-treated group: pre-treatment, 163 ± 6/94 ± 3 mmHg; post-treatment, 128 ± 5/71 ± 3 mmHg; p < 0.05; amlodipine-treated group: pre-treatment, 169 ± 8/98 ± 3 mmHg; post-treatment, 131 ± 3/74 ± 3 mmHg; p < 0.05; values are the mean ± SEM). There were no significant differences in systolic or diastolic blood pressure between the enalapril-treated group and amlodipine-treated groups.

Absolute FBF data recorded in the infused limbs at baseline were not significantly different between groups (normotensive, 4.2 ± 0.6; hypertensive, 5.7 ± 0.5; enalapril-treated group, 4.3 ± 0.7; amlodipine-treated group, 4.9 ± 0.3 ml/100 ml forearm/min; values are the mean ± SEM; p = 0.15). But during the infusion of acetylcholine at maximal dose level, absolute FBF increased after antihypertensive treatment (normotensive, 17.6 ± 1.9; hypertensive, 15.9 ± 1.6; enalapril-treated group, 26.4 ± 4.2; amlodipine-treated group, 26.1 ± 4.3 ml/100 ml forearm/min; values are the mean ± SEM; p = 0.02).

Table 2 shows the absolute basal forearm blood flow data after L-NMMA infusion. There were no significant differences in basal FBF after L-NMMA infusion between normotensive controls and hypertensive patients. However, administration of vitamin C caused a significant increase in basal FBF after L-NMMA infusion in normotensive controls.

Blood pressure did not change significantly during any of the drug infusions.

**FBF in the HT and NC Groups**

Endothelium-dependent vasorelaxation (vasodilatory re-
response to acetylcholine) was significantly greater in the normal control group than in the hypertensive group before antihypertensive treatment (maximum FBF in NC group, 448 ± 63%; in HT group, 302 ± 58%; p < 0.05; Fig. 1).

Co-infusion of L-NMMA, an inhibitor of NO synthase, blunted the FBF response to acetylcholine, that is, the endothelium-dependent vasorelaxation (maximum FBF in the L-NMMA (−) NC group: 448 ± 63%; in the L-NMMA (+) NC group: 210 ± 30%; p < 0.05; maximum FBF in the L-NMMA (−) HT group: 302 ± 58%; in the L-NMMA (+) HT group: 188 ± 25%; p < 0.05; FBF during co-infusion of L-NMMA in the NC group vs. the HT group, p = 0.23).

**FBF Responses to Sodium Nitroprusside Infusion**

Endothelium-independent vasorelaxation (vasodilatory response to sodium nitroprusside) was similar in the normal control group and hypertensive group (maximum FBF in NC: 424 ± 58%; in HT: 396 ± 64%). No significant differences in the response to sodium nitroprusside were observed between the normotensive and hypertensive groups.

**FBF Response to Intra-Arterial Vitamin C Infusion**

FBF response to acetylcholine was significantly enhanced with intra-arterial infusion of vitamin C (Vit C) in the hypertensive group before antihypertensive treatment (maximum FBF in Vit C (−): 302 ± 58%; in Vit C (+): 446 ± 43%; p < 0.05; Fig. 1A). Such an enhanced response was not observed in the normal control group (maximum FBF in Vit C (−): 448 ± 63%; in Vit C (+): 383 ± 51%; p = 0.11; Fig. 1B). Co-infusion of L-NMMA, an inhibitor of NO synthase, blunted forearm blood flow response to acetylcholine (maximum FBF in Vit C (+): 446 ± 43%; in Vit C + L-NMMA (+): 229 ± 23%; p < 0.05; Fig. 1A).

**FBF after Antihypertensive Treatment in HT Group**

In the hypertensive group, antihypertensive treatment with enalapril for 2 months significantly improved endothelium-dependent vasorelaxation (vasodilatory response to acetylcholine) (maximum FBF: pre-treatment, 361 ± 64%; post-treatment, 643 ± 78%; p < 0.05; Fig. 2A). Intra-arterial infusion of vitamin C in the enalapril-treated hypertensive group did not change the forearm blood flow response to acetylcholine (maximum FBF in Vit C (−): 643 ± 78%; in Vit C (+): 468 ± 52%; p = 0.13; Fig. 2B).

Co-infusion of L-NMMA, an inhibitor of NO synthase, blunted FBF response to acetylcholine in the enalapril-treated hypertensive group (maximum FBF in L-NMMA (−): 643 ± 78%; in L-NMMA (−) HT group: 332 ± 21%; p < 0.05; Fig. 2C). In the hypertensive group, antihypertensive treatment with amlodipine for 2 months significantly improved endothelium-dependent vasorelaxation (vasodilatory response to acetylcholine) (maximum FBF: pre-treatment, 219 ± 23%; post-treatment, 539 ± 69%; p < 0.05; Fig. 3A). Intra-arterial infusion of vitamin C in the amlodipine-treated hypertensive group did not change the forearm blood flow response to acetylcholine (maximum FBF in Vit C (−): 539 ± 69%; in Vit C (+): 448 ± 67%; p = 0.21; Fig. 3B).

Co-infusion of L-NMMA, an inhibitor of nitric oxide synthase, blunted FBF response to acetylcholine in the amlodip-
Discussion

The present study demonstrates that the endothelium-dependent vasodilation is impaired in essential hypertensive patients as compared with normotensive control subject. In addition, short-term intra-arterial administration of the antioxidant vitamin C restores endothelium-dependent vasodilation in patients with essential hypertension. A NO synthase inhibitor, L-NMMA, blunted the improvement of endothelium-dependent vasodilation. These findings suggest that oxygen-derived free radicals may decrease the bioavailability of endothelium-derived NO and impair endothelium-dependent vasodilation in patients with essential hypertension. These results were consistent with previous observations that, in essential hypertensive patients, impaired endothelium-dependent vasodilation of forearm circulation could be improved by the antioxidant vitamin C (3, 18, 19).

After L-NMMA infusion, absolute basal FBF data may constitute an index of basal NO activity. There were no significant differences in basal FBF after L-NMMA infusion between normotensive controls and hypertensive patients. And there were also no significant differences between the pre- and post-hypertensive treatment values of basal FBF during L-NMMA infusion. This finding suggest that basal NO activity in both groups was negligible or too variable to constitute a trend for detection. However, after administration of vitamin C to normotensive controls, there was a significant increase of basal FBF during L-NMMA infusion compared to the level in saline-infused controls. Similar findings were seen after hypertensive treatment. However, such an increase of basal NO activity after vitamin C infusion was not seen in hypertensive patients. The increase in FBF during co-infusion of L-NMMA and vitamin C may indicate that vitamin C increased basal FBF via endothelium-independent mechanisms; further studies will be needed to resolve this matter.

In essential hypertension, impaired endothelium-dependent vasodilation seems to be a primary phenomenon, and the endothelial vasomotor dysfunction is not normalized by the mere reduction of blood pressure (12, 20, 21).
Several investigators have addressed the possibility that antihypertensive treatment could restore or at least improve endothelial function. Particular attention has focused on ACE inhibitors, because of the well-known effect of ACE on degradation of bradykinin. Despite the positive experimental evidence, data on humans are controversial. Higashi et al. reported that ACE inhibitors augmented reactive hyperemia of forearm circulation, an index of endothelium-dependent vasodilation, in patients with essential hypertension (10). Several other studies have demonstrated the restoration of endothelial function of the brachial artery in essential hypertensive patients through the administration of temocapril (9), cilazapril (11), captopril (22), or perindopril (23). However, other studies have reported that therapy with captopril, enalapril (13), or cilazapril (24) did not improve endothelial dysfunction in the forearm vessels of patients with hypertension. Taddei et al. reported that antihypertensive treatment with lisinopril did not improve vasodilation in response to infusion of acetylcholine during acute, prolonged (1 month), or chronic (12 months) treatment (25).

The present study demonstrates that antihypertensive treatment with enalapril for 8 weeks restored endothelial dysfunction in essential hypertensive patients. These findings are consistent with those of Higashi et al. (10), but are inconsistent with those of Creager and Roddy (13). One possible explanation of this discrepancy is that the magnitude of the blood pressure reduction was insufficient to affect endothelium-dependent vasodilation in the study of Creager and Roddy (13). Higashi et al. reported that a comparable blood pressure reduction with thiazide or β blocker did not improve the endothelial function (10). However, the blood pressure reduction induced by the antihypertensive drugs in their study may simply have been insufficient to improve endothelial function. Thus it is important to ensure that an adequate blood pressure reduction is achieved by the antihypertensive drug with antioxidant activity. Accordingly, normalization of the systolic and diastolic blood pressures of all hypertensive patients in the present study was confirmed.

In patients with coronary artery disease, quinapril therapy selectively improves the endothelium-dependent vasodilator responsiveness of the brachial and coronary arteries by increased NO bioactivity (26, 27). Angiotensin II-induced hypertension has been associated with increased vascular superoxide production, and angiotensin II has been shown to stimulate endothelial superoxide production in the human forearm vasculature (28). In another study, oxidative stress appeared to be involved in hypertension in rats and humans (29). Thus ACE inhibitors could exert a beneficial effect on endothelial function by diminishing superoxide production and reducing NO breakdown. This idea is supported by the finding of Kakoki et al. that an ACE inhibitor had a beneficial effect on endothelial function in rats (30).

Calcium antagonists have been shown to be effective in reversing endothelial dysfunction of angiographically normal and stenotic epicardial coronary vessels in essential hypertension (31). In agreement with these results, calcium antagonists have also shown a beneficial effect on endothelial function in the forearm microcirculation (32).

Taddei et al. found that nifedipine increased endothelium-dependent vasodilation by restoring NO availability, an effect probably determined by antioxidant activity (19).

But several other studies have demonstrated that treatment with a calcium antagonist did not improve forearm vasodilator response to reactive hyperemia or to acetylcholine (9, 10, 22). In our findings, prolonged (8 weeks of oral treatment) amlodipine administration did improve endothelium-dependent vasodilation in essential hypertensive patients. And intra-arterial infusion of vitamin C to the treated patients did not increase forearm vasodilation in response to acetylcholine. L-NMMA blunted the effect of amlodipine on endothelium-dependent vasodilatation. These data indicate that amlodipine is effective in improving endothelial dysfunction in essential hypertension, and amlodipine appears to act specifically on the NO pathway by a mechanism that is probably related to antioxidant activity.

In addition, experimental data indicate that calcium antagonists exert an antioxidant effect and thus could protect endothelial cells against free radical injury and diminish oxidative breakdown of NO (33, 34). Taddei et al. found that nifedipine treatment significantly lowered plasma oxidative stress and increased the vasodilatation to acetylcholine (19).

In the present study, however, there were no differences in endothelium-independent vasodilation between the normotensive and hypertensive groups. Because we did not assess FBF responses to sodium nitroprusside infusion after antihypertensive treatment, it may appear that this finding is inconclusive. However, Taddei et al. found that the response to sodium nitroprusside was not changed after antihypertensive treatment (19), and other studies have also reported that endothelium-independent vasodilation did not change after treatment (9, 10, 12, 22). We therefore consider that our findings on endothelium-dependent vasodilatation could be conclusive.

Conclusion and Implications

Even though the relative importance of the various possible mechanisms leading to depressed endothelial function in essential hypertension remain to be elucidated, our study shows that an ACE inhibitor or a calcium antagonist can achieve a demonstrable improvement of endothelial-dependent vasodilatation via a mechanism that is probably related to antioxidant activity.

References


