Comparative Study of ACE-Inhibition, Angiotensin II Antagonism, and Calcium Channel Blockade on Flow-Mediated Vasodilation in Patients With Coronary Disease (BANFF Study)

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OBJECTIVES
To determine the effect of angiotensin-converting enzyme (ACE) inhibition on brachial flow-mediated vasodilation.

BACKGROUND
Quinapril, an ACE inhibitor with high affinity, has been shown to improve coronary endothelial dysfunction in patients with coronary artery disease. The effectiveness of different vasoactive agents to improve human endothelial function is unknown.

METHODS
High resolution ultrasound was used to assess endothelium-dependent brachial artery flow-mediated vasodilation (FMD) in patients with coronary disease. We studied 80 patients (mean age 58 ± 0.9 years) in a partial-block, cross-over design trial. Patients were randomized to one of four different drug sequences to receive quinapril 20 mg, enalapril 10 mg, losartan 50 mg or amlodipine 5 mg daily. Each patient received three drugs with a two-week washout period between treatments. The primary end point was the absolute difference in FMD after eight weeks of each study drug compared with their respective baselines analyzed in a blinded fashion.

RESULTS
There was mild impairment of FMD at baseline (7.3 ± 0.6%). The change in FMD from baseline was significant only for quinapril (1.8 ± 1%, p < 0.02). No change was seen with losartan (0.8 ± 1.1%, p = 0.57), amlodipine (0.3 ± 0.9%, p = 0.97) or enalapril (−0.2 ± 0.8%, p = 0.84). No significant change in nitroglycerin-induced dilation occurred with drug therapy. The improvement in quinapril response was not seen in those with the DD ACE genotype (0.5 ± 2.1%) but was seen in those with the ID and II genotype (3.3 ± 1.2 and 3.2 ± 1.9%, respectively, p = 0.03).

CONCLUSION
Only quinapril was associated with significant improvement in FMD, and this response is related to the presence of the insertion allele of the ACE genotype. (J Am Coll Cardiol 2000; 35:60–6) © 1999 by the American College of Cardiology

Angiotensin-converting enzyme (ACE) inhibitors have been shown to be highly effective against a variety of cardiovascular disorders (1). A functional ACE system present in the vascular endothelium contributes to the regulation of vascular tone (2). The healthy endothelium releases autocrine and paracrine factors such as nitric oxide (NO) which maintain vascular integrity (3). Endothelial dysfunction occurs early in the course of atherosclerosis in response to cardiovascular risk factors (4,5) and contributes to the morbidity of coronary disease.

Angiotensin-converting enzyme inhibition has a favorable effect on endothelial function in animal models (6). Studies have suggested that bradykinin is the mediator responsible for the beneficial effects of ACE inhibition on endothelial function and atherosclerosis development (7,8). However, angiotensin II blockers, agents that have no effect on bradykinin, have demonstrated beneficial vascular effects comparable to ACE inhibitors in some studies (9,10).
Recently, six months of therapy with the tissue-specific ACE inhibitor, quinapril, has been shown to improve coronary endothelium-dependent vasodilation in patients with coronary atherosclerosis (11). The effect of angiotensin II blockade on endothelial function has not been studied in humans. In addition, the ACE gene I/D polymorphism is well described, and the deletion genotype has been associated with higher levels of circulating ACE. Some studies have suggested that the effects of ACE inhibition differ according to this gene polymorphism.

The present study was designed to compare the effect of ACE inhibition with quinapril or enalapril, angiotensin II blockade with losartan, and calcium-channel blocking therapy with amlodipine on brachial artery endothelial function in patients with coronary artery disease. Amlodipine is an anti-hypertensive drug without significant effect on the angiotensin system, but it may have favorable effects on the endothelium in vitro (12). In addition, we sought to study the relationship between the ACE genotype and the change in vasomotor responses with drug therapy.

METHODS AND ANALYSIS

Patient population. The study population consisted of 80 patients recruited from the University of Calgary and McGill University. Patients were eligible for the study if they had at least one >50% stenosis in a major vessel verified by angiography within the preceding six months. Patients were excluded for the following reasons: history of coronary bypass grafting, myocardial infarction within 28 days, left ventricular dysfunction (ejection fraction <40%), total cholesterol >6 mmol/liter, uncontrolled hypertension (>160/95), significant valvular heart disease, type I diabetes mellitus or smoking within six months before screening. Patients were excluded if they had received ACE inhibition, angiotensin II blockade or treatment with dihydropyridines within six months of randomization. Also, treatment with lipid-lowering agents, hormone replacement therapy or antioxidant therapy within three months of randomization was an exclusion. Most patients received aspirin (95%), and some were taking long-acting nitrates (36%).

Study protocol. RANDOMIZATION. Written, informed consent was obtained at both institutions in accordance with the guidelines established by the Committee for the Protection of Human Subjects. Subjects were randomized to one of four treatment groups according to a randomization code card. Patients received three study drugs for eight weeks each with a two-week washout period between each, in a crossover design. Patients were randomized to groups 1 and 2 on a 2:1 basis versus groups 3 and 4. The four groups were as follows: Group 1: quinapril, enalapril, losartan (n = 25); Group 2: enalapril, quinapril, amlodipine (n = 28); Group 3: losartan, amlodipine, quinapril (n = 13); Group 4: amlodipine, losartan, enalapril (n = 14). The study medication was open-label and consisted of quinapril 20 mg, enalapril 10 mg, losartan 50 mg, and amlodipine 5 mg each taken daily.

BRACHIAL ENDOTHELIAL FUNCTION STUDIES. Assessment of brachial artery endothelial function was performed at baseline, after each eight-week study period and at the end of each washout period (six studies) by a previously validated technique (13,14). A 7.5 MHz linear phase arrayed ultrasound transducer attached to a Hewlett-Packard ultrasound machine was used. Pulsed wave doppler was used to record brachial artery velocity for each of the interventions. The study medications were withdrawn 72 ± 12 h before brachial studies, and vasoactive calcium-channel blockers and nitrates were discontinued at least 12 h before the brachial studies.

ACE GENOTYPING. A separate informed consent was obtained from each participant, and DNA was isolated from peripheral blood lymphocytes as previously described (15). The polymerase chain reaction was performed using a modified method of Rigat et al. (16). Reactions were performed using 1.0 to 1.7 µg of template DNA, 25 pmol of each primer and five units of TAQ polymerase (Pharmacia).

Data analysis. BRACHIAL ARTERY ANALYSIS. Brachial artery analysis was performed at a core laboratory at the University of Calgary by a single technician blinded to the study group assignment or study sequence. Three sequential systolic frames (taken at the end of the T wave on the electrocardiogram) for each were digitized via an analog to digital converting board. A software algorithm automatically calculates the average diameter (100 points) over the operator-selected segment. Flow-mediated vasodilation (FMD) was calculated from the diameters as: (reactive hyperemia − baseline)/baseline × 100%. The intra- and interobserver variability in our laboratory is 1%. Systolic frames were used as previously validated (13). In 20 studies selected randomly, measurements were made at both end-diastole and systole, and the FMD was exactly the same. In addition, to normalize for changes in nitroglycerin (NTG) effect, a normalized ratio was calculated as reactive hyperemia diameter/NTG diameter. The percentage change in this ratio from baseline to eight-week study was determined. Brachial artery flow was calculated as the product of velocity and cross-sectional arterial area.

STATISTICS. All data presented are mean ± standard error of the mean. The primary efficacy parameter, change from baseline in flow-mediated brachial artery vasodilation, was compared among the four treatments by analysis of covari-

**Abbreviations and Acronyms**

- **ACE** = angiotensin-converting enzyme
- **ANCOVA** = analysis of covariance
- **FMD** = flow-mediated vasodilation
- **NO** = nitric oxide
- **NTG** = nitroglycerin
The study was powered to be able to detect a difference of 2% (SD 4%) between the quinapril and enalapril treatment groups. Change from baseline or zero change using ANCOVA. The SAS 6.08 procedure GLM was used to generate the analysis. Adjusted means for each treatment group were generated by the procedure. The adjusted means were compared to baseline or zero change using t tests. The ANCOVA model included study site, treatment sequence, subject, period and treatment. The baseline measure was included as a covariate. No adjustments to alpha level were made, as these were the pre-specified comparisons. When comparisons were made between treatments, the Bonferroni method was used to preserve the experiment-wide alpha level of 0.05. Demographic data among the four groups were compared by analysis of variance, and changes during the study period among parameters (blood pressure) were examined by repeated measures.

RESULTS

Patient demographics and events. The study population consisted of 80 patients with coronary artery disease. Complete brachial ultrasound data were available from 194 treatment periods (of a possible 240). In total, 17 patients withdrew before completion of the study (six for adverse events). There was no difference in the demographics compared with those patients completing the study. Baseline demographic parameters between different randomization groups were well-matched (Table 1). The mean age of the study population was 58 ± 0.9 years, with 82% male. Risk factors were present as follows: hypercholesterolemia 54%, hypertension 26% and diabetes mellitus 10%. Previous myocardial infarction was present in 47%, and 95% had previous angioplasty. There was no change in serum lipids during any of the treatment periods from baseline values: total cholesterol 5.1 ± 0.1, low-density lipoprotein cholesterol 3.1 ± 0.1, high-density lipoprotein cholesterol 1.1 ± 0.1, or triglycerides 2.2 ± 0.1 (mmol/liter). There was no significant change in blood pressure during any of the treatment periods, but the measurements were made 72 h after drug withdrawal (Table 2). The mean blood pressure at entry was 125/75 mm Hg.

During the course of the study there were no deaths, and there were 17 hospitalizations for chest pain. The frequency of reported hospitalizations and angina was similar for the four treatments. The only associated adverse event more prevalent in specific treatment groups was “increased cough” noted more commonly with both ACE inhibitors.

Treatment effect on brachial artery characteristics. Flow-mediated vasodilation is inversely related to baseline brachial artery diameter in this and other studies (4). The baseline brachial artery diameter was the same for each of the drugs tested, and the study medications, despite being

### Table 1. Baseline Demographics

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 25)</th>
<th>Group 2 (n = 28)</th>
<th>Group 3 (n = 13)</th>
<th>Group 4 (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>60 ± 2</td>
<td>57 ± 1</td>
<td>59 ± 2</td>
<td>57 ± 2</td>
</tr>
<tr>
<td>Male (%)</td>
<td>21 (84)</td>
<td>23 (82)</td>
<td>12 (92)</td>
<td>10 (71)</td>
</tr>
<tr>
<td>Female (%)</td>
<td>4 (16)</td>
<td>5 (18)</td>
<td>1 (8)</td>
<td>4 (29)</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>7 (28)</td>
<td>16 (57)</td>
<td>9 (69)</td>
<td>5 (36)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>9 (36)</td>
<td>5 (18)</td>
<td>2 (15)</td>
<td>5 (36)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>4 (17)</td>
<td>3 (10)</td>
<td>1 (8)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Lipids (mmol/liter)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>4.9 ± 0.1</td>
<td>5.2 ± 0.2</td>
<td>5.0 ± 0.3</td>
<td>5.3 ± 0.1</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.1 ± 0.05</td>
<td>1.0 ± 0.04</td>
<td>1.0 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>LDL-C</td>
<td>2.9 ± 0.1</td>
<td>3.2 ± 0.2</td>
<td>3.1 ± 0.2</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>2.1 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>2.0 ± 0.3</td>
<td>2.1 ± 0.3</td>
</tr>
</tbody>
</table>

HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein cholesterol. To convert mmol/liters to mg/dl multiply cholesterol values by 38.67, and triglycerides by 88.57.

### Table 2. Baseline Parameters at Initial and Eight Week Study

<table>
<thead>
<tr>
<th></th>
<th>Baseline Dia (mm)</th>
<th>Baseline Dia (mm)</th>
<th>Delta in Systolic BP (mm Hg)</th>
<th>Delta in Diastolic BP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinapril (n = 56)</td>
<td>3.9 ± 0.1</td>
<td>3.8 ± 0.1</td>
<td>3 ± 3</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>Enalapril (n = 55)</td>
<td>3.8 ± 0.1</td>
<td>3.9 ± 0.1</td>
<td>2 ± 2</td>
<td>0 ± 2</td>
</tr>
<tr>
<td>Losartan (n = 38)</td>
<td>4.0 ± 0.1</td>
<td>3.8 ± 0.1</td>
<td>3 ± 3</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>Amlodipine (n = 45)</td>
<td>3.8 ± 0.1</td>
<td>3.8 ± 0.1</td>
<td>−2 ± 3</td>
<td>0 ± 2</td>
</tr>
</tbody>
</table>

Pre = before initiation of medication; Post = following eight weeks of study medication (off medications for 72 h).
vasodilators, had no significant effect on baseline brachial diameter after eight weeks of therapy (Table 2). Upper arm occlusion resulted in an increase in forearm blood flow of approximately 500%, which was the same at baseline for each drug and was not affected by drug treatment.

**Treatment effect on FMD.** At baseline, the study population had FMD of 7.3 ± 0.6% and NTG-mediated vasodilation of 14.2 ± 0.8%. Baseline FMD and NTG-mediated vasodilation was the same for each of the drugs tested. Only treatment with quinapril resulted in a significant increase in brachial flow-mediated vasodilation (delta 1.8 ± 1.0%; p < 0.02) (Fig. 1). The improvement in FMD was no longer evident when baseline measurements were repeated after two weeks of quinapril withdrawal. As a secondary assessment, quinapril’s effects on FMD were compared with the other four treatments. There was only a trend for a difference in quinapril compared with enalapril (p = 0.12) and no difference when compared with losartan or amlodipine.

There was no significant effect of treatment on NTG-mediated vasodilation. Since there was a trend (p = 0.10) for improvement in the NTG response after quinapril, the FMD/NTG ratio was analyzed. This did not change the results. Only quinapril demonstrated an improvement from baseline in the FMD/NTG ratio, while the other agents did not (Fig. 1b). The improvement in this ratio with quinapril implies improvement in endothelium-dependent vasodilation and not simply vascular smooth muscle responsiveness.

**TREATMENT EFFECT AND ACE GENOTYPE.** There was no difference in the baseline flow-mediated vasodilation among the three genotypes. In response to eight weeks of therapy, there was no improvement in brachial responses noted in the DD genotype with any of the four agents. Significant improvement was attributed only to quinapril in the ID (delta 3.3 ± 1.2%) as well as the II genotype (delta 3.2 ± 1.9%). The improvement with quinapril in the ID and II genotypes was significantly different from the quinapril response in the DD genotype (Fig. 2).

**DISCUSSION**

The current study demonstrated that eight weeks of quinapril improves impaired endothelium-dependent FMD in the brachial circulation of patients with atherosclerotic risk factors. This was not seen with the other drugs tested, suggesting that there may be a difference between the ability of quinapril and the ability of the other drugs to modulate vascular function.

**Current Study**

The patients in the present study had endothelial dysfunction at baseline as a result of coronary disease, as has been demonstrated in other studies (13). A significant improvement from baseline endothelial dysfunction was seen with eight weeks of quinapril therapy only. Patients were studied after the drug had been withdrawn for 72 h, avoiding the confounding acute hemodynamic effects of the medication as in other studies (11).

In addition to an improvement in endothelial function with quinapril, there was a trend for an improvement in NTG-mediated vasodilation. Because measurements were
made during systolic frames, a beneficial effect of quinapril on vascular smooth muscle (compliance) cannot be excluded. Interestingly, a corresponding improvement in NTG-mediated vasodilation was not observed with enalapril. Angiotensin-converting enzyme inhibition in hypertensive patients has been previously shown to have a beneficial effect on the arterial wall morphology of resistance vessels, although this effect probably takes longer than eight weeks (18). However, after correcting for changes in NTG response, the FMD was still augmented, demonstrating an endothelial effect.

**Endothelial Function and ACE-inhibition**

Angiotensin converting enzyme-inhibition is thought to improve vascular function by several mechanisms. It decreases the concentration of angiotensin II and hence endothelin, increases the concentration of bradykinin, which is a vasodilator and stimulator of NO, endothelial derived hyperpolarizing factor and prostacyclin, and decreases superoxide anion concentration (19). An augmentation of endothelium-dependent vasodilation has been demonstrated for several ACE inhibitors in animal studies (20). In the recently published TREND (Trial on Reversing Endothelial Dysfunction) study, six months of therapy with quinapril (40 mg/day) attenuated acetylcholine-induced vasoconstriction in patients with coronary disease (11). Hornig et al. (21) have recently demonstrated that the acute arterial administration of quinaprilat augmented brachial FMD, and that this effect is predominantly mediated via the bradykinin-2 receptor. Animal studies had also suggested that the beneficial vascular effect of ACE inhibition was mediated through bradykinin and NO (22).

It is not clear from the present study why augmentation of FMD was seen with quinapril but not enalapril. Although the improvement from baseline was not statistically different among the drugs tested, only quinapril showed a difference from baseline, and there was a trend for a difference between the two ACE inhibitors (p = 0.12). The lack of effect of enalapril, losartan and amlodipine may be related to choice of dose or length of treatment. However, quinapril has been shown to have high tissue specificity for ACE, and the dissociation of the drug from the enzyme is markedly prolonged compared with other converting enzyme inhibitors (23). In addition, the enhanced lipophilicity of the drug may allow better cellular penetration with beneficial effects on enzymatic processes such as cNOS activity, for example (24). Greater inhibition of vascular ACE has also been demonstrated for quinapril compared with enalapril in a recent human study of forearm blood flow (25). A more recent study by Hornig et al. (26) demonstrated improved FMD acutely in response to quinaprilat but not to increasing doses of enalaprilat in patients with heart failure. Studies of enalapril in diabetics with endothelial dysfunction have shown mixed results. One month of treatment with enalapril was able to improve forearm blood flow in response to acetylcholine in patients with type I diabetes (27), whereas 24 weeks of enalapril had no statistically significant effect on FMD in other patients with type I diabetes (28). Further work is required to contrast the effects of different ACE inhibitors on vascular function in different disease states.

**Endothelial Function and ACE Genotype**

Polymorphism of the ACE gene has been demonstrated, and the presence of the deletion allele has been associated with higher levels of circulating and tissue ACE (29). The DD genotype has also been associated with increased risk of coronary restenosis and myocardial infarction in some but not all studies (30,31). In addition, some studies have suggested a relationship between the genotype and physiological effects from ACE inhibition with attenuation of beneficial effect noted with the deletion allele (32). In the present study we noted no difference in baseline brachial FMD between the different genotypes, as was seen in one other study (33). However, the improvement in FMD with quinapril was restricted to the ID and II genotypes. The reason for this observation is not explained by this study. It may relate to increased tissue levels of ACE, attenuated interaction with quinapril and the tissue ACE, or increased levels of oxidative stress in these subjects. Down-regulation of the AT1 receptor in those with the DD genotype has also recently suggested (34). The duration of effect of ACE inhibitors may also be related to the genotype, affecting the results seen (35).

**Endothelial Function and Angiotensin-II Blockade**

Farhy and colleagues (36) demonstrated that both ramipril and losartan reduced neointimal proliferation in a rat balloon injured model, but that ramipril was more effective. Concomitant bradykinin blockade with HOE-140 nullified this advantage, suggesting that kinins were important in the beneficial effect of ramipril in this model. However, a similarly designed study in rabbits comparing perindopril and losartan demonstrated equal efficacy in reducing neointimal formation (10).

This is the first human study to assess endothelial function with an angiotensin-II receptor blocker in humans. Although there was no difference in the response between quinapril and losartan, losartan did not augment FMD from baseline in our study. Although this might suggest that vasodilator kinins are important in augmenting endothelium-dependent vasodilation, further studies are required to clarify the impact of angiotensin-II blockade on endothelial function (37,38).

**Endothelial Function and Calcium-Channel Blockers**

Acute administration of calcium-channel blockers has been shown to block exercise-induced coronary artery vasoconstriction in patients with hypertension (39). Amlodipine has been demonstrated to have antioxidant properties in atherosclerotic animal models, but its effect on endothelial function has not been assessed in humans (40). In addition,
amlodipine has been recently shown to increase NO production from canine microvessels (41). However, amlodipine (2.5 to 5 mg daily for six months) did not improve reactive hyperemic blood flow in peripheral arteries of patients with hypertension by contrast to the improvement observed with a potent ACE inhibitor, temocapril (42).

**Study Limitations**

The lack of effect of enalapril, losartan and amlodipine may be related to improper dosage or length of treatment. Full dose-ranging and time-course studies for each drug would be required before concluding that these agents have no effect on endothelial function. Drugs were withdrawn for 72 h before the brachial studies to avoid hemodynamic effects, and this may have influenced results as well. However, in the current model, quinapril did demonstrate improved vasodilator responses.

Other measures of endothelial function, such as adhesion molecules or platelet function, were not assessed. In addition, the relationship between endothelial function and clinical events has yet to be proven.

There is variability in the ultrasound assessment of FMD in the acquisition, repeatability and measurement of multiple studies per patient. However, previous brachial studies with a cross-over design had demonstrated the feasibility of this approach (43).

Finally, the study was open-label. However, the single technician who analyzed the more than 500 brachial studies was blinded to randomization and phase of the study.

**Conclusions**

In a comparative trial of four vasoactive agents, only quinapril was associated with significant improvement in FMD in patients with coronary disease, and this response appeared to be related to the ACE genotype. There may be differences in vasoactive drugs in their ability to improve vascular endothelial function, although further dose-ranging and time-course studies are required.

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