OVERVIEW OF CLINICAL TRIALS TO IMPROVE ENDOTHELIAL FUNCTION

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Published under the auspices of the Vascular Biology Working Group

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College of Medicine
Fundamental views on the progression of cardiovascular disease are evolving toward a clearer understanding that the endothelium plays a central role in health and disease.

The endothelial lining of the vasculature sustains damage in the presence of several conditions including hypertension, dyslipidemia, diabetes and smoking. Each of these conditions causes oxidative stress that leads to abnormal functioning of the endothelium. One of the primary alterations is an imbalance between vasodilator compounds, such as nitric oxide (NO), and vasoconstrictor compounds, such as angiotensin II (A II) and endothelin-1. These vasoactive compounds are part of auto-, para-, and endocrine systems that interact at the vessel wall-blood interface. Within the vessel wall A II up-regulates endothelin-1 production while NO down-regulates it, thus maintaining a delicate balance in blood vessel tone.

When this delicate balance within the endothelium is disturbed, pathophysiologic changes can occur that often lead to the development or progression of atherosclerosis.
The Importance of the Renin-Angiotensin System and ACE

As research reveals more of the complex interaction occurring within the endothelium, approaches are emerging that are beneficial in improving endothelial function. One area of study focuses on inhibitors of angiotensin converting enzyme (ACE).

Historically, ACE inhibitors are known to restore the balance within the renin-angiotensin system (RAS) once it is activated. It is now known that there are actually two RASs on which ACE inhibitors can act. One system, the classic RAS, is responsible for acute blood pressure regulation. A second system, the tissue RAS, regulates vascular and cardiac function and structure over the long term. ACE inhibitors suppress production of AII thereby reducing vasoconstriction and, consequently, blood pressure in hypertensives.

However, it is becoming clear that inhibition of ACE does more than lower blood pressure by reducing AII formation. ACE inhibition also increases production of NO via its effect on bradykinin, as shown in Figure 2, leading to vasodilation. Use of these agents also appears to reduce proliferation and migration of smooth muscle cells, neutrophils and mononuclear cells in the subintimal layer. ACE inhibitors seem to have antiplatelet effects and enhance endogenous fibrinolysis by increasing tPA and reducing PAI-1 levels. Research suggests that the sum effect of ACE inhibitors is to improve arterial compliance and tone, ameliorate endothelial dysfunction, exert an antiatherogenic effect and thereby decrease probability of plaque rupture.

“When this delicate balance is disturbed, pathophysiologic changes can occur that often lead to clinical outcomes...”
IN PATIENTS WITH CAD: THE TRENDS STUDY (TRIAL ON REVERSING ENDOTHELIAL DYSFUNCTION)

The evidence for these beneficial effects of ACE inhibitors on endothelial function comes from a number of recently completed clinical studies. One of these studies is TRENDS. TRENDS was a randomized, double-blind, placebo-controlled study that followed up 105 adults with coronary artery disease (CAD) for 6 months. Subjects randomized to the therapy group received quinapril (40 mg/day). The primary study end point was the net change in coronary artery diameter in response to acetylcholine, with responses at baseline compared to those seen after treatment.

After 6 months of treatment, the group receiving quinapril demonstrated significant improvement in arterial function compared with the placebo group (P<0.0003). Out of all the clinical and angiographic variables included at baseline (such as smoking status, stenosis severity, blood pressure, gender, initial response to acetylcholine, and lipid values), assignment to the quinapril group was the only independent predictor of improved endothelial function (P =0.022).

THE BANFF STUDY (BRACHIAL ARTERY NORMALIZATION OF FOREARM FUNCTION)

The BANFF trial also examined the effectiveness of ACE inhibition at improving endothelial function. In this study, the effectiveness of 4 anti-hypertensive agents was compared. Study subjects included patients with documented CAD but without previous coronary artery bypass graft (CABG) surgery, lipid-lowering therapy, uncontrolled hypertension, a left ventricular fraction of <40%, or a total cholesterol level >6.0 mmol/L. Two ACE inhibitors were administered in the study: quinapril (20 mg/day) and enalapril (10 mg/day). The angiotensin receptor antagonist losartan was given (50 mg/day). The fourth treatment was the calcium channel antagonist, amlodipine (5 mg/day). Endothelial function was assessed in these patients by measuring flow-mediated dilation (FMD) of the brachial artery during reactive hyperemia.

The only treatment to show significant improvement in FMD was quinapril (P<0.02). A nonsignificant trend toward improvement was observed with losartan and amlodipine. The study authors concluded that some antihypertensive agents are more effective than others at improving endothelial function.
While the TREND and the BANFF studies focused on patients with CAD, a recent investigation by Hornig et al examined the effect of ACE inhibition in patients with congestive heart failure (CHF). FMED was used as a measure of endothelial function. High-resolution ultrasound and Doppler were used to measure radial artery diameter and blood flow in patients given intra-arterial infusions of either quinaprilat 1.6 µg/min or enalaprilat 5 µg/min. Measurements were made at rest and during reactive hyperemia both before and after administration of L-nomonomethyl-L-arginine (L-NMMA), a substance that inhibits endothelial synthesis of NO. The investigators found that quinaprilat improved overall FMED by >40% (P<0.01) while enalaprilat had no detectable effect. Through use of L-NMMA, the investigators were able to isolate the portion of FMED attributable to NO. They found that quinaprilat increased NO-dependent FMED by >100% (P<0.01).

### In patients undergoing CABG

Quo Vadis study (Effects of Quinapril on Vascular ACE and Determinants of Ischemia)

The effect of ACE inhibition on endothelial function via reduction of A II was the focus of one portion of the Quo Vadis study.

<table>
<thead>
<tr>
<th>Quinapril</th>
<th>Captopril</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>delta pEC50</td>
<td>1.0 ± 0.12*</td>
<td>0.86 ± 0.1</td>
</tr>
<tr>
<td>ABC</td>
<td>91 ± 8*</td>
<td>84 ± 4</td>
</tr>
</tbody>
</table>

Mean ± SEM
*P < 0.05 (quinapril compared to placebo)

In this part of the study, 187 patients scheduled for coronary bypass surgery were randomized at least 7 days prior to surgery. Patients were treated with quinapril (40 mg/day), captopril (150 mg/day) or placebo prior to surgery. Portions of the internal mammary artery not used for CABG were exposed to increasing doses of A I. A I to A II conversion was measured using 2 methods: the difference between pEC50 of the dose-response curves to A I and A II and as the area between the curves (ABC) of A I and A II. The data indicated that quinapril was more effective than captopril or placebo at inhibiting endothelial A II production.

*These results of the Quo Vadis study support those of the BANFF and Hornig investigations which suggest that ACE inhibitors work by differing mechanisms and that not all are equally effective at altering endothelial function.*
ACE inhibition and VEGF: Effects on Angiogenesis

ACE inhibition may affect more than vascular tone; a recent investigation by Fabre et al suggests that it may induce angiogenesis as well. A rabbit model of chronic hindlimb ischemia was used in the Fabre investigation to test the influence of ACE inhibition on new blood vessel growth. Vascular endothelial growth factor (VEGF) was used as a positive control in the same model. VEGF was first isolated in 1989 and appears to facilitate angiogenesis as well as induce migration and proliferation of endothelial cells, enhance vascular permeability and modulate thrombogenicity. VEGF is produced by endothelial cells, macrophages, smooth muscle cells and tumor cells. In the Fabre study, several measurements of angiogenesis, including angiographic score, capillary density and an estimation of endothelial function, were taken 10 days after inducing ischemia to obtain a baseline. Either placebo, VEGF, quinapril or captopril was then administered and the same measurements were taken at day 40. Based on all measurements, both VEGF and quinapril significantly improved endothelial function assessed by the response to acetylcholine and enhanced angiogenesis that was demonstrated as increased blood vessel density (\( P < 0.01 \)).

Figure 8: Coronary artery responsiveness increased with HMG-CoA reductase inhibition

The authors concluded that cholesterol-lowering with a statin significantly improved endothelial function in patients with atherosclerosis. Potentially, this improvement in endothelial activity could influence blood flow to relieve symptoms of ischemia and may reduce progression of atherosclerotic plaques.

HMG-CoA Reductase Inhibitors and Antioxidants Enhance Endothelial Activity in Hypercholesterolemic Patients

HMG-CoA reductase inhibitors have been combined with other agents such as antioxidants to reduce endothelial dysfunction. In a study by Anderson et al, 23 patients with hypercholesterolemia were randomized to 1 of 3 groups: those consuming an American Heart Association Step 1 diet (7 patients), those taking lovastatin and cholestyramine (7 patients) or those taking lovastatin and probucol (9 patients). They maintained these treatments for 1 year at which time endothelium-dependent vasomotion was studied using acetylcholine and quantitative angiography.
Susceptibility of low-density lipoprotein (LDL) to oxidation was determined by measuring the formation of conjugated dienes by Cu²⁺ and calculating the lag phase. A prolonged lag phase indicates low susceptibility to oxidation. Taking all of the groups together, the lag time of conjugated diene formation correlated closely with coronary vascular response to acetylcholine (r=0.62, P=0.002). Assignment to a treatment group was also a significant predictor of responsiveness to acetylcholine (P=0.002). The group treated with lovastatin and probucol had a substantially increased lag phase.

Figure 9: HMG-CoA reductase inhibitors and antioxidants enhance endothelial activity in hypercholesterolemic patients

Based on data from this investigation and related studies by the authors, they conclude that treatment with an HMG-CoA reductase inhibitor combined with an antioxidant provides marked improvement in endothelial function by protecting LDL from oxidation as well as by reducing levels of LDL.

“Based on all measurements, both VEGF and quinapril significantly improved endothelial function and increased blood vessel density (P<0.01).”

Future clinical trials: Identifying treatments for CVD and CHD based on mechanisms of action

The studies highlighted in this monograph are only a few of the clinical trials that have investigated therapies to ameliorate endothelial dysfunction. This monograph has focused on trials of ACE inhibitors as well as 2 trials of HMG-CoA reductase inhibitors that studied the efficacy of these agents at reducing endothelial dysfunction. Overall, the results from the numerous studies conducted so far are varied and may suggest mechanistic differences between ACE inhibitors.

This concept was proposed in the study discussed earlier by Hornig; the authors stated, “...differences exist among ACE inhibitors with regard to their ability to improve endothelial function.” However, a clear understanding of the mechanistic and functional differences among ACE inhibitors is lacking. New clinical studies with larger patient populations are needed to elucidate differences in the ways ACE inhibitors reduce hypertension by attacking its underlying endothelial pathology. Some ongoing or recently completed trials—such as QUIET, HOPE, ALLHAT and PEACE—that have yet to be published may further knowledge in this area.

<table>
<thead>
<tr>
<th>ACE-I</th>
<th>PC</th>
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<th>Disease</th>
<th>Results</th>
<th>Reference</th>
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<td>CAD</td>
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<tr>
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</table>

Figure 10: Selected studies of ACE inhibition in endothelial function
REFERENCES


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