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Scientific Update™

The Living Lining: The Endothelium as a Strategic Target of Therapy for Cardiovascular Disease

Originally presented by: VICTOR DZAU, MD, MICHAEL WEBER, MD, HELMUT DREXLER, MD, CARL PEPINE, MD, and TODD ANDERSON, MD.

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Reported and discussed by:
JUAN CARLOS MONGE, MD

Endothelial dysfunction is arguably the main factor underlying the number one cause of morbidity and mortality in the western world — atherosclerotic cardiovascular disease. Nitric oxide (NO), a key molecule synthesized by the endothelium, is essential to good vascular health and abnormalities in NO-mediated functions are at the root of endothelial dysfunction. Cardiovascular risk factors elicit a state of oxidative-stress in endothelial cells which induce the expression of multiple pro-atherogenic genes. Angiotensin II also stimulates oxidative-stress and a vicious cycle in the vascular wall leading to the early lesions of atherosclerosis. Antioxidants such as vitamin C have been shown to ameliorate endothelial function in various conditions, including the oxidative-stress states present in smokers and patients with congestive heart failure. Drugs such as the HMG-CoA reductase inhibitors — statins — and the ACE inhibitor quinapril have been shown to improve endothelial function in patients with coronary artery disease (CAD). The Brachial Artery Normalization of Forearm Function (BANFF) study examined the effects of four medications on endothelial function in patients with CAD using a brachial artery noninvasive assessment of NO-mediated vasodilatation. The results showed that quinapril significantly increased flow-mediated vasodilatation from baseline, whereas enalapril had a neutral effect and flow increases with losartan and amlodipine did not achieve statistical significance. Differences may exist, therefore, between vasoactive drugs in their ability to improve endothelial dysfunction, both within and between drug classes.

Endothelial dysfunction in cardiovascular disease

The endothelium is a monolayer of flat, elongated cells aligned in the direction of blood flow. Under normal circumstances, there is no cell adhesion to the endothelium and it forms a thrombo-resistant interface between the circulation and the rest of the vessel wall and tissues. The endothelium is a dynamic entity that senses changes in the circulation, transmits signals and elicits responses. As well, the endothelium influences vascular tone and structure, regulates cell growth, prevents the adhesion of leukocytes and platelets, modulates lipid oxidation and regulates vascular permeability (Tables 1 and 2).

A key molecule synthesized by the endothelium is nitric oxide (NO), a diffusible gas synthesized by the enzyme nitric oxide synthase which uses the amino acid L-arginine as its substrate.[1,2] NO has multiple functions. It mediates vasorelaxation in vascular smooth muscle, has potent antiproliferative effects, inhibits platelet aggregation and adhesion, and is important in preventing the formation of the platelet thrombus and the adhesion of monocytes to the endothelium. In fact, the term "endothelial dysfunction" refers, in the clinical context, to anomalies in NO-mediated actions such as flow-dependent or acetylcholine-induced vasorelaxation (Table 3).

It is well established that risk factors for cardiovascular disease are associated with endothelial dysfunction. Creager and co-workers demonstrated that young individuals with hypercholesterolemia exhibited impaired NO-mediated endothelial function (vasorelaxation) very early and prior to the onset of overt atherosclerosis.[3] Furthermore, these risk factors have an additive effect. They also impair other

St. Michael's Hospital
30 Bond St., Suite 701A
Toronto, Ontario M5B 1W8
Fax: (416) 864-5330

Table 1: Normal endothelial function: A balance between opposing states

- Dilation vs. constriction
- Growth inhibition vs. growth promotion
- Antithrombosis vs. prothrombosis
- Anti-inflammatory vs. proinflammatory
- Anti-oxidant vs. pro-oxidant

normal functions of the endothelium such as the prevention of the adhesion of leukocytes and the deposition of lipids. The abnormal endothelium allows the adhesion of monocytes which then migrate to the subintima of the vessel where they take up minimally modified-LDL to become macrophages or oxidized LDL to become foam cells, leading to the formation of the fatty streak, the first visible manifestation of atherosclerosis.

Endothelial dysfunction is of great clinical relevance as it is arguably the main factor underlying the number one cause of morbidity and mortality in the western world — atherosclerotic cardiovascular disease. The causes of endothelial dysfunction are multiple. It is clear, however, that all the risk factors for atherosclerosis elicit an oxidative-stress state in the endothelial cells resulting in significant changes in their oxidation-reduction (redox) state that are mediated by the generation of superoxide anions and other free-oxygen radicals (Table 4). This process leads to the activation of redox-sensitive genes within the vascular cells which facilitates the process of atherosclerosis.[4]

Angiotensin II is a very powerful inducer of oxidative-stress in the endothelial cells where it can stimulate monocyte adhesion, macrophage and foam cell formation, and loss of the inhibition of smooth muscle cell proliferation.

In keeping with the role of oxidative stress in endothelial dysfunction, it has been demonstrated that in some instances, antioxidants can restore the normal function of the endothelium. For example, the administration of vitamin C in smokers can inhibit the increased interaction of the monocytes with the endothelial cells.[5,6] As well, vitamin C has been shown to improve endothelial function in diabetics, presumably also by restoring the normal redox state in the endothelial cells.

Table 2: Vasoactive substances released by the endothelium

Vasodilators (growth inhibitors)	Vasoconstrictors (growth promoters)
NO	Angiotensin II
Bradykinin	Endothelin
Prostacyclin	Thromboxane A_2
Endothelium-derived hyperpolarizing factor (EDHF)	Superoxide anion

Table 3: Vasculoprotective functions of NO

- Vasodilator (via relaxation of smooth muscle cells – SMC)
- Growth inhibitor (via actions on SMC, endothelial and mononuclear cells)
- Inhibitor of platelet adherence/aggregation
- Inhibitor of endothelial/leukocyte interactions

Endothelial dysfunction and hypertension

As our understanding of endothelial dysfunction improves, there is a growing awareness that it is not only present in atherosclerosis but in other cardiovascular conditions such as arterial hypertension.[7] There is evidence that even relatively early in the course of the disease, the function of the endothelium is significantly impaired. Besides angiotensin II, potent endothelium-derived vasoconstrictors such as endothelin are likely to play a role in hypertension. This has been demonstrated by a recent report showing that bosentan, an endothelin receptor antagonist, was as effective as enalapril in lowering blood pressure in mild- to moderate-hypertensive subjects.[8] Endothelin and the renin-angiotensin system are closely interrelated and the ACE inhibitor quinapril has been shown to decrease endothelin expression in the uninephrectomized rat model of hypertension.[9] This ACE inhibitor has been shown to have a very low rate of dissociation from the enzyme, a property that could explain its relatively early and full inhibition of circulating and tissue ACE.

There have been few studies of the effects of ACE inhibitors on endothelial function in hypertensive patients and the results have been mostly disappointing. This is an area awaiting a definitive study. An ongoing trial, Q-TENSE, will compare the effects of two ACE inhibitors which are quite different in their ability to bind ACE, quinapril and enalapril, and assess endothelial function by measuring flow-dependent vasodilatation in the forearm of hypertensive patients.

Endothelial dysfunction and congestive heart failure

There is growing evidence that abnormalities in endothelial function are associated with congestive heart failure (CHF).[10] Patients with this condition exhibit impaired vasodilator responses mediated by NO. Flow-

Table 4: Cardiovascular risk factors associated with increased oxidative stress

- Hypercholesterolemia
- Hypertension
- Cigarette smoking
- Diabetes mellitus
- Estrogen deficiency in women
- Hyperhomocysteinemia
- Mechanical injury

dependent vasodilatation, a marker of NO-mediated endothelial function, is significantly impaired in patients with CHF. ACE inhibitors have been shown to have differential effects in this condition, with quinapril practically normalizing this response, an effect not seen with enalapril.[11] As well, a state of increased oxidative stress is present in the cardiac myocytes and the endothelial cells in heart failure. In a double-blind, randomized study, the use of vitamin C as an antioxidant restored endothelial function in heart failure patients as measured by flow-mediated vasodilatation in the forearm.[12]

The endothelium: A therapeutic target in coronary disease

In view of its clinical relevance and implications, endothelial dysfunction has been studied as a potential target for therapy in coronary artery disease (CAD). Both lipid-lowering drugs (such as the statins) and the ACE inhibitor quinapril have been shown to improve endothelial function in the coronary arteries of patients with CAD.

The TREND study was a placebo-controlled trial that evaluated the effects of six months of therapy with quinapril on the coronary endothelial function of patients with documented coronary disease. The methodology of the trial was based on the experimental observation that in normal endothelium, the intravascular administration of acetylcholine stimulates NO synthase to release NO and causes vasorelaxation. In contrast, in dysfunctional endothelium, the release of NO does not occur and acetylcholine acts instead on constrictor receptors in the vascular smooth muscle causing a narrowing of the vessel. In the TREND trial, the CAD patients exhibited constriction of the coronary arteries at baseline. After six months of therapy, the patients on quinapril exhibited a significant improvement in coronary endothelial function characterized by vasodilatation in response to acetylcholine. In contrast, no change was observed in the placebo-treated patients.[13] In a subsequent analysis, the patients were divided in two groups: those with an LDL-cholesterol below the mean for the study which was 3.30 mmol/L, or those with an LDL ≥3.30 mmol/L. The beneficial effect of quinapril was observed in both groups, but was particularly striking in the patients with the higher cholesterol levels.

Another study, the QUIET trial, evaluated the effect of quinapril versus placebo in patients with stable, mild-to-moderate, low-risk coronary disease. The study randomized 750 patients who also had normal left ventricular ejection fraction and were normotensive. There was no significant difference in the intention-to-treat analysis for all deaths, MI and repeat revascularization during the scheduled follow-up period. However, when the analysis was limited to cardiac deaths, nonfatal MI and resuscitated ventricular tachycardia or fibrillation, there was a strong trend – a 13% reduction – in these events in the patients treated with quinapril.

A post-hoc analysis also divided patients in a subgroup of the QUIET study according to their LDL-cholesterol levels (<3.25 mmol/L or ≥3.25 mmol/L). Quantitative coronary angiography demonstrated a significantly greater progression of the coronary disease in placebo-treated patients (an increase of 7.5 % in diameter stenosis compared with no progression in the quinapril treated patients, p=0.004).[14] Quinapril has been shown, therefore, to improve coronary endothelial function and prevent progression of coronary disease independently of lipid-lowering or effects in blood pressure. These results strongly correlate with previous evidence of the benefits of quinapril in other models of endothelial dysfunction and suggest that not all ACE inhibitors are equal in their ability to influence endothelial function.

The BANFF Study

The BANFF Study was a Canadian trial conducted by primary investigators Dr. T. Anderson and Dr. F. Charbonneau at the University of Calgary and McGill University.[15] Designed to address the paucity of data regarding the effects of different vasoactive medications on endothelial function, the study was a randomized, open-label trial with four medications: quinapril, enalapril, losartan and amlodipine. Each medication was given for eight weeks with a two-week washout period between medications. All the patients had documented CAD, 50% had a previous MI, and 97% had previous coronary angioplasty. Excluded from the trial were patients with severe underlying disease, previous coronary artery bypass surgery, heart failure, current cigarette smoking, hypertension or total cholesterol >6 mmol/L.

The study utilized a brachial artery noninvasive assessment of endothelial function. In this method, upper arm occlusion is performed to generate ischemic metabolites and the release of the occlusion is followed by reactive hyperemia which is a manifestation of flow-dependent vasodilation and an endothelium-dependent, NO-mediated response. Endothelium-independent vasodilatation is assessed with sublingual nitroglycerin. At baseline, the increase in flow-mediated vasodilatation was 7% in patients enrolled in the study. Since the normal value is a 11% to 12% increase, the lower levels

in these patients indicate the presence of endothelial dysfunction at baseline.

Quinapril treatment resulted in an absolute improvement in flow-mediated vasodilatation of 1.8% (or an absolute increase of more than 25%, p=0.02). Quinapril was the only one of the four medications that significantly increased flow from baseline, the primary end-point of the study, and a reflection of improved endothelial function not seen with the other agents. Enalapril had a neutral effect; losartan and amlodipine increased vasodilatation by 0.7% and 0.3%, respectively, but neither of these values achieved statistical significance. Thus, differences may exist between vasoactive drugs in their ability to improve endothelial function, both within and between drug classes.

Conclusion

The endothelium has been called the "maestro" of the circulation. Like the conductor of an orchestra, the endothelium is responsible for the simultaneous performance of many functions that maintain the overall health and normal homeostasis of the vascular tree. By secreting NO and other vasodilators, the endothelium maintains normal blood flow. By its inhibitory properties on smooth muscle cells, the normal structure of the vessels is preserved. Through its antithrombotic, antioxidant and anti-inflammatory properties, the normal endothelium prevents the development of the atherothrombotic process at the root of the most common causes of morbidity and mortality in our times.

Given the multiplicity and complexity of its functions, it should come as no surprise, therefore, that endothelial dysfunction is associated with, and in some cases may be the initiating factor of, many cardiovascular conditions. Indeed, endothelial dysfunction is present in CAD, hypertension, heart failure, and hyperlipidemia, to name but a few "endotheliopathies." Reversing endothelial dysfunction could result in prevention of the progression of the underlying disease and could alter its natural history. Available evidence demonstrates that some of the present cardiovascular therapies, such as the statins, antioxidants and some ACE inhibitors, have the potential of reversing endothelial dysfunction.

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