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Scientific Update™

The Endothelium as a Modulator of Atherosclerotic Risk

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The endothelium, the single-cell layer that lines the interior of blood vessels, had been considered until not long ago, a relatively inert entity without many known functions. Pivotal discoveries in the 1980s led by Furchgott's description of endothelium-derived relaxing factor[1] — later identified by Moncada as nitric oxide (NO)[2] — opened the way for a completely different understanding of the endothelium. It was recognized as being one of the most metabolically active tissues in the body, responsible for numerous critical functions that maintain the integrity and homeostasis of the circulation. Indeed, the endothelium has been called the "maestro" of the circulation,[3] and because of its crucial roles, it is increasingly identified as the prime modulator of atherosclerotic risk factors.

The endothelium has the ability to respond, via specific receptors or other sensing mechanisms, to multiple physiological or pharmacological stimuli. Many of these stimuli lead to an increase in the intracellular calcium concentration and the activation of the endothelial NO synthase that can generate NO, utilizing as substrate the amino acid L-arginine. NO is a short-lived volatile gas which can diffuse to the vascular smooth muscle cells where it interacts with soluble guanylyl cyclase to increase the concentration of cGMP and cause relaxation. It is precisely this NO-mediated (or endothelium-dependent) vasorelaxation that is used to measure endothelial function.

In response to pathological conditions or adverse stimuli such as the atherosclerotic risk factors, the endothelium increases the production of pro-oxidant substances. This gives rise to a state called oxidative stress in which superoxide and other reactive oxygen species (free radicals) inactivate NO and suppress its biological effects.

Although endothelial function can be assessed routinely by measuring NO-mediated vasodilatation, the endothelium, through the actions of NO and other vasoactive factors, also modulates a variety of anti-atherogenic activities. The normal endothelium inhibits platelet adhesion and aggregation, restricts permeability, inhibits smooth muscle cell proliferation, promotes fibrinolysis, inhibits coagulation and has anti-inflammatory properties. Indeed, there is a growing body of evidence that in endothelial dysfunction, cellular adhesion molecules are expressed on the surface of the endothelium, allowing leukocytes to adhere to it and subsequently migrate into the subendothelial space. This process is important, not only in the early stages of atherosclerosis, but also when there is rupture of atherosclerotic plaques and in the genesis of unstable coronary syndromes. Thus, when endothelial function is evaluated by NO-mediated vasodilatation, the other properties of the endothelium are also assessed indirectly as they depend on the normal biological activity of NO.

Measuring endothelial function

Endothelial function can be assessed invasively in the coronary arteries by quantitative coronary angiography (Figure 1). With this technique, acetylcholine (ACh) is infused in the coronary artery where, if endothelial function is normal, it binds to receptors on the endothelial surface and leads to activation of NO synthase and release of NO. This results in vasodilatation or endothelium-dependent relaxation. In contrast, in patients with atherosclerosis or its risk factors, there is a decrease in the production or activity of NO and ACh binds instead to receptors on smooth muscle cells

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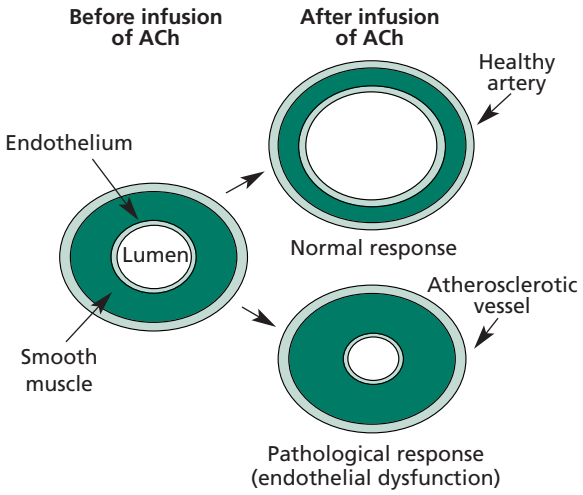
Figure 1: Assessment of endothelial function



leading to paradoxical vasoconstriction, a hallmark of endothelial dysfunction (Table 1).[4] However, quantitative coronary angiography to assess endothelial function is not practical in most patients because of its invasive nature.

Subsequently, Celermajer and co-workers described a noninvasive technique to assess endothelial function in which an ultrasound probe is used to measure brachial artery diameter very accurately.[5] In this technique, a blood pressure cuff is inflated around the arm to suprasystolic levels for five minutes to make the limb ischemic. When the cuff is deflated, there is a compensatory 5- to 10-fold increase in blood flow, a process called "reactive hyperemia." The increase in blood flow stimulates receptors on the endothelium that results in the release of NO and vasodilatation. The increase in diameter of the brachial artery, or flow-mediated dilatation (FMD), is a measure of endothelial function and under normal circumstances, it is approximately 10%. A potential limitation of this approach is that the brachial artery is not prone to developing atherosclerosis so it is not immediately clear how the endothelial function of this artery relates to that of the coronaries. This issue was addressed by Anderson and co-workers who assessed endothelial function in the coronary arteries and the brachial artery of 50 patients.[6] A close correlation was found between endothelial function measured invasively in the coronary circulation and that measured by ultrasound in the brachial artery. In patients with normal coronary artery function, the brachial arteries dilated by 10% to 12%, whereas in those with impaired coronary endothelial function, there was brachial FMD of only approximately 4%. These results confirmed that patients with coronary atherosclerosis had evidence of endothelial dysfunction in other vascular territories and validated the use of brachial artery FMD as a non-invasive method to assess endothelial function.

Table 1: The effects of endothelial function

Normal Endothelial function	Endothelial dysfunction
• Vasodilation	• Vasoconstriction
• Growth inhibition	• Growth promotion
• Antithrombosis	• Prothrombosis
• Anti-inflammation	• Proinflammation
• Anti-oxidation	• Pro-oxidation

Endothelial function and risk factors for atherosclerosis

Later studies evaluated whether endothelial dysfunction was associated with risk factors for atherosclerosis in:

• preclinical stages in asymptomatic individuals and therefore could represent a mechanism for the development of this condition, or

• if it only developed in patients in the late stages of the disease when the clinical consequences were already obvious.

The scientific evidence that emerged from these studies provides solid support for the former. Indeed, in children under the age of 10 who are homozygous for familial hypercholesterolemia, marked impairment of flow-mediated dilatation has been reported. This finding is remarkable because these children are decades away from developing symptomatic atherosclerosis and it suggests that hypercholesterolemia impairs endothelial function independently of atherosclerosis.[7] Moreover, it has been demonstrated that although there is a correlation between endothelial dysfunction and total cholesterol levels, the correlation is even better with the number of small dense LDL particles. This suggests that these particles have a greater potential to be injurious to the endothelium, perhaps because they are more susceptible to oxidation.[8] Indeed, when the LDL particles were examined for their susceptibility to oxidation, it was demonstrated that patients whose LDL was more resistant to oxidation tended to have the best endothelial function.[9]

Additional studies have demonstrated that the presence of other risk factors significantly worsens the endothelial dysfunction associated with hypercholesterolemia. For instance, smokers have marked endothelial dysfunction, but the combination of smoking and hypercholesterolemia causes significantly worse endothelial dysfunction than either risk factor alone. This suggests a synergism of atherosclerotic risk factors in their ability to injure the endothelium.[10] Smoking, by increasing oxidative stress, could act on LDL and increase

Table 2: Cardiovascular risk factors associated with endothelial dysfunction

- Hypercholesterolemia
- Hypertriglyceridemia
- Small dense LDL
- Hypertension
- Smoking
- Diabetes mellitus
- Estrogen deficiency in women
- Hyperhomocysteinemia

its oxidation making it more injurious to the endothelium; this mechanism would be potentiated when LDL levels are elevated, especially more susceptible small dense LDL particles. As well, higher levels of LDL have been shown to increase the production of oxygen free radicals, such as superoxide, by the endothelium.[11] Oxygen free radicals inactivate NO and impair its many important functions such as vasodilation.

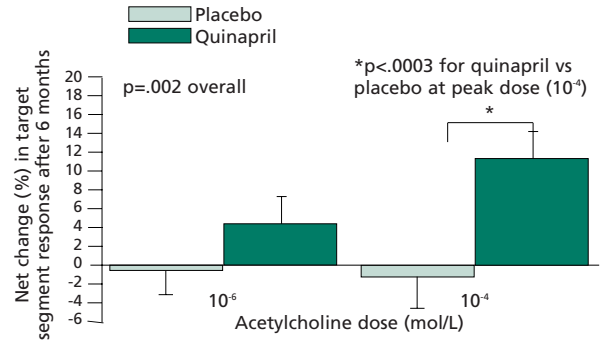
Recent studies have demonstrated that even a short exposure to high lipid levels is enough to significantly impair endothelial function.[12] Human studies evaluating the effects of high-fat, high-calorie meals on flow-mediated dilatation in normal individuals demonstrated that normal endothelial function can be converted into endothelial dysfunction only four hours after such a meal. Interestingly, supplementation with vitamins E and C resulted in attenuation of the impairment on endothelial function, presumably because of their antioxidant effects.[13]

Endothelial dysfunction has also been associated with other atherosclerotic risk factors. In patients with type II diabetes mellitus and insulin resistance, significant impairment of endothelial function has been demonstrated.[14] Moreover, in healthy individuals, an infusion of glucose for 6 hours raised glucose levels to >16 mmol/L on average, and resulted in acute impairment of FMD of the brachial artery.[15] This could be related to increases in superoxide production and oxidative stress caused by the hyperglycemia. As well, abnormalities in endothelial function measured by FMD of the brachial artery have been identified in hypertensive individuals.[16]

Recently, elevated levels of homocysteine have been identified as an additional risk factor for the development of atherosclerosis and have been associated with marked impairment of endothelial function.[17]

In summary, all of the known risk factors for coronary atherosclerosis are associated with, or have been shown to cause, endothelial dysfunction, often acutely and in some

Figure 2: The TREND results



cases, many years before overt atherosclerosis develops (Table 2). This evidence, and the crucial role of the endothelium in maintaining vascular health, strongly suggest that the endothelium is the main modulator of the activity of those risk factors. The common mechanism whereby the risk factors act on the endothelium appears to be the induction of a state of oxidative stress. This also seems to be an important component of the actions of strong vasoactive peptides that are known to be mediators of cardiovascular disease such as angiotensin II and endothelin.

Endothelial dysfunction: A therapeutic target?

In view of the central role that the endothelium plays in the modulation of the effects of atherosclerotic risk factors, endothelial dysfunction has been studied as a potential target for therapy in coronary artery disease (CAD). Lipid reduction with statins, as well as antagonism of the renin-angiotensin system with high tissue-affinity angiotensin-converting enzyme (ACE) inhibitors, have been shown to improve endothelial function in patients with CAD.

The TREND study was a placebo-controlled trial that evaluated the effects of six months of therapy with the ACE inhibitor quinapril on coronary endothelial function in patients with CAD. At baseline, marked abnormalities were demonstrated in the response of the coronary arteries to ACh, consistent with an impairment of NO activity. After six months of therapy, patients on quinapril had a significant improvement in their endothelial function, whereas no change was observed in placebo-treated patients (Figure 2).[18]

A recently concluded Canadian trial, BANFF, studied the effect of several vasoactive medications on endothelial function in patients with CAD. The study was a randomized, open-label trial with four medications: quinapril, enalapril, losartan and amlodipine. All participants in the trial had CAD, but patients with severe underlying disease, previous coronary artery bypass surgery, heart failure, current cigarette

smoking, hypertension, or total cholesterol >6 mmol/L that required statin therapy, were excluded from the trial.

Utilizing brachial artery noninvasive assessment of endothelial function, the investigators demonstrated that at baseline, patients with CAD exhibited a FMD of only 7%, compared with normal values of 11% to 12%, indicating the presence of endothelial dysfunction. Quinapril treatment resulted in an improvement of FMD of more than 25% (p=0.02) and was the only one of the four medications to significantly increase flow from baseline, which was the primary endpoint of the study.[19] These results may be interpreted as indicating that quinapril significantly improved endothelial function, whereas the other agents did not. Enalapril had a neutral effect, whereas losartan and amlodipine resulted in slight flow-mediated vasodilatation which did not reach statistical significance. These findings may be due to differences between vasoactive drugs in their ability to improve endothelial function, both within, and between drug classes.

Pharmacogenetics: Can DNA testing predict the therapeutic response of endothelial dysfunction?

Gene polymorphisms are DNA sequence differences, deletions or insertions that can be detected by the polymerase chain reaction (PCR) among other techniques. In some cases polymorphisms are associated with differences in the level of expression of a particular gene, or the susceptibility to a pathological condition in which the gene product is involved. In the case of ACE, polymorphisms allow the detection of two types of alleles: the D and I alleles. With the normal genetic complement being two alleles of each gene, there are three possible genotypes for the ACE gene: DD, ID and II. The DD genotype has been associated with increased levels of ACE and angiotensin II in some studies. As well, some clinical studies suggest that individuals with the DD genotype are at an increased risk of restenosis following coronary angioplasty. Some reports have also suggested that the DD genotype is associated with a higher risk of myocardial infarction.

In a substudy of the BANFF trial, investigators evaluated whether the effectiveness of the ACE inhibitor quinapril in reversing endothelial dysfunction was related to the ACE genotype. As previously discussed, quinapril resulted in a significant improvement in endothelial function, but there was no significant change in FMD with enalapril. Interestingly, patients with the II and the ID genotypes accounted for the improvement in endothelial function seen with quinapril, while patients with the DD genotype did not improve.[20] Differences may exist, therefore, in the ability of ACE inhibitors to improve endothelial function and this may be mediated, in part, by local vascular effects related to the ACE genotype. Pharmacogenetic studies could represent an exciting new approach in which molecular analyses are utilized to identify which treatment modalities are most appropriate for specific individuals, depending on the characteristics of the genes or regulatory pathways that are being targeted by the intervention.

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