ROLE OF ENDOTHELIAL DYSFUNCTION IN CARDIOVASCULAR DISEASE: A REVIEW

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Introduction

Cardiovascular disease remains to be the chief cause of death in industrialized countries. Although the cause of cardiovascular disease remains unknown, it is now clear that an impairment of tissue perfusion represents the primary problem. Three main factors contribute to the impairment of tissue perfusion, which are: enhanced vasoconstrictor responses, increased interaction of circulating blood cells and structural changes of the arterial intima.

Endothelium, due to its strategic anatomical location between the circulating blood and vascular smooth muscle is a primary target and mediator of cardiovascular disease. The importance of endothelium in modulating the activity of vascular smooth muscle and therefore in regulating vascular tone was first recognized by the pioneering studies of Furchgott and Zawadzki (1). Functional integrity of the endothelium is crucial for the maintenance of blood flow and antithrombotic capacity because, endothelium releases humoral factors that control muscle relaxation and contraction, thrombogenesis and fibrinolysis and platelet activation and inhibition.

Physiology of the endothelium

Stimulation of intact monolayer of endothelial cells by neurotransmitters, hormones, and substances derived from platelets and the coagulation system causes release of a substance, that in turn induces relaxation of the underlying vascular smooth muscle. Furthermore, shear forces generated by circulating blood induce endothelium-dependent vasodilation, which is an important adaptive response of the vasculature during exercise. This endothelium-derived relaxing factor (EDRF), a diffusible substance with a half-life of a few seconds has been identified as the free radical, nitric oxide (NO). Nitric oxide is formed from L-arginine by oxidation of the guanidine-nitrogen terminal (2). The NO-synthesizing enzyme exists in several isoforms in endothelial cells, platelets, macrophages, vascular smooth cells, nerves and the brain (3). The activity of NO synthase can be inhibited by the circulating amino acid, asymmetrical dimethylarginine (ADMA) indicating that endogenous substances also regulate the activity of L-arginine NO pathway.

Endothelium-dependent relaxations due to NO involve an increase in cyclic 3',5'- guanosine monophosphate (cGMP) in vascular smooth muscle via the soluble enzyme guanylyl cyclase (4) (Fig 1). Soluble guanylyl cyclase is also present in platelets and, if activated by NO, increases cGMP in platelets and in turn reduces adhesion and aggregation. NO induced endothelium-dependent relaxation can be pharmacologically inhibited by analogues of L-arginine such as L-NG-monomethyl arginine (L-NMMA) or L-nitroarginine methylester (L-NAME), which compete with the natural precursor L-arginine at the catalytic site of the enzyme (3). When infused, L-NMMA induces long lasting increases in blood pressure indicating that the vasculature is in a constant state of vasodilation due to continuous basal release of NO by the endothelium.

In addition to NO, endothelial cells release two other relaxing substances (Table 1). Prostacyclin increases cyclic 3',5'-adenosine monophosphate(cAMP) in smooth muscles and platelets. Its platelet-inhibitory effects play a greater physiologic role than its contribution to endothelium-dependent relaxation. NO and prostacyclin synergistically inhibit platelet aggregation suggesting that both the mediators are required for maximal inhibition of platelet aggregation. In the epicardial coronary circulation, inhibitors of the L-arginine pathway do not prevent all endothelium-dependent relaxations (5). Because vascular smooth cells become hyperpolarized during NO-independent relaxation, the existence of endothelium-dependent hyperpolarizing factor (EDHF) has been proposed (6,7). EDHF appears to activate ATP-sensitive K+ channels and/or Na+-K+-ATPase in smooth muscle cells (8).

Soon after EDRF was discovered, it became clear that endothelial cells can also mediate contraction. Endothelium-derived contracting factors (EDCF) include the 21-aminoacid peptide endothelin-1 (ET-1), vasoconstrictor prostanoids such as thrombaxane A2 and prostaglandin H2, and components of the rennin-angiotensin system such as angiotensin 2. Translation of messenger RNA generates preproendothelin, which is converted to big endothelin that is further converted by endothelin-converting enzyme to the mature peptide

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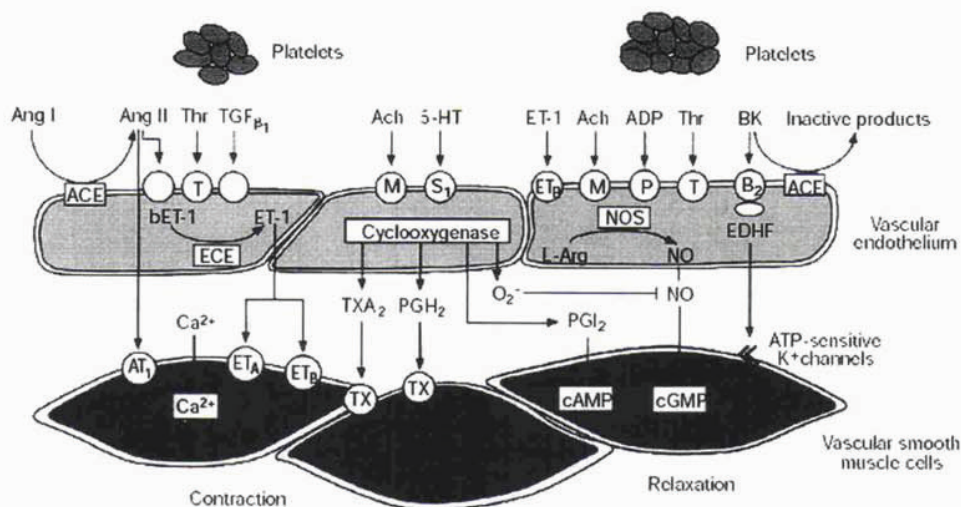50603 Kuala Lumpur, Malaysia.

FIG. 1 Vasoactive mediators released by the endothelium. The endothelium produces factors that promote both relaxation (right) and contraction (left). Ang = angiotensin, ACE = angiotensin-converting enzyme, Ach = acetylcholine, ADP = adenosine diphosphate, ATP = adenosine triphosphate, Bk = bradykinin, cAMP/cGMP = cyclic adenosine/guanosine monophosphate, ECE = endothelin-converting enzyme, EDHF = endothelium-derived hyperpolarizing factor, ET = endothelin-1, 5HT = 5-hydroxytryptamine (serotonin), L-Arg = L-arginine, NO = nitric oxide, NOS = nitric oxide synthase, O_2^- = superoxide, PGH_2 = prostaglandin H_2, PGI_2 = prostacyclin, $TGF\beta_1$ = transforming growth factor β_1, Thr = thrombin, TXA_2 = thromboxane A_2. Circles represent receptors (AT = angiotensinergic, B = bradykinergic, ET = endothelin receptor, M = muscarinic, P = purinergic, S = serotonergic, T = thrombin receptor, TX = thromboxane receptor).

Fig Source: TF Luscher et al., Clin Cardiol. Vol.20(Suppl 2), 1997

Table 1. Relaxing and contracting factors released by endothelium

Relaxing factors	Contracting factors
1. Endothelium derived nitric oxide	1. Cyclo-oxygenase dependent endothelium derived constricting
2. Prostacyclin (PGI2)	2. Endothelin 1
3. Endothelium-derived hyperpolarizing factor (EDHF)	3. Thromboxane A2
4. Atrial natriuretic peptide	4. Prostaglandin H2
5. Adrenomedullin	5. Angiotensin 2

ET-1. Expression of messenger RNA and release of ET-1 are stimulated by thrombin, transforming factor beta, interleukin-1, epinephrine, angiotensin 2, arginine vasopressin, calcium ionophore and phorbol ester (9,10). Endothelin-1 causes vasodilation at lower concentrations but marked and sustained contractions at higher concentrations (9). The cyclooxygenase pathway also produces endothelium-derived vasoconstrictors. Agonists such as arachidonic acid, acetylcholine, histamine and serotonin can evoke endothelium-dependent contractions mediated by thromboxane A2 or prostaglandin H2. In addition, endothelium also plays an important role in regulating the activity of renin-angiotensin system.

Endothelial dysfunction: marker or mediator ?

An imbalance between endothelium-derived contracting and relaxing factors results is endothelial dysfunc-tion. It may be the cause or consequence of vascular disease and is a hallmark of known cardiovascular risk factors. Endothelial dysfunction precedes structural vascular alterations indicating a protective role of the functionally intact endothelium.

Endothelial dysfunction and atherosclerosis are particularly common in epicardial coronary arteries and large arteries such as the aorta and iliac artery while others such as internal mammary artery appear to be protected. This difference could be related to selective alterations in endothelial function in different areas of the vascular tree or due to pulse pressure alterations. Atherosclerosis and plaque rupture are associated with endothelial cell denudation in late stages. Such morphological changes in endothelium are invariably associated with functional alterations and intimal thickening, accumulation of vascular smooth muscle cells and white blood cells and fibroblasts and matrix deposition.

Endothelial dysfunction in Hypertension

Hypertension is associated with functional and morphological alterations of the endothelium (11). In hypertensive blood vessels, endothelial cells have an increased volume and bulge into the lumen. The subintimal space exhibits structural changes with increased fibrin and cell deposition. And the interaction of endothelium with platelets and monocytes is increased in hypertensives compared with normotensives.

Studies have shown that patients with essential hypertension (EH) have impaired response to acetylcholine,

an endothelium-dependent vasodilator (12,13) and a normal response to sodium nitroprusside, an endothelium-independent vasodilator. These results indicated that patients with EH have a specific deficit in the endothelium-derived nitric oxide system and this defect could partly be responsible in determining both the increased vascular resistance and the impaired response to endothelium-dependent agents in these patients. Subsequent studies have shown that blunted endothelium-dependent vasodilator responses in essential hypertensives is largely due to reduced bioactivity of nitric oxide (14), is not related to decreased availability of the natural NO precursor, L-arginine (15), is not due to impaired responsiveness of the vascular smooth muscle to nitrovasodilators (12,13) and is not related to abnormolaties of any specific intracellular signal transduction pathways (16).

A recent study has demonstrated the presence of impaired endothelial function in normotensive subjects with a family history of hypertension (17) and also in the offsprings of EH patients (18). This shows that the onset of endothelial dysfunction may be an important pathogenic event preceeding the development of clinically evident vascular disease.

Endothelial dysfunction in congestive heart failure

The pathogenesis of heart failure is determined by the ventricular and vascular responses to myocellular injury. Studies indicate that vascular endothelium may play an important role in modulating the progression of ventricular and vascular remodeling in heart failure. Abnormalities of vascular endothelial function characterized by increased basal vasomotor tone and decreased vasodilatory reserve could be of particular relevance to the pathophysiology of congestive cardiac failure (19).

Endothelium-dependent vasodilation has been investigated in various experimental models and clinical studies of congestive heart failure. In experimental studies on rats and dogs with induced heart failure, agonist-stimulated and flow-stimulated nitric oxide-mediated vasodilation was found to be decreased in both conduit and resistance vessels compared with normal controls (20). Similarly in clinical studies, endothelium dependent vasodilation in response to hormonal agonists and increased flow were impaired in the coronary and skeletal muscle circulations of patients with heart failure compared to normal subjects (20). Studies have also shown that endothelin-1, an endothelium-derived contracting factor is increased in both experimental subjects and clinical heart failure patients compared with normal control subjects (20). These findings demonstrate that heart failure is associated with generalized endothelial dysfunction, which is partly described by impaired nitric oxide-mediated vasodilation and increased plasma concentration of endothelin-1.

Increased peripheral vascular resistance is known to be a hallmark of congestive cardiac failure. The impaired functional capacity of peripheral blood vessels to dilate in response to sheer stress is a major determinant of the degree of exercise intolerance, which is an important clinical feature in patients with heart failure (21,22). This deficit in peripheral vasodilator capacity that results from attenuated vascular endothelial function has been attributed to the loss of ability of the endothelium to release nitric oxide in response to physiologic stimuli (23,24).

Immunologic and inflammatory responses may also play a role in the development of heart failure (25). Elevated circulating levels of pro-inflammatory cytokines such as interleukin-1, interleukin-6 and tumour necrosis factor-alpha, as well as certain chemokines has been noted in patients with congestive heart failure (26-28). These cytokines cause endothelial dysfunction either directly or through the generation of free radicals (29,30). In addition, changes in regional flow and pressure patterns seen commonly in patients with heart failure may also contribute to increased free radical production (31) providing a potential link between endothelial dysfunction and congestive heart failure. A very recent study has shown that the serum of patients with congestive heart failure potently induced apoptosis of endothelial cells through the activation of caspase cascade, thus providing another possible mechanistic clue in the pathogenesis of endothelial dysfunction in these patients (32).

Endothelial dysfunction in atherosclerosis

Endothelial injury, either physical trauma or more subtle cellular damage, is now regarded as an important initial event in atherogenesis (33,34). Physical damage to the endothelium has been shown to cause atherosclerotic lesions even in normocholesterolemic animals (35). Hypertension has been shown experimentally to disrupt endothelial integrity (36). Hyperhomocysteinemia, that causes chemical endothelial injury, is associated with premature atherosclerosis and thrombosis (37). The finding of these insults associated with the clinical progression of vascular disease, being related to endothelial injury has added more glamour to the "response to injury" hypothesis proposed by Ross and Glomset (38).

The consequences of endothelial damage that initiate fatty streak and plaque formation include increased adherence of monocytes, increased permeability to monocytes/macrophages and lipoproteins that accumulate in the vessel wall, increased platelet adherence and increased smooth muscle cell migration and proliferation (39). Endothelial dysfunction may also be accompanied by decreased availability of local NO. This may be due to decreased endothelial production of NO or to excess production of superoxide anions or both with consequent degradation of NO before it can reach its target tissues. Because NO is a local vasodilator that

also inhibits platelet adherence and aggregation, smooth muscle proliferation and endothelial cell leucocyte interactions, reduced NO activity may also contribute to the initiation and progression of atherogenesis (40). In hypercholesterolemia, superoxide production is enhanced with consequently decreased bioavailability of NO (41). Studies have shown that supplementation with oral L-arginine, the precursor of NO has profound antiatherogenic effects in cholesterol-fed animals (42) and was associated with decreased platelet aggregation (43) and monocyte/endothelial cell adhesion (44) in humans.

Endothelial dysfunction has been demonstrated in asymptomatic children and young adults with risk factors for atherosclerosis (45). Hypercholesterolemia is associated with endothelial dysfunction in children as young as 7 years old, with significant correlations between the degree of endothelial impairment and the levels of lipoprotein (46). In the coronary circulation, endothelial dysfunction is not only observed at the sites of obstructive stenosis, but has also been documented in angiographically smooth arteries of subjects with risk factors for atherosclerosis (47,48). However no longitudinal studies in humans have yet shown that those young subjects with endothelial dysfunction will go on to develop advanced atherosclerosis. Traditional risk factors (Table 2) interact to damage the endothelium in symptomatic subjects in the same way, as they are known to interact in determining the clinical cardiovascular end points (49).

Table 2. Common conditions associated with endothelial dysfunction

Atherosclerosis	Type 1 and 2 diabetes mellitus
Hypercholesterolemia	Hyperglycemia
Low HDL cholesterol	Active and passive cigarette smoking
Hypertension	Heart Failure
Aging	Family history of coronary disease
Insulin resistance	Postmenopausal status

HDL = high-density lipoprotein

In coronary arteries, endothelial dysfunction occurs first at branch points and precedes occlusive arterial disease in both the experimental models and in human heart transplant recipients (50,51). Studies have shown that impaired endothelium-dependent dilation at the site of coronary plaques may result in paradoxic vasoconstriction during exercise or mental stress. This phenomenon was first demonstrated by Ludmer et al. in human coronary arteries where, stenotic arteries showed paradoxic vasoconstriction in response to intra coronary acetylcholine (52). Endothelial dysfunction has also been noticed in the coronary microcirculation and may play a significant role in the pathogenesis of myocardial ischemia (53). The clinical correlate

of endothelial dysfunction in the coronary arteries may be episodic myocardial ischemia, either with or without chest pain. Taken together, these data from in vivo human studies indicate the importance of impaired endothelial function in both the early and late stages of atherosclerotic disease.

Assessment of endothelial dysfunction

A large number of studies have assessed arterial endothelial function in health and disease over the past two decades. The ability of normal endothelium to release the vasorelaxing factor NO in response to physiologic or pharmacologic stimuli was tested in most of these studies. Although this is only one of many endothelial functiuons, NO release is particularly important because of its actions on platelets, monocytes and smooth muscle cells.

Coronary artery testing. In vivo assessment of coronary endothelial function was first reported in humans in the mid 1980s (52). Coronary artery diameter was measured by quantitative angiography before and after intracronary infusion of acetylcholine. In normal arteries, acetylcholine stimulated the endothelial release of NO, resulting in vasodilation, whereas in subjects with endothelial dysfunction, vasoconstriction was observed due its direct smooth muscle constrictor effect. This response was contrasted with the response to nitroglycerin, an exogenous source of NO and therefore an endothelium-independent vasodilator. Invasive testing of coronary microvascular endothelium was also described by measuring the response of coronary flow to administration of endothelium-dependent and endothelium-independent small vessel-dilator substances using Doppler wires or catheters (54). The potential for reversibility of endothelial dysfunction in the coronary arteries has been assessed using this technique to assess novel therapeutic strategies such as angiotensin-converting enzyme inhibition and cholesterol-lowering therapy (55,56). The major disadvantage of intra-coronary testing is its invasive nature and is therefore generally unsuitable for use in children and adults who are at risk atherosclerosis but with no clinical signs or symptoms of the disease.

Peripheral artery testing. Non-invasive detection of endothelial dysfunction in the brachial and femoral arteries was first described in 1992 (45). In this technique, arterial diameter is measured in response to an increase in shear stress, which causes endothelium-dependent dilation and in response to sublingual nitroglycerin, an endothelium-independent dilator. This technique has been shown to be reproducible (57) and to correlate well with invasive testing of coronary endothelial dysfunction (58). Endothelial function has also been investigated in the forearm microcirculation by intra-arterial infusion of endothelium-dependent and inde-

pendent vasodilator substances, followed by measurement of forearm flow using plethysmographic techniques (59,60). These techniques have provided important insights into the risk factors for atherogenesis in children and in young adults and are being used in various studies of endothelial dysfunction in asymptomatic subjects.

Reversibility of endothelial dysfunction

Studies over the past decade have demonstrated that endothelial dysfunction can be attenuated by a variety of therapeutic interventions. To date, most interventions attempting to improve endothelial dysfunction have targeted one or more of the numerous risk factors that can cause endothelial damage: hypertension (ACE-inhibition), hypercholesterolemia (lipid-lowering agents), cigarette smoking (cessation), sedentary life style (increased physical activity), menopause (hormone replacement therapy), and diabetes mellitus (control of associated metabolic abnormalities). Several pharmacologic agents have been suggested to achieve vascular protection through various mechanisms. Beneficial changes to the endothelium might result from promotion of vascular relaxation, inhibition of vasoconstriction, reduction in the production of free radicals, or other mechanisms that protect the endothelium from injury.

Lipid lowering agents. Cholesterol lowering therapy has been associated with a decreased risk of coronary ischemic events and an improvement in coronary endothelial function (61). Reduction of LDL cholesterol alone failed to improve vasodilation in coronary arteries but was significantly improved with the addition of antioxidant therapy (62), thus highlighting the importance of oxidative stress in the pathogenesis of endothelial dysfunction. Improvement in the vasomotor response to acetylcholine was significantly greater in the combined therapy (lovastatin and probucol) group than with diet or LDL cholesterol lowering alone. Tamai *et al.* demonstrated that endothelial vasodilator function could be improved immediately after plasmapheresis in patients with familial hypercholesterolemia (63).

Angiotensin-converting enzyme (ACE) inhibition. The role of rennin-angiotensin system in endothelial dysfunction relates primarily to angiotensin 2 as a potent endothelium derived contracting factor. One of the first studies to demonstrate an improvement in endothelial dysfunction with an ACE inhibitor was the Trial on Reversing Endothelial Dysfunction (TREND) (64). This trial demonstrated significant improvement in endothelial vasomotor function in normotensive patients with coronary heart disease treated with an ACE inhibitor (quinapril 40g/day). The beneficial mechanisms of quinapril in this trial probably relate to the effects of ACE inhibition on both angiotensin2

and bradykinin, which is a potent vasodilator. In the TREND study quinapril improved endothelial dysfunction without altering lipids or reducing bloodpressure (64). More recently, the Heart Outcome Prevention Evaluation (HOPE) trial has shown favorable effects with ramipril in highrisk group of patients with preexisting vascular disease (65).

Antioxidants. Because oxidation of low-density lipoprotein (LDL) cholesterol contributes to endothelial dysfunction, investigators have reasoned that a diet rich in antioxidants may be protective. However results of clinical studies have not consistently shown a benefit. Levine *et al.* reported that vitamin C reversed endothelial dysfunction in the brachial circulation of patients with coronary artery disease (66).

Hormone replacement therapy. The finding that estrogen receptors are localized on endothelial and smooth muscle cells of several mammalian species has suggested that the hormone may directly influence vascular function (67). Estrogen receptor expression has also been demonstrated in human endothelial cells suggesting, estrogen may act directly on human vascular tissue (68). Estrogen therapy has been shown to have a beneficial effect on endothelial function in postmenopausal women with atherosclerotic coronary arteries (69). This protective effect of estrogen may be due to an antioxidant effect, or an estrogen-induced enhancement of NO synthase expression.

Other interventions. Augmentation of NO production by L-arginine supplementation has been shown to improve vascular relaxation in certain conditions (70). A recent study demonstrated improved brachial artery flow mediated dilation (FMD) in hypercholesterolemic subjects after four weeks of oral L-arginine supplementation (71). Future longterm oral studies will clarify the usefulness of L-arginine in modulating endothelial dysfunction.

Conclusion

Over the past decade, knowledge regarding the versatile functions of the endothelium has advanced enormously. Experimental and clinical evidence suggest that endothelial dysfunction is a major determinant for the development and progression of cardiovascular diseases. A major goal of therapy in these patients should be to improve or preserve endothelial function. Furthermore, since endothelial dysfunction occurs prior to structural vascular changes, therapy should be initiated early in patients at risk eg., familial hypercholesterolemia, hypertension, diabetes mellitus etc. Prevention or correction of endothelial dysfunction in cardiovascular disease with agents targeting the endothelium are likely to improve the clinical outcome in these patients and may have important public health benefits in the future.

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