

ENDOTHELIAL DYSFUNCTION - METHODS OF ASSESSMENT AND PHARMACHOLOGICAL APPROACH IN CARDIOVASCULAR DISEASES

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REZUMAT

Endoteliul vascular este unul dintre cele mai bine reprezentate țesuturi din organism. În ultimii ani se observă un interes sporit în elucidarea rolului jucat de endoteliu în vasomotricitate, precum și pentru disfuncția endotelială ca precursor al bolii vasculare aterosclerotice. Lucrarea de față încearcă o trecere în revistă a principalilor factori endoteliali cu rol în vasomotricitate, cuprinzând factorii relaxanți derivați din endoteliu (NO, PG I₂, EDHF) și factorii constrictori derivați din endoteliu (ET-1, CODCF, ACE). Disfuncția endotelială a fost identificată precoce în hipertensiunea arterială sau hipercolesterolemie ca rezultat al dereglării echilibrului fiziologic între factorii vasodilatatori și cei vasoconstrictori endoteliali. Evaluarea disfuncției endoteliale reprezintă la ora actuală o provocare atât pentru clinicieni cât și pentru cercetători; există variante de determinare ultrasonografice, angiografice sau imunologice, dar la ora actuală se impune o standardizare a acestor metode. Se încearcă elaborarea de noi produse care să intervină în metabolismul NO, placa turnantă în disfuncția endotelială, considerându-se astfel endoteliul una dintre principalele ținte terapeutice în tratamentul sau prevenția aterosclerozei.

Cuvinte cheie: disfuncție endotelială, NO, ateroscleroză, hipertensiune, hipercolesterolemie

ABSTRACT

Vascular endothelium is the most extensive tissue in the body. An increased interest has been noticed recently in elucidated the role played by the endothelium in vasomotricity and in endothelial dysfunction as precursor of atherosclerotic vascular disease. The present paper tries to make an overview on major endothelial factors with a role in vasomotricity, comprising endothelium-derived relaxing (NO, PG I₂, EDHF) and constricting factors (ET-1, CODCF, ACE). Endothelial dysfunction has been identified in early stages of hypertension or hypercholesterolemia due to an alteration in the physiological balance between endothelial dilating and constricting factors. Evaluation of endothelial dysfunction is nowadays a challenge for both clinicians and researchers; there are various ultrasonographical, angiographical and immunological methods, and a standardization of these methods would be welcome. The focus is now in developing new products which would interfere in NO metabolism, which plays a key role in endothelial dysfunction. We can consider the endothelium one of the major therapeutic targets in the treatment and prevention of atherosclerosis.

Key Words: endothelial dysfunction, NO, atherosclerosis, hypertension, hypercholesterolemia

BACKGROUND

Atherosclerosis is the major contributor to cardiovascular disease development, and risk factors such as hypercholesterolemia, hypertension, smoking, diabetes mellitus, and obesity have been associated with its progression.

In particular, a large accumulation of data has established a strong association between hypercholesterolemia and the physiopathology of atherosclerosis. This relationship has traditionally been thought to involve the oxidative modification

of LDL-cholesterol deposited in vessel walls, followed by cholesterol uptake by scavengers such as macrophages. Then, the recent formed foam cells release inflammatory and growth factors; as a consequence an atherosclerotic plaque forms in the vessel wall. Atherosclerosis is the major contributor to coronary vascular disease progression, where thrombosis following plaque disruption can lead to clinical events.¹

It has been generally recognized that endothelial dysfunction plays an early pathogenic function in cardiovascular disease. After numerous studies, now it is known that the vascular endothelium is a multifaceted organ, which controls the vascular tone, inflammation and hemostasis through the release of vasoactive substances.

Few years ago, the endothelium was known as a semipermeable membrane, separating the smooth muscle from the blood; now is recognized as a large, complex organ with endocrine, autocrine and paracrine

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regulatory and anti-atherosclerotic effects.² It becomes more evident that the developing of atherosclerosis may be due to a dysfunctional endothelium.

The vascular endothelium not only acts as a selective barrier, controlling permeability and transport, but it is also strategically positioned to detect haemodynamic forces or hormonal signals. The endothelium response through release of vasoactive mediators and growth factors which in turn modulate vascular smooth muscle tone and proliferation, inflammation and allow hemostasis through maintenance of a non-adhesive, anti-thrombotic surface.³

THE VASCULAR ENDOTHELIUM

Endothelial cells actively regulate basal vascular tone and reactivity under both physiological and pathological conditions by responding to mechanical forces and neuro-hormonal mediators with the release of a variety of relaxing and contracting factors. (Fig.1)

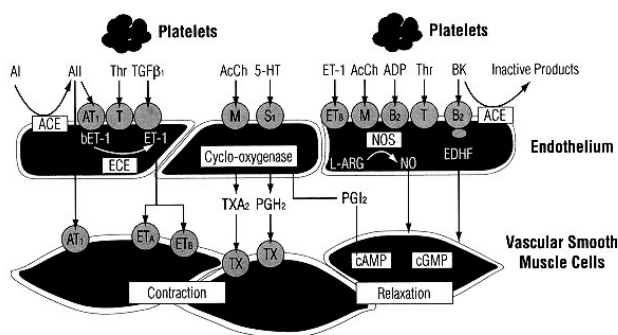


Figure 1. Endothelium derived vasoactive substances: relaxing factors such as nitric oxide (NO), prostacyclin I₂ (PGI₂), and hyperpolarizing factor (EDHF) and contracting factors, including endothelin-1 (ET-1), angiotensin II (AII), thromboxane A₂ (TXA₂) and prostaglandin H₂ (PGH₂). (Adapted from Luscher and Noll in: Braunwald E. Heart Disease, Philadelphia 2004)

ENDOTHELIUM-DERIVED RELAXING FACTORS (EDRFs)

Nitric oxide (NO)

It is widely distributed throughout the body, in smooth muscle, platelets, kidneys, the nervous system and the endothelium, where it plays a key role in the regulation of vascular tone. NO was originally described as the endothelium-derived relaxing factor, it is released from endothelial cells in response to the shear stress produced by blood flow and the activation of a variety of receptors. It is a free-radical gas with an in vivo half life of a few seconds, and is capable of crossing biological membranes. After its diffusion from endothelial to vascular smooth muscle cells, it increases intracellular cGMP concentrations by activating the guanylate cyclase enzyme, thus leading

to the relaxation of smooth muscle cells.⁴

NO is synthesized from L-arginine in the presence of NO synthase, but this conversion can be inhibited by L-arginine analogues, such as NG monomethyl- L-arginine monoacetate (L-NMMA).⁵ The continuous basal release of NO determines the tone of peripheral blood vessels, systemic inhibition of NO synthesis causes an increase in arterial blood pressure. NO also has antithrombotic, antiproliferative, leukocyte-adhesion inhibiting effects and influences myocardial contractility.⁶

Prostacyclin (PG I₂)

It is released by endothelial cells in response to shear stress, hypoxia and a number of other NO releasing substances, and increases cAMP in smooth cells and platelets. In platelets, NO and prostacyclin synergistically inhibit platelet aggregation, thus suggesting that the activity of both is required to exert full anti-platelet activity.⁷

Endothelium-Derived Hyperpolarizing Factor (EDHF)

Not all endothelium-dependent relaxations in the coronary circulation are prevented by inhibitors of the L-arginine pathway.⁸ It has been suggested the existence of an endothelium-dependent hyperpolarizing factor with an unknown chemical structure.

ENDOTHELIUM-DERIVED CONTRACTING FACTORS

Endothelin-1 (ET-1)

It is highly recognized that endothelin-1 (ET-1) is the most potent endogenous vasoconstrictor.^{9,10} The endothelin (ET) family consists of three closely related peptides (ET-1, ET-2, ET-3) that are converted by endothelin converting enzymes from "big endothelin" originating from the large proendothelin peptides cleaved by endopeptidases.¹¹

The ET peptides are not only synthesized in vascular endothelial and smooth muscle cells, but also in neural, renal, pulmonary and some circulatory cells carrying the genes for endothelins.¹² The factors modulating the expression of ET-1 include shear stress, epinephrine, angiotensin II, thrombin, inflammatory cytokines (tumor necrosis factor α , interleukin-1 and interleukin-2), transforming growth factor β and hypoxia. It is metabolized by a neural endopeptidase, which also cleaves natriuretic peptides.

ET-1 has a paracrine rather than endocrine mode of action, which is reflected by plasma ET-1 levels in the picomolar range. The infusion of an ET-1 receptor antagonist into the brachial artery of healthy humans

leads to vasodilatation, thus indicating that ET-1 plays a role in the maintenance of basal vascular tone.¹³

Cyclooxygenase-derived contracting factor (COD CF)

In veins, in cerebral and opthalmic circulation, agonists such as arachidonic acid, acetylcholine, histamine and serotonin can evoke endothelium-dependent contractions that are mediated by thromboxane A2 or prostaglandine H2.¹⁴

The cyclooxygenase pathway is a source of superoxide anions that indirectly inactivate NO and can also directly cause vasoconstriction.

Angiotensin-converting enzyme (ACE)

The endothelium regulates the activity of renin-angiotensin system. The angiotensin-converting enzyme (ACE), which activates angiotensin I to angiotensin II, is expressed on endothelial cell membranes.¹⁵ ACE is identical to kinase II, which breaks down bradykinin. It is still not clear whether other components of the renin-angiotensin system are produced in endothelial cells.

ENDOTHELIAL DYSFUNCTION

Under normal physiological conditions, the endothelium carefully regulates vasoactive substances release, creating a dynamic balance of opposite actions. Disruptions of this equilibrium can generate endothelial dysfunction, which is now widely implicated as an early event in the pathogenesis of various cardiovascular diseases. Many cardiovascular risk factors, including hypertension or hypercholesterolemia are characterized by a dysfunctional endothelium, providing a contributing factor for atherosclerosis.

Endothelial dysfunction in hypercholesterolemia

Studies using animal models have associated hypercholesterolemia with impaired endothelium-dependent vasodilation of blood vessels.^{16,17} This vasodilator dysfunction has been shown both before and after atherosclerotic lesion development in both animal and human hypercholesterolemia.

Increased ET-1 Action

Recent studies have suggested that vascular dysfunction in hypercholesterolemia might be due to an increased production and action of potent vasoconstrictor ET-1. Elevated circulating and tissue ET-1 in hypercholesterolemic patients with coronary atherosclerosis is also evident. Suggestions have been made implicating increased LDL as a cause of this upregulation in gene expression.¹⁸

Further atherogenic and injurious implications of increased ET-1 may relate to its effect of promoting

smooth muscle cell and fibroblast proliferation, resulting from growth factor synthesis stimulation, enhancement of adhesion molecule expression, chemoattractant and macrophage activating properties.¹⁹

Reduced NO synthesis

The administration of the NOS substrate, L-arginine, has been demonstrated to improve endothelial function and blood flow in experimental models of hypercholesterolemia, suggesting the presence of a substrate deficiency or impaired L-arginine metabolism.²⁰ Direct effects of hypercholesterolemia on the NO-pathway include the down-regulation of eNOS expression by oxidized LDL, leading to reduced NO production.

Increased inactivation of NO

In endothelial dysfunction from hypercholesterolemia, there is a crucial role of both native (nLDL) and oxidized (oxLDL).²¹ In particular, the oxidized LDL has been demonstrated to be injurious to the endothelium, and acutely inhibit the release of both NO and endothelium derived relaxing factors (EDHF) in vitro. It seems that a diminished endothelium-dependent vascular relaxation, suggesting the involvement of the recognized oxygen free radical destruction of NO, may be the main contributor to the decreased NO bioactivity.²²

Endothelial dysfunction in hypertension

Nitric oxide in hypertension

In patients with arterial hypertension, endothelium-dependent vasodilation in response to acetylcholine is impaired in the forearm circulation and in the coronary vascular bed, and there is a closed correlation between the two.²³⁻²⁵ Whole body NO production in patients with essential hypertension is therefore diminished under basal conditions. The vasoconstrictor response to the NG monomethyl -L-arginine monacetate (L-NMMA) inhibitor of NO synthesis has been found to be significantly reduced in hypertensive patients than in normotensive subjects, whereas there is no difference

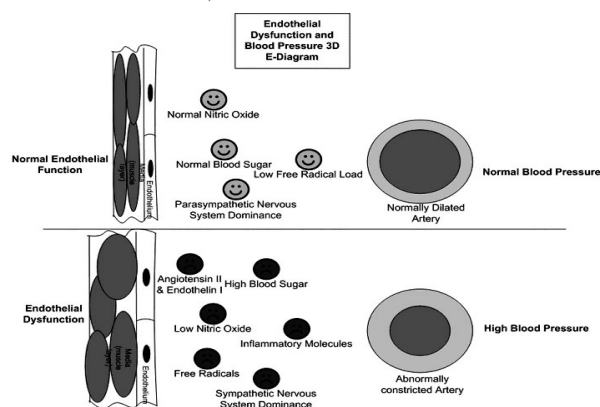


Figure 2. Endothelial dysfunction in hypertension. (Adapted from C. Michael Wright MD, in www.cardiology.lifescore.com)

in their responses to noradrenaline, an endothelium-independent vasoconstrictor.²⁶

Oxidative stress in hypertension

Oxidative stress plays an important role in the pathogenesis of hypertension. Superoxide anion, (O⁻²) an oxygen radical, can scavenge NO from peroxynitrite (ONOO⁻) and thus effectively reduce the bioavailability of endothelium-derived NO.²⁷ It can also act as a vasoconstrictor.²⁸

The renin-angiotensin system plays a major role in hypertension. In addition to the direct vasoconstricting effects of angiotensin II (AII), there are important interactions between AII, oxygen radicals and NO. AII stimulates the generation of O⁻² by increasing the expression of the NADPH oxidase gene and the activity of NADPH oxidase.²⁹ Furthermore, AII increases the production of endothelin (ET) in the blood vessel walls, which induces vasoconstriction and the proliferation of vascular smooth muscle cells.³⁰

Endothelium-derived hyperpolarizing factor

There is evidence that a calcium-dependent potassium channel on endothelial or smooth muscle cells is important in mediating endothelium-dependent hyperpolarization, a mechanism that is impaired in patients with arterial hypertension. Endothelium-dependent hyperpolarization may also be involved in compensating for the impaired NO-system in patients with essential hypertension.³¹

Endothelin

In addition to increasing arterial blood pressure, ET-1 induces vascular and myocardial hypertrophy, that are independent risk factors for cardiovascular morbidity and mortality. In patients with essential hypertension, carotid wall thickening and left ventricular mass correlate with reduced endothelium-dependent vasodilatation.^{32,33} Plasma ET levels have been reported to be correspondingly high in some patients with essential hypertension.³⁴

ASSESSMENT METHODS

Quantitative coronary angiography can be used to examine the change in diameter in response to intracoronary infusions of endothelium-dependent vasodilators such as acetylcholine. In healthy vessels, acetylcholine evokes a NO-mediated vasodilatory response; in patients with endothelial dysfunction, this effect is blunted or paradoxical vasoconstriction is produced instead.

Endothelial function of the coronary microvasculature can be assessed with intracoronary Doppler techniques to measure coronary blood flow in response to pharmacological or physiological

stimuli. Noninvasive tests for assessment of coronary endothelial function include Doppler echocardiography, positron emission tomography, and phase-contrast magnetic resonance imaging.

Brachial artery ultrasound is a widely used noninvasive measure of endothelial function. Upper-arm occlusion for 5 minutes results in reactive hyperemia after the cuff is released; this increase in shear stress results in endothelium-dependent flow-mediated vasodilatation. Importantly, endothelial dysfunction assessed by this technique correlates with measurements of coronary endothelial dysfunction.³⁵

Peripheral resistance vessel function can be assessed by strain-gauge venous impedance plethysmography.³⁶ This technique examines the change in forearm blood flow in response to direct intra-arterial (brachial artery) administration of agonists.

A number of circulating markers of endothelial dysfunction and vascular inflammation have been studied over the past few years. We limit our discussion to soluble cellular adhesion molecules (CAMs) and C-reactive protein (CRP).

CAMs are expressed on the surface of endothelial cells and leukocytes in response to endothelial dysfunction. The three major classes of CAMs include the selectins (P-selectin, L-selectin, E-selectin),^{2,37} the beta-2 integrins (CD11/CD18), and immunoglobins (intercellular adhesion molecule-1 [ICAM-1], vascular cell adhesion molecule-1 [VCAM-1], and platelet endothelial cell adhesion molecule-1 [PECAM-1]). CAMs orchestrate the complicated process of leukocyte rolling, adhesion, and transmigration into the subintimal space. Circulating levels of CAMs have been examined as surrogate markers of endothelial function. Elevated levels of CAMs have been observed in patients with cardiovascular risk factors and may predict the development of cardiovascular disease. High-sensitivity CRP has been widely studied as a predictor of cardiovascular disease.³⁸ Accumulating evidence suggests that atherosclerosis represents a chronic inflammatory process, and hence, inflammatory markers like CRP may provide an adjunctive method for global assessment of cardiovascular risk. Several large-scale studies have shown that plasma levels of CRP are a strong independent predictor of endothelial dysfunction, future myocardial dysfunction, stroke, peripheral artery disease, and vascular death.

PHARMACOLOGICAL MODULATION OF ENDOTHELIAL FUNCTION

Endothelium-dependent coronary artery vasodilatation, which largely depends on endothelium

derived NO and is impaired in atherosclerosis, is a prognostic factor in patients with coronary artery disease.³⁹

Lipid-lowering therapies have been shown to induce substantial declines in cardiovascular morbidity and mortality. Administration of statins has been shown to cause significant improvement in forearm endothelial function.⁴⁰ In addition to their beneficial lipid lowering properties, it seems that statins may have protective antioxidant properties. Statins are known to increase the expression of NO synthase, which is considerably reduced in patients with atherosclerotic vascular disease.

ACE-inhibitors can similarly improve endothelial function in patients with coronary artery disease, and also improve endothelium dependent vasomotion in hypertension and heart failure. Certain ACE-inhibitors increase the expression of endothelial NO synthase.

There are newer NO releasing β -blockers, causing concentration-dependent relaxation that are prevented by removal of the endothelium.⁴¹

Calcium-channel antagonists represent a further class of vasodilating drugs, capable of improving endothelial function in hypertension.⁴²

CONCLUSIONS

As a result of its widely-recognized role in atherosclerotic development and hence cardiovascular health and disease, studies on the endothelium and its potential as a therapeutic target have been extensively conducted. Investigations, ranging from elucidating the mechanisms involved in atherosclerotic pathogenesis to discovering ways to rectify vascular tone imbalance continue to be a priority, in order to find a way to prevent clinical cardiovascular disease.

Endothelial dysfunction also plays an important role in the clinical course of atherosclerosis. Impaired endothelium-dependent vasodilatation in coronary arteries with established atherosclerosis results in paradoxical vasoconstriction, which may result in reduced myocardial perfusion and myocardial ischemia. Additionally, endothelial dysfunction actively modulates plaque architecture and portends the vulnerability of the lesion and the likelihood of rupture. Through this vasoconstrictor and inflammatory mechanism, endothelial dysfunction in atherosclerotic vessels may lead to the development of unstable coronary syndromes.

Dysfunction of endothelial cells is probably the earliest event in the process of lesion formation - hence, the concept that assessment of endothelial function may be a useful prognostic tool for coronary artery disease. Heterogeneity of vascular dysfunction

must be appreciated. Nonetheless, coronary endothelial cell perturbations often are reflected in peripheral vasodilator abnormalities, thereby allowing the assessment of peripheral endothelial function as a measure of coronary vasomotricity. Recent studies also suggest that there is a correlation between endothelium-dependent vasodilation and CRP levels.

Thus, endothelial dysfunction may be reflected systemically, thereby allowing for a less invasive approach to the assessment of overall endothelial cell biocompatibility.

DISCUSSIONS

It is generally accepted that endothelial dysfunction occurs in response to cardiovascular risk factors and precedes the development of atherosclerosis. The balance of published information supports the paradigm of endothelial dysfunction as the common link between risk factors and atherosclerotic burden. Endothelial dysfunction actively participates in the process of lesion formation by promoting the early and late mechanisms of atherosclerosis. These include up-regulation of adhesion molecules, increased chemokine secretion and leukocyte adherence, increased cell permeability, enhanced LDL oxidation, platelet activation, cytokine elaboration, and vascular smooth muscle cell proliferation and migration.

We will witness an increasing number of therapeutic strategies aimed at improving endothelial function in a variety of cardiovascular disease states. The next decade will witness an exponential interest in developing reliable methods of testing endothelial function as a potential predictor of cardiovascular disease. Several large noninvasive studies currently are underway to determine the predictive value of brachial ultrasound testing. Inflammatory markers, such as CRP, likely will find their way into risk assessment algorithms. As measures of endothelial dysfunction become clinically applicable, this may translate into improved methods of risk assessment and equip physicians with yet another tool to predict, prevent, and treat cardiovascular disease.

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