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Endothelial dysfunction reversibility

Reverzija disfunkcije endotela

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Introduction

Coronary artery disease, the leading cause of morbidity and mortality, is a continuum of events that can lead to end stage heart disease [1]. Common to the pathogenesis of most cardiovascular diseases are the processes, called risk factors for atherosclerotic coronary artery disease, that cause pathologic change and dysfunction in the blood vessels and lead to damage of the heart. The central role played in circulatory homeostasis is by the cells that line the vascular system - the endothelium. Originally considered an inert diffusional barrier between blood and vascular smooth muscle, it is now known that the endothelium has many important functions. Probably the most significant advance in cardiovascular medicine over the last two decades has been the identification of endothelial cells as a vasoactive organ. After the pioneering report [2] by the 1998 Nobel Prize winner Robert Furchgott, an impressive array of evidence has made it possible to state today that the endothelium plays a primary autocrine/paracrine regulatory role by secreting substances that control both vascular tone and structure. Moreover, accumulating evidence has indicated that the dysfunctioning endothelium, characteristic for the majority of cardiovascular risk factors, is a major promoter of atherothrombosis and, consequently, cardiovascular events. That's why endothelial dysfunction is now considered an important target for cardiovascular treatment.

Endothelium in vascular health

The endothelium is the largest organ in the body. In a 70-kg man, the total surface of the endothelium is about six tennis courts, total mass is as five normal hearts, total weight about 1 800 g with around 1 trillion of cells.

The endothelium serves as endocrine and paracrine organ, with numerous regulatory functions. The specific location of endothelium allows it to "sense" changes in hemodynamic forces and signals from blood and "respond" by realizing a number of substances [3]. One of the primary functions of the endothelium is to maintain vascular tone [2, 3]. In the response to different signals (such as shear force on the vessel wall, hormonal and other regulatory substances) endothelial cells produce and secrete (table 1) vasoactive substances that

Table 1

Factors released by endothelium

Vasoactive Substances
• Vasodilatators
– Endothelium-derived relaxing factor (EDRF)
– Endothelium-derived hyperpolarising factor (EDHF)
– Prostacyclins (PGI_2)
– Bradykinin
– Acetylcholine, serotonin, histamine, substance P
• Vasoconstrictors
– Endothelin
– Angiotensin II
– Tromboxane A2, acetylcholine, arachidonic acid, prostaglandin H2, thrombin, nicotine, high potassium levels
Growth Modulators/Mediators
• Promotors
– Platelet derived growth factor (PDGF)
– Basic fibroblast growth factor
– Insulin-like growth factor-1
– Interleukin 1
– Endothelin, angiotensin II
• Inhibitors
– Heparin sulfate
– Transforming growth factor 8
– NO, prostacyclin, bradykinin
Inflamatory Modulators/Mediators
• Adhesion molecules
– Endothelial leucocyte adhesion molecule (ELAM)
– Intracellular adhesion molecule (ICAM)
– Vascular adhesion molecule (VCAM)
• Antigens
– Major histocompatibility complex
Hemostasis and Thrombolytic Factors
– Tissue plazminogen activator
– Plasminogen activator inhibitor (PAI-I)
– Thrombomodulin

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can dilate or constrict blood vessels [4]. Endogenous vasodilatators are: nitric oxide (NO) [5], prostacyclin [6, 7], endothelium-derived hyperpolarizing factor (EDHF). Endothelium-derived vasoconstrictors are tromboxane A2, endothelin and angiotensin II (A II) [8].

Endothelial cells produce and release factors that promote or inhibit the growth of smooth muscle.

A number of antithrombotic, anticoagulant and fibrinolytic factors: tissue plasminogen activator (t-PA), plasminogen activator inhibitor (PAI-1) and prostacyclin are released by the endothelium. These factors help to keep the endothelial surface nonadhesive and keep the blood stream flowing smoothly. Endothelial cells also release anti-inflammatory and antioxidant agents.

The endothelium modulates vascular smooth muscle function and structure providing a smooth nonthrombogenic surface and a permeability barrier by secreting and releasing numerous substances, i. e. a balanced release of these bioactive factors facilitates vascular homeostasis. Endothelial cell dysfunction disrupts this balance, thereby predisponding vessel wall vasoconstriction, cell promotion, prothrombosis, proinflammation and pro-oxidation.

The endothelium works to maintain vascular health through a variety of mechanisms [3, 9].

Notable among vasoactive substances is endothelium – derived relaxing factor – the potent endogenous vasodilatator now identified as nitric oxide (NO) [5]. Endothelium-derived NO is released in response to receptor-dependent agonists such as bradykinin and acetylcholine, or physical factors such as increased shear stress. In normal endothelial cells, NO is produced by conversion of L-arginine to L-citruline. This reaction is mediated by the endothelial isoform of NO synthase (eNOS) in the presence of the cofactor tetrahydrobiopterin (BH4) in response to agonist stimulation or shear stress. NO diffuses to the vascular smooth muscle cell (SM) and stimulate guanylate cyclase (IGz) to convert guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP). This leads to a reduction in intracellular calcium (Ca^{2+}) culminating in vasorelaxation [10]. In normally functioning endothelium, low levels of NO are continuously released to keep the blood vessel in the state of dilatation. In this way, the endothelium maintains an appropriate blood pressure. The ability of the endothelium to release NO is often used in research studies as a test of endothelial function.

Risk factors in producing endothelial dysfunction

The mechanisms by which cardiovascular disease risk factors lead to endothelial malfunction have gradually being revealed. A common denominator among these risk factors is oxidative stress [11, 12].

Several conditions such as hypertension, dyslipidemia, heart disease, diabetes, smoking, depression and some others, cause physiological and structural changes that can lead to cardiovascular diseases. In each disorder, one of the earliest changes to occur is an alteration of the oxidative metabolism in the endothelium leading to an increase in the level of the oxidative stress. This change causes the endothelial cells to decrease production of some compounds and increase production of others. Thus, NO production is decreased, facilitating vasoconstriction. Other compounds are released that allow plaque and thrombosis formation.

What is happening in atherosclerosis?

Degeneration of healthy endothelium *via* the pathogenesis of atherosclerosis occurs slowly over decades [13]. It begins with the subtle form of endothelial injury that alters function. Foam cells are the earliest sign of endothelial dysfunction. They are macrophages that contain oxidized low density lipoprotein cholesterol (LDL-C) and are most frequent in infants and children. Foam cells may then infiltrate the vessel, progressing to a fatty streak. As the lesion progresses to an intermediate lesion, small pools of extra cellular lipid form with the smooth muscle layers, disrupting the intimal lining of the vessel. Progression to an advanced lesion occurs when the accumulated lipid, cells, and other components of the plaque disrupt the artery wall. This lesion is termed an atheroma. Once the plaque becomes fibrous, it is primed to rupture. This type of advanced lesion can be found from the forth decade of the life onward. The endothelium itself appears to participate in some of this remodeling through secretion of specific compounds. Atherosclerosis is now recognized as an inflammatory process where immune mechanisms interact with metabolic risk factors to initiate, propagate and activate lesions in the arterial tree [14]. Endothelial function is regulated by 2 renin-angiotensin systems (RAS) [15, 16]. Angiotensin II is produces by conversion of A I with the help of angiotensin converting enzyme (ACE). Angiotensin II is at the center of both RASs. It exerts physiologic control over the cardiovascular system. The hemodynamic effects of the circulating RAS increase blood pressure. Tissue RAS causes long-term changes in vascular tone that create alterations in vascular structure and function. Only about 10% of the ACE in the body circulates in the plasma [15]. Approximately 90% of the ACE is found in the tissue: blood vessels, the heart and the central nervous system. In this system circulating or local A I is converted to A II by local ACE. This local production of A II by vascular ACE is thought to be involved in vascular and cardiac structure and function over the long term [15].

Endothelial injury or dysfunction is associated with the increased local production of A II, with subsequent smooth muscle cell migration and proliferation, induced expression of photo-oncogenes and growth factors, impaired platelet activity and endogenous fibrinolysis, and a reduction in bradykinin levels. Together, these effects of local (tissue) ACE contribute to the development and progression of coronary artery disease.

How can we assess endothelial dysfunction?

Our understanding of endothelial cell responses led to the development of tests that are believed to reflect endothelial cell dysfunction or integrity *in vivo*. The growing body of evidence supports a prognostic role of endothelial function assessment [17–20]. For example, impaired endothelium-

dependent vasodilatation is an adverse prognostic parameter in patients with risk factors such as hypertension and atherosclerotic vascular disease.

Endothelial dysfunction could be assessed by different stimuli (table 2): mediator stimuli as is acetylcholine, or shear/flow stress stimuli – pharmacological stimuli: papaverin, adenosine or non pharmacological – ischemia, exercise, or cold exposure [4]. The methods for noninvasive assessment of endothelial function are: for peripheral arteries ultrasound and pletismography; for coronary arteries transthoracic and transoesophageal echocardiography, magnetic resonance imaging; for endothelial integrity – contrast echocardiography; for myocardial perfusion – contrast echocardiography and positron emission tomography. Assessment of endothelial function clinically is based on the coronary vasodilatator response to pharmacologic or mechanical stimuli. The rationale for measuring the vasodilatator response is based on the obligatory role of the endothelium and EDRF in modulating vascular tone and of the paradoxical vasoconstrictor response to acetylcholine in atherosclerotic coronary arteries [21]. The vasoconstrictor response to acetylcholine and other vasoactive stimuli distinguishes patients with endothelial dysfunction (i.e., those with risk factors for coronary disease or atherosclerosis) from those with normal endothelium and a vasodilatator response [21].

Table 2

Endothelial function assessment

Vasodilatation
Invasively
QCA/ICUS after ”endothelial stimulation“ during angiography
Noninvasively
Direct M mode 2D measurement after ”endothelial stimulation“
Peripheral arteries
Coronary arteries
Flow
Invasively
CSA(QCA/ICUS)xVTI (ic. Doppler) of CA during angiography
Noninvasively
ECHO-Doppler
Pletismography
Approprate perfusion
Stress wall motion
Contrast echocardiography
Positron emission tomography (PET)
Phase-contrast magnetic resonance imaging

VTI – velocity time integral, QCA – quantitative coronary angiography,
CSA – coronary spastic angina, ICUS – intracoronary ultrasound

As endothelial dysfunction is a generalized vasculopathy, both coronary and peripheral arteries could be used for endothelial function assessment. But, while accuracy is nearly the same for both invasive and noninvasive methods, patients comfort, a viability, serial assessment and cost are much better for noninvasive methods. Vascular ultrasound and flow mediated dilatation (FMD) of peripheral arteries are now most frequently used.

Hypertension is a well-recognized risk factor for coronary artery disease. The mechanisms of endothelial dysfunction differ in various models of hypertension. In most forms of experimental, as in human hypertension, the formation of NO is reduced. The results of various studies tend to confirm impaired endothelium-dependent vasodilatation in essential hypertension presumably due to defects in the synthesis or release of NO [22–24]. Clinical data demonstrate that forearm endothelial dysfunction is a marker of future cardiovascular events in patients with essential arterial hypertension [25]. Hypertensive patients also present with increased levels of inflammation and oxidative stress parameters.

Like hypertension, one of the earliest abnormalities detected in hypercholesterolemia is an abnormal endothelial function and a reduced availability or activity of NO [22]. Hypercholesterolemia impairs endothelium-dependent relaxation, an effect that occurs before the formation of atherosclerotic lesions, suggesting that NO dysfunction is central to the pathogenesis of the atherosclerotic process. Zeiher [24] demonstrated a progression of the endothelial dysfunction in coronary arteries that begins with hypercholesterolemia and angiographically normal coronary arteries to patients with atherosclerotic coronary arteries. All stages were angiographicaly defined. The degree of endothelial dysfunction was parallel to worsening stages to atherosclerotic process.

Studies in patients with type 1 diabetes have found impaired endothelium dependent dilatation in forearm resistance vessels [26] as well as in conduit arteries [27], due to the reduced synthesis, release, or activity of NO. There are also other evidences for endothelial dysfunction in diabetic patients: fibrinolytic activity is decreased, PAI activity is increased [28]. Type 2 diabetes is associated with the increased generation of reactive oxygen species (ROS), and the addition of antioxidants and BH4 improves endothelial function in experiments [29].

The metabolic syndrome is defined as the coexistence of several risk factors for atherosclerosis (central obesity, hyperglycemia, dyslipidemia and hypertension) [30, 31]. Endothelial dysfunction associated with the metabolic syndrome

and other insulin resistant states is characterized by the impaired insulin-stimulated production of NO from the endothelium and decreased blood flow to the skeletal muscle. Drugs which improve insulin sensitivity also improve endothelial function. So, drugs for the control of single components of the metabolic syndrome might have some additional effects on endothelial function [32].

Vasoconstriction, platelet aggregation and increased monocyte adhesion are a few effects of cigarette smoking that lead to the increased risk for atherosclerosis and other cardiovascular diseases [33]. This effect is related to the decreased function of eNOS and to the increased synthesis of oxidative stress resulting in the reduced NO bioavailability. Besides activating platelets, smoking activates coagulation cascade, and increases oxidative stress and inflammation [34].

The threshold of smoking and endothelial dysfunction appeared to be ≥ 20 pack-years. Flow mediated dilatation is significantly impaired in passive as well as in active smokers [35].

Although the specific components of cigarette smoke that are responsible for vascular damage are not yet defined, cigarette smoking is strongly associated with impaired arterial dilatation.

Cigarette smoke introduces reactive oxygen intermediates into the circulation which vitamin C scavenges, thereby preventing lipid peroxidation. Endothelial vasodilatator dysfunction observed in smokers is partially reversible by the administration of L-arginine.

There is also a synergistic effect of smoking and hypercholesterolemia because the oxidation of LDL cholesterol is enhanced in a long-term hypercholesterolemic smokers.

Besides these classical risk factors, endothelial dysfunction was found in patients with heart failure, proven atherosclerosis, family history for coronary disease, hiperhomociteinemia, and hard working doctors in night shifts [36] and some other rare conditions.

Modalities for reversing endothelial dysfunction

The most important question when endothelial dysfunction is proven is what we should do next, i.e. if there are methods for reversing endothelial dysfunction.

Several types of interventions, both nonpharmacologic and pharmacologic, have been shown to improve endothelial dysfunction. These include diet and lifestyle changes, antioxidants, lipid lowering agents, ACE inhibitors etc. Most of these interventions have the impact on the balance between nitric oxide activity and oxidative stress. Some of them have parallel benefits on endothelial function and clinical events. Table 3 shows the list of endothelial modulating approaches and their way of acting [4].

Table 3

Therapeutic strategies for treating endothelial dysfunction

Nonpharmacological treatment
Dietary lowering of cholesterol
Life style changes
Smoking cessation
Weight loss
Specific treatment
Angiotensin converting enzyme (ACE) inhibitors
Angiotensin receptor blockers (ARB)
HMG-CoA reductase inhibitors
Calcium channel blockers
Beta blockers
Estrogen
Metformin
Folate
Antioxidants, C/E vitamins
L-arginine supplements
Attractive treatment
Red wine
Black chockolate
Sauna treatment

HMG-CoA – 3-hyproxy-3metylglutaryl coenzyme A

Calcium channel blockers, such as verapamil and nifedipine, reverse vasoconstriction caused by endothelin-1. They exert activity in different vascular beds, including the coronary macrocirculation and peripheral microcirculation [37]. Nifedipine and cerivastatin together have a positive effect on recovery of endothelial function in coronary circulation [38].

It has been demonstrated that cholesterol feeding increases the production of superoxide anion from the endothelium of rabbit aorta, and that dietary lowering of cholesterol improves endothelial function and attenuates super- oxide formation. Since than, at least 20 studies [39] have demonstrated the beneficial effects of lipid lowering on endothelial function in humans. The underlying mechanisms are pleotropic, as HDL mediates reverse cholesterol transport and has additional anti-inflammatory, profibrinolytic and antioxidant properities [40].

Forearm endothelial function could be modulated within hours of LDL pheresis, demonstrating the dynamic nature of the process [41]. *In vitro* studies have suggested that HDL can enhance the expression and promote the activation of eNOS, the rate limiting enzyme for synthesis of NO [42]. *In vivo* studies provide an additional support to this concept [42]. The infusion of HDL into the forearm resulted in an improved endothelial function through an increase in the bioactivity of NO [43].

An investigation of effects of statin therapy [44] has showen that an improvement in endothelial dysfunction is one of the pleotrophic effects of statins, independent of their lipid lowering capabilities. Statins ameliorate endothelial dysfunction by the inhibition of LDL oxidation and up-regulating of eNOS. In a porcine model of hypercholesterolemia, simvastatin preserved endothelial function while decreasing serum markers for oxidative stress in the absence of lipid lowering [45]. Statin improves endothelial dysfunction as well as mortality from cardiovascular death independent of lipid lowering effects.

A treatment with classical beta blockers (atenolol) [46] did not improve the impaired endothelium- dependent vasodilatation, but there are evidences that novel agents, selective beta-1 blockers with vasodilating properties (nebivolol) [47], cause endothelium- dependent vasodilatation and other selective beta-1 blockers with alfa-1 adrenoceptor antagonist properties (carvedilol) could restore endothelial dysfunction in patients with hypertension.

Experimental and clinical evidences suggest that ACE inhibitors (ACE1) may protect against the development and/or progression of atherosclerosis. Vasculoprotective and antiatherosclerotic effects of ACEI are the result of decreasing the level of AII and increasing bradykinin levels which facilitates release of NO, which leads to relaxation of smooth muscle cells around blood vessels. ACEI improve endothelial function by variety of mechanisms including: reducing vascular smooth muscle growth and cell migration, decreasing platelet aggregation and PAI-1 levels, inhibiting matrix synthesis and increasing t-PA levels.

In humans, acute administration of ACEI augmented endothelium-dependent vasodilatation in both coronary and peripheral circulation [48]. Tissue-specific ACEI quinapril attenuated coronary endothelial dysfunction in patients with coronary disease [49] These observations were extended to the peripheral circulation in the BANFF study [50] and suggested potential differences between ACEI with respect to their effect on the endothelium. ACEI with a high tissue affinity might protect the endothelium more effectively than those with primary affinity for plasma ACE [49, 51]. The HOPE trial firmly established ACEI as anti-atherogenic therapy [52]. The dramatic reduction in major cardiovascular events in the HOPE study was attained with only a modest reduction in blood pressure. This may be explained by the inhibition of tissue ACE- mediated processes that are related to atherosclerotic and ischemic complications. An EUROPA trial gave more aprovement [53].

A II contributes to the development of atherosclerosis in various ways: it increases the uptake and oxidation of low-density lipoprotein (LDL) cholesterol by macrophages and endothelial cells, promotes vascular inflammation, stimulates and recruits macrophages and monocytes, increases matrix metalloproteinases [54]. ACEI has been showen to have antiatherogenic effect: without changing blood pressure or lipid profile [55].

A II has a negative effect *via* AT1 receptor on endothelial function by releasing endothelin-1, vasoconstrictor prostanoids, inhibiting NOS activity and increasing oxygen-free radical production. Angiotensin receptor blockers (ARB) have a different form of blockade of the RAS and according to some data ARB are superior to ACEI in restoring normal endothelial function [56].

Although not prescribed for their antioxidant potential *per se*, many conventional cardiovascular therapies directed at cardiovascular risk factors reverse endothelial dysfunction (ameliorating vascular oxidant stress).

Despite preclinical evidence advocating vitamin antioxidant therapy, multiple large clinical trials have failed to validate the efficacy of this approach [57].

Similar, some clinical outcome data did not show that estrogen replacement therapy improves cardiovascular health [58].

Newer reports suggest that consuming dark chocolate may have positive effect on endothelial function [59].

Improved endothelial function appears to be possible *via* a variety of currently available methods with novel approaches still to come. It seems reasonable to expect that future therapeutic strategies and agents will be directly targeted to this monolayer of cells that regulates vascular tone and structure. Early detection of endothelial dysfunction may be a useful measure to guide therapy prior to the development of symptomatic atherosclerosis.

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