Nitric oxide and cardiovascular system

Nitrik oksit ve kardiyovasküler sistem

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ABSTRACT

Endothelium has many important functions including the control of blood-tissue permeability and vascular tonus, regulation of vascular surface properties for homeostasis and inflammation. Nitric oxide is the chief molecule in regulation of endothelial functions. Nitric oxide deficiency, which is also known as endothelial dysfunction, is the first step for the occurrence of many disease states in cardiovascular system including heart failure, hypertension, dyslipidemia, insulin resistance, diabetes mellitus, hyperhomocysteinemia and smoking. This review deals with the importance of nitric oxide for cardiovascular system. It also includes the latest improvements in the diagnosis and treatment of endothelial dysfunction. *(Anadolu Kardiyol Derg 2006; 6: 364-8)*

Key words: Nitric oxide, cardiovascular system

ÖZET

Endotel, vasküler fonksiyonların normal bir şekilde yürüyebilmesinde son derece önemli görevlere sahip bir organdır. Endotel disfonksiyonu kardiyovasküler sistemi ilgilendiren birçok hastalığın bir parçasıdır. Endotel fonksiyonlarının düzenlenmesinde en önemli aracı moleküllerden biri nitrik oksittir. Bu derlemede nitrik oksidin kardiyovasküler sistem açısından önemi anlatılmıştır. Nitrik oksit eksikliği endotel disfonksiyonuna yol açarak, kardiyovasküler sistemi hedef alan birçok hastalığa zemin hazırlayabilir. Bu yazıda ayrıca, endotel disfonksiyonunun tanısı ve tedavisindeki son gelişmeler üzerinde durulmuştur. *(Anadolu Kardiyol Derg 2006; 6: 364-8)*

Anahtar kelimeler: Nitrik oksit, kardiyovasküler hastalıklar

Introduction

Endothelium, which is the inner layer of vascular surface, is a dynamic organ with many properties for the continuity of the normal vascular functions. Its main functions include the control of blood-tissue permeability and vascular tonus, regulation of vascular surface properties for homeostasis and inflammation (1). Many vasoactive molecules, secreted from endothelium are involved in the control of these functions. The most important of these molecules is probably nitric oxide (NO). Nitric oxide is a potent vasodilator and performs a pivotal role in the normally functioning cardiovascular system.

Nitric oxide is synthesized by endothelial cells from L-arginine and molecular oxygen. The vascular flow and the shear stress caused by vascular flow induce NO synthesis by phosphorylation of nitric oxide synthase (NOS). Nitric oxide synthase catalyzes the reaction which converts L-arginine to citrulline and NO and requires help of calmodulin and pteridin tetrahydrobiopterin (BH4)as cofactors. There are three different forms of NOS: endothelial NO synthases (e NOS), neuronal NO synthase (nNOS) and inducible NO synthase(iNOS). The eNOS, the Ca-dependent form of the enzyme, is found in many types of cells and responsible from the production of most of the NO in the healthy blood vessel. The nNOS is a special type of eNOS that functions in nervous system. The iNOS, the inducible form of the enzyme, is found in myocytes, macrophages and endothelial cells and inducible by immunological stimuli (2). Nitric oxide synthases are formed from two distinct catalytic units as C-terminal reductase domain and N-terminal oxygenase domain (3). In the presence of sufficient amount of BH4 these units work together and synthesize NO, otherwise or in cases of increased oxidative stress, cause production of peroxynitrite.

Resultant NO induces guanilate cyclase for cGMP synthesis from cGTP. cGMP provides the hyperpolarization of cells due to activation of K channels. These reactions cause calcium inhibition and results in vasodilatation in cardiovascular system.

Endothelial Dysfunction and NO

Normal vascular tonus depends on the equilibrium between the vasoconstrictor and vasodilator molecules released from the endothelium. In healthy endothelium, the balance is shifted towards vasodilatation due to NO. Endothelial dysfunction is synonymous with the insufficiency of endothelium dependent vasodilatation and results in the failure of vasoactive, anticoagu-

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lant and anti-inflammatory effects of healthy endothelium. The most important mechanism for endothelial dysfunction is the decrease in NO availability. The insufficiency of substrate like the decrease in L-arginine in endothelial cells or any defect in the transport of L-arginine into the cell, the existence of NOS inhibitors like asymmetrical dimethylarginine (ADMA) and NG-monomethyl- L- arginine (L-NMMA), increase in the reactive oxygen molecules, the decrease in the diffusion of NO due to intimal thickening, the mutations in the eNOS gene expression, increase in the catabolism of NO, cofactor insufficiency and increase in the vasoconstrictor molecules released from endothelium are the other mechanism that must be considered in endothelial dysfunction. Endothelial dysfunction coexists with many disease states in cardiovascular system and known as the first step of atherosclerosis, which is probably the most important disease of the age. In cardiovascular system, other clinical conditions, which are related with endothelial dysfunction are hypertension, hyperglycemia- insulin resistance, dyslipidemia, menopause, heart failure, variant angina, cardiac syndrome X, and hyperhomocysteinemia.

Heart Failure and NO

In physiological doses NO results in positive inotropic, positive chronotropic and positive lusinotropic effects in myocardium. Besides, NO paradoxically decreases the oxygen requirement of the heart by inhibiting the mitochondrial metabolism. The cardiac NO release is cyclic, and increases in early diastolic filling period. When the preload increases, NO release increases too. Nitric oxide is also effective in Frank-Starling mechanism. Moreover, in low doses, NO increases the β-adrenergic activity in myocardium. In heart failure both iNOS and nNOS increases. In idiopathic dilated cardiomyopathy it is shown that 80% of NOS activity in myocardium is dependent on nNOS (4). The increase in iNOS and nNOS is positively correlated with increase in oxidative stress in patients with heart failure, and moreover NO produced by iNOS can result in peroxynitrite production and contractile dysfunction (5). The high doses of NO released in heart failure results in negative inotropic, negative chronotropic effect and decrease the β-adrenergic stimulation (6). Saito et al. (7) reported that administration of selective iNOS inhibitor resulted in a significant decrease in mortality, infarct size and cardiomyocyte hypertrophy in patients with postinfarction heart failure. In patients with dilated cardiomyopathy, it is also shown that the contractile effect of dobutamine is increased with NMMA administration. In cases that developed resistant cardiogenic shock, despite intraaortic balloon pumping and percutaneous coronary intervention after myocardial infarction; the administration of L-NMMA significantly decreased the mortality rate (8). These findings suggest that NO play a major role in the pathogenesis of heart failure and inhibition of this NO with deleterious effects may be helpful in treatment.

Hypertension and NO

In case of hypertension, the release of vasoconstrictor mediators from endothelium increases. Hypertension is characterized by an increase in the production and activity of angiotensin II. Angiotensin II is one of the most potent vasoconstrictors besides; it induces endothelin production by way of mitogen activated protein kinase (MAPK) pathway. It also stimulates production of superoxide anions and reactive oxygen radicals (9) and increases the consumption of BH4, and inhibits NO production.

In rats with salt sensitive hypertension it is shown that the NOS activity and NO levels are low, but the ADMA level is high. In these animals, endothelial dysfunction was treated by L-arginine infusion (10).

These findings lead us to one of the most popular arguments of hypertension pathogenesis: ``Is the endothelial dysfunction in hypertension, a cause or a result?`` Cardillo et al. (11) suggested that a special defect in the phosphoinositol pathway, which causes activation of NOS, is responsible for the endothelial dysfunction in essential hypertension. Moreover Zizek showed endothelial dysfunction in the normotensive children of hypertensive patients (12). Since the endothelial dysfunction in hypertension was shown to have a genetic dimension, hypertension is thought to be the cause of endothelial dysfunction. However; since the endothelial dysfunction can also be seen in patients with secondary hypertension and it is treatable with antihypertensive medication; endothelial dysfunction may be the result of hypertension. Under the highlights of these findings, it seems endothelial dysfunction contributes to both the cause of hypertension and the clinical condition that occurs as a result of hypertension.

Dyslipidemia and NO

Low-density lipoprotein (LDL) causes the occurrence of a situation characterized by an increase in angiotensin II, surface adhesion molecules and reactive oxygen molecules, which results in a low grade inflammation. This situation provides a base for endothelial dysfunction. Moreover, the oxygen radicals react with NO and cause the production of peroxynitrite in the existence of oxidized LDL. Peroxynitrite inhibits eNOS production and also changes the mission of eNOS from synthesis of NO to synthesis of oxygen radicals (13).

The increase in LDL and decrease in high density lipoprotein (HDL) causes the disruption of caveola complex, which are the specialized invaginations of endothelial membrane, containing eNOS (14). Since the cofactors, essential for NO synthesis, are oxidized due to dyslipidemia, the function of eNOS is affected in a negative manner. ADMA, the endogenous NO inhibitor, also increases in dyslipidemia probably due to inhibition of dimethyl diamino hydrolase (DDAH) which is the enzyme responsible for ADMA catabolism because of low grade chronic inflammation.

Diabetes Mellitus, Insulin Resistance and NO

The metabolic abnormalities like, hyperglycemia, increase in free fatty acids and insulin resistance cause endothelial dysfunction by inhibiting NO synthesis or increasing the catabolism of NO.

In healthy humans, insulin increases the NOS activity by stimulating phosphotidylinositol-3 kinase and Akt kinase. In insulin resistant patients the signal transduction by insulin through phosphotidylinositol-3 kinase pathway impairs. Insulin stimula-

tes NOS less and the NO production decreases . However the signal transduction by insulin through MAPK remains intact. As a result of this pathway more endothelin is produced and inflammation and thrombosis increase (15). Phosphotidylinositol-3 kinase pathway is also responsible of the insulin-mediated glucose uptake in the cells. So insulin resistance aggravates in case of endothelial dysfunction resulting in a vicious cycle. It is shown that, after administration of L-NMMA, which is a NOS inhibitor, both the endothelium-dependent vasodilatation and insulin mediated glucose uptake are impaired (16). The clinical studies with ACE inhibitors and statins demonstrated that these agents did not only decreased coronary artery disease and death due to cardiovascular events but also prevented occurrence of type II diabetes mellitus (17,18). These findings suggest a role for endothelial dysfunction in the pathophysiology of insulin resistance.

Hyperglycemia increases production of the superoxide anion due to mitochondrial electron transport (17). Superoxide activates protein kinase C. The activation of protein kinase C, stimulates membrane bounded NAD(P)H oxidases to produce more superoxide. The reactivity of superoxide and NO results in peroxynitrite production. Peroxynitrite oxides the BH4 which is a cofactor for NOS. This situation causes NOS to produce superoxide instead of NO. Superoxide anion also increases the production of advanced glycation end products (AGEs)(19). The AGEs increase superoxide and reactive oxygen radical production.

Moreover, the oxidative stress caused by hyperglycemia inhibits DDAH (20). This increases ADMA levels. As a result, NO synthesis decreases.

The increase in the amount of free fatty acid seen in diabetes mellitus and insulin resistance, affects the NO balance in an opposite manner by increasing free oxygen radicals, activating protein kinase C and causing dyslipidemia.

Another mechanism for endothelial dysfunction in diabetes mellitus and insulin resistance is the increase in the release of vasoconstrictor prostanoids and endothelin (21).Even, in healthy humans, the administration of insulin resulted in an increase in plasma concentrations of endothelin-1 (20,22).

Hyperhomocysteinemia and NO

Homocysteine is an amino acid metabolized from methionine or taken to the body by diet. It has toxic effects on endothelial cells (23). There are two pathways for the metabolism of homocysteine: 1) vitamin B6 dependent transsulfuration, which leads to formation of cysteine irreversibly, 2) folate and vitamin B12 dependent remethylation to form methionine by help of methionine synthase. In vascular endothelial cells, the only pathway for metabolism of homocysteine is resynthesis of methionine by methionine synthase (24). Since this reaction requires folate and vitamin B12, sufficient amounts of these cofactors are mandatory for the prevention of endothelium from toxic effects of homocystein.

Hyperhomocysteinemia is a situation characterized by increased production of reactive oxygen radicals, and folate deficiency, which leads to reduced bioavailability of NO and endothelial dysfunction. Homocysteine increases the production of proinflammatory cytokines and expression of adhesion molecules and chemotactic factors. This effect is caused by stimulation of the activation of transcription factors like nuclear factor-κ B (NF-κ B) and sterol regulatory element binding protein (SREBP), and inhibition of peroxisome proliferator- activated receptors α and γ (PPAR-α and γ) (25). Since increased methionine levels results in increased levels of S-adenosyl methionine (SAM); hyperhomocysteinemia leads to increased synthesis of ADMA (26). Homocysteine also cause the inhibition of DDAH, which is the enzyme responsible for the catabolism of ADMA, leading further increase in plasma ADMA level (27). Hyperhomocysteinemia is found to be related with coronary artery disease (28).

Tobacco Use and NO

Smoking decreases NO activity directly and indirectly. It decreases NO production by decreasing BH4 levels (29). The decreased BH4 bioavailability causes uncoupling of eNOS. This leads to an increase in peroxynitrite formation and further suppression of eNOS activity.

On the other side, smoking is one of the major risk factors for atherosclerosis. It increases triglycerides and LDL, while decreasing HDL. It induces platelet activation and expression of surface adhesion molecules causing a pro-thrombotic state. Smoking also increases homocysteine, which has direct toxic effects on vascular endothelium. Smoking also stimulates the insulin resistance.

These effects result in a low grade inflammatory state characterized by an increase in free oxygen radicals, fibrinogen, high sensitive C- reactive protein (CRP) and eventually a decrease in NO bioavailability. The detrimental effects of smoking are shown to be independent of the dosage. So both the heavy and light cigarette smokers have similar effects on endothelium (30).

Diagnosis of Endothelial Dysfunction

The serum markers for endothelial dysfunction includes endothelin-1, Von Willebrand factor, tissue plasminogen activator, plasminogen activator inhibitor-1, intracellular adhesion molecules, vascular cell adhesion molecules, E-selectin, P-selectin, ADMA and CRP.

The functional tests for diagnosis of endothelial dysfunction can be examined in two distinct groups as the tests related with coronary circulation and the tests related with peripheral circulation. The tests related with coronary circulation include measurement of coronary flow reserve by inducers like acethylcholine during coronary angiography or positron emission tomography (PET). The tests related with peripheral circulation include the flow mediated dilatation (FMD) measurement by brachial artery ultrasonography, impedance pletismography, measurement of pulse wave velocity and carotid-intima-media thickness.

Since peripheral endothelial dysfunction shows a close relation with coronary endothelial dysfunction and coronary ischemia; documentation of peripheral endothelial dysfunction is a very important clue for coronary endothelial dysfunction. Kuvin et al suggested (31) a relation between peripheral endothelial dysfunction and coronary artery disease in their study. Moreover; in comparison with the diagnostic tests related with coronary circulation, the tests related with peripheral circulation are safer, less invasive and less expensive.

Treatment of Endothelial Dysfunction

There are experimental and clinical studies that show the benefit of antioxidants, ACE inhibitors, statins, estrogen, antidiabetics and L-arginine in the treatment of endothelial dysfunction.

Although theoretically antioxidant vitamins are thought to be beneficial in the treatment of endothelial dysfunction; the results of clinical studies are not clear. The results of only a few clinical studies show the benefit of antioxidant vitamins, were positive. CHAOS study, which is a secondary prevention study, suggested a 47% decrease in nonfatal myocardial infarction incidence but no effect on mortality with α-tocopherol (32). SPACE trial, which includes end stage renal failure patients showed antioxidant vitamins treated endothelial dysfunction (33). But; most of the studies do not support these results. The possible explanations for disapproving results of the studies includes the choice of incorrect vitamin form (α-tocopherol), insufficient dose, combined treatment with β-carotene, improper choice of study population and short treatment period. However, Kinlay et al showed that, long-term, high-dose combined vitamin C and vitamin E treatment failed to improve endothelial functions and did not decrease LDL oxidation (34).

Folic acid may be a useful treatment strategy in endothelial dysfunction. Administration of folic acid decreases homocysteine concentrations inhibits NOS uncoupling and shows direct antioxidant effect (35). Wilmink et al (36) showed that folic acid inhibited postprandial lipid-induced endothelial dysfunction and caused an increase in urinary excretion of oxygen radicals in healthy volunteers.

Another group of drugs used in endothelial dysfunction is ACE inhibitors. ACE inhibitors inhibit the NADPH oxidase by both causing bradykinin accumulation and direct effect. By this way the production of oxygen radicals decrease. With BANFF study, quinapril is shown to treat the endothelial functions in 80 patients with coronary artery disease after 8 weeks of treatment (37). It is found that the effect of ACE inhibitors on endothelial function is related with genotype. Quinapril is found to be ineffective on endothelial functions in patients with ACE DD genotype (37). Ghiadoni et al (38) investigated the effect of different antihypertensive medications on FMD in 168 hypertensive patients and only perindopril was found to increase FMD. HOPE and EUROPE studies showed a decrease in clinical cardiovascular end points with ramipril and perindopril (39,40).

In primary and secondary prevention studies, statins are found to be effective in endothelial dysfunction treatment independent of their cholesterol lowering effect (41,42). Statins inhibit the activity of plasminogen activator inhibitor-1, tissue factor, growth factor and matrix metalloproteinases; terminate the smooth muscle cell proliferation and migration and decrease the low-density-lipoprotein (LDL) oxidation. They increase eNOS and NO levels, decrease apoptosis and inflammation and increase angiogenesis.

Another agent, which is thought to be effective in treatment of endothelial dysfunction, is estrogen. Estrogen increases NO release and NO dependent vasodilatation. It inhibits vascular smooth muscle cell proliferation and decrease LDL oxidation. However; the results of clinical studies like HERS and WHI suggested an increase in major cardiovascular end points with estrogen treatment (43,44).

The studies showed that L-arginine treatment increases the NO synthesis , treats endothelial vasodilator functions , inhibits platelet aggregation , decreases cell adhesion and slow the atherosclerosis (45).

Thiazolidinediones are peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists and they are known to be effective in type II diabetes mellitus by decreasing insulin resistance. In recent studies, these agents are shown to decrease inflammation (46) and ADMA levels (47), and have good effects on blood pressure (48). As insulin resistance causes a decrease in endothelium dependent vasodilatation, the drugs that increase insulin sensitivity, like thiazolidinediones and metformin may be beneficial in treatment of endothelial dysfunction.

For treatment of endothelial dysfunction, the only drug groups that have a proven benefit with large clinical trials is ACE inhibitors and statins. All the other groups are still being investigated and their use is limited to experimental situations.

In summary, endothelial dysfunction is an important component of many clinical disease states in cardiovascular system. The main problem in endothelial dysfunction seems to be deficiency in NO bioavailability. Any strategy targeting an increase in NO bioavailability will be helpful in prevention of many cardiovascular diseases by treatment of endothelial dysfunction.

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