

Endothelial Dysfunction-Its Molecular Mechanism and Potential Implication in Therapy

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ABSTRACT

Endothelial dysfunction is known to be early stage of atherosclerosis and its presence leads to poor prognosis. Recently many basic researchs were done to elucidate the basic mechanism related to endothelial nitric oxide synthase (eNOS). eNOS is composed of dimer, between which calmodulin binding domain is located. This area plays important role in producing NO with BH4 (tetrahydrobiopteryn as a co factor). In the presence of excess of superoxide from NADPH oxidase, NO binds with superoxide to be peroxynitrite. BH4 is converted to BH2 in the presence of peroxynitrite and eNOSdimer dissociates to monomer, which is called uncoupling. Uncoupled eNOS produces superoxide and it results in endothelial dysfunction. NO deficiency is related to the decreased number of peripheral endothelial progenitor cell and HDL-cholesterol is known to stimulate eNOS. This would be new field of research relating eNOS to atherogenesis and eNOS's role in prevention of cardiovascular disease. (Korean Circulation J 2006;36:609-611)

KEY WORDS : eNOS ; BH4 ; Superoxide ; Nitric oxide.

Endothelial dysfunction can be defined as impaired vasodilation due to the impaired secretion of smooth muscle relaxing substances from the endothelium. Traditional risk factors for cardiovascular disease make the endothelium less active leading to endothelial dysfunction. Endothelial dysfunction leads to accelerated atherosclerosis by losing the ability to inhibit the proliferation of smooth muscle cells and by being more susceptible to monocyte adhesion to endothelium and its migration to subendothelium. Many vasoactive substances are secreted from the endothelium but nitric oxide(NO) is the most important factor regulating smooth muscle tone and inhibiting its proliferation.

Structure of NOS and Its Stimulation

Nitric oxide is made from conversion of arginine to citrulline by NO synthase (NOS) activity. Endothelial NOS (eNOS) is a calcium/calmodulin dependent enzyme that uses tetrahydrobiopteryne as a cofactor, whereas inducible NOS is calcium/calmodulin independent. eNOS possesses two dimers, each of which are composed of three domains. Reductase domain

(FMN, FAD and NADPH binding domain) is linked to oxygenase domain(arginine, heme, iron, tetrahydrobiopterin binding domain) via calmodulin binding domain.¹⁾

NO production is stimulated by both physical and humoral factors. The most important physical factor is increased shear stress to the endothelium to make the vascular tone be regulated. A long list of humoral factors was found to stimulate NOS among which acetylcholine, bradykinin, VEGF (vascular endothelial growth factor), insulin, angiopoietin and estrogen are well known stimulants. Endothelial NOS was localized both in a membrane and in the Golgi complex. eNOS in a membrane is mainly located in caveolae, where many regulatory proteins such as HSP (heat shock protein) 90 interact with eNOS. eNOS in a membrane is more susceptible to cholesterol than that in the Golgi complex.²⁾ Also posttranslational modification such as phosphorylation at Serine 1,177 by Akt potentiates the activity of eNOS.

Mechanism of Endothelial Dysfunction

Arginine paradox

Immediately after endothelial dysfunction was found in hypercholesterolemic rabbits, large doses of arginine were reported to reverse the endothelial dysfunction.³⁾ So, arginine deficiency was raised as a basic effect in

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endothelial dysfunction. But in reality the amount of arginine in endothelial cell is far beyond the maximal activity of eNOS. This phenomenon was called the arginine paradox, although the main mechanism for arginine paradox is not known yet. Recently, arginase which converts arginine to ornithine to initiate the urea cycle was found to be present in endothelial cells. eNOS and arginase may compete for arginine as substrate. If the activity of arginase were high, NO production from eNOS would be low.⁴⁾

Decreased bioavailability of BH4

Tetrahydrobiopterine (BH4) binds iron and oxygen to act as a cofactor for eNOS. The availability of BH4 is reduced by peroxynitrite,⁵⁾ which is made by inactivating NO using superoxides. So, increased production of superoxide can result in the diminished activity of eNOS.

Increased production of superoxide by NADH/NADPH oxidase

Superoxide is a very potent reactive oxygen species and is produced by NADH/NADPH oxidase in endothelial cells, vascular smooth muscle cells, and macrophages. NADH/NADPH oxidase is stimulated by G protein coupled receptor activators such as angiotensin II or by receptor tyrosine kinases such as epidermal growth factor. Superoxide, once produced, stimulates cell growth by MAP kinase.

Hypertension by angiotensin II infusion in animals was accompanied by endothelial dysfunction and it is reversed with microsomal SOD infusion, suggesting that superoxide plays a pivotal role in the development of hypertension and endothelial dysfunction.⁶⁾ The source for angiotensin II would be the monocyte, smooth muscle cells in subintima of the vessel. The mechanism on how superoxides can lead to endothelial dysfunction would be the binding to NO to become peroxynitrite and by inhibition of the K channel opening, suggesting that superoxide would be the inhibitor of endothelial dependent hyperpolarizing factor.

A recent report showed that endothelial NADH/NADPH oxidase is related to lipid raft in cell membranes and can participate in redox signaling by death receptor and also in endothelial dysfunction.⁷⁾

Besides angiotensin II, aldosterone is reported to stimulate NADH/NADPH oxidases to produce superoxide mainly by enhanced transcription of the subunit p47phox and translocation to the membrane.⁸⁾

NOS uncoupling and production of superoxide by NOS

NO has been thought to be an important mediator for maintaining vascular tone but genetic deletion of eNOS had little effect on basal vasodilation and ad-

hesion of leukocyte to endothelium, suggesting the less essential role of NO in basal condition.⁹⁾¹⁰⁾ At basal state, NO competes with oxygen to play a more important role as an electron acceptor at the level of mitochondria and inhibitor of metabolism. But in case of stressful condition, eNOS may act as a defensive mechanism by increased transcription and producing free radicals as described below.¹¹⁾

Actually in case of deficiency of arginine or BH4, an electron in NOS is transferred to make superoxide instead of NO.¹²⁾ This is usually called NOS uncoupling. For NOS to be uncoupled, homodimeric form of NOS dissociates to monomeric form. Superoxide once produced rapidly reacts with NO to form peroxynitrite reducing the availability of NO. Peroxynitrite is very toxic to cells and cause oxidative damage to lipids, proteins, and DNA.

Basal mechanism for uncoupled NOS would be the depletion of BH4. As mentioned above, peroxynitrite rapidly decreases the BH4 to inactive BH2 and decreases NOS activity to produce NO. Another explanation is that peroxynitrite leads to disrupt zinc-thiolate cluster in oxygenase domain instead of BH4 inactivation.

What the source of superoxide to uncouple NOS would be is not clearly determined but NADH/NADPH oxidase is thought to play a major role. As described above, angiotensin II and aldosterone stimulate NADH/NADPH oxidase to produce superoxide and positive feedback to peroxynitrite starts to move.

A recent report on the role of aldosterone to NOS suggested that it reduced VEGF induced eNOS phosphorylation at Ser 1177 and intracellular concentration of cGMP but did not alter AktSer473 phosphorylation, highlighting the importance of rennin-angiotensin-aldosterone system in eNOS regulation.

Potential Implication of NO Deficiency for Future Therapeutics

NO deficiency and endothelial progenitor cell (EPC) mobilization

Cardiovascular diseases, in addition to other risk factors, have been associated with impaired number and function of EPC's.¹³⁾ A strong correlation between a number of circulating EPC's and patient's combined Framingham risk factor score has been demonstrated and the level of circulating EPC's represented a better predictor of endothelial function than conventional risk factors.¹⁴⁾ Actually NO is known to stimulate mobilization of EPC's from bone marrow. Mice lacking eNOS failed to upregulate MMP-9 and were incapable of EPC mobilization.¹⁵⁾ Improved availability of NO would lead to vascular protection and anti-atherosclerosis as well as improvement of endothelial function.

Practical methods to improve NO availability and vascular protection

Factors such as statin, angiotensin converting enzyme inhibitor, angiotensin receptor blocker, aldosterone antagonist, estrogen and physical exercise can improve NO availability. HDL may stimulate eNOS activity by binding to SR-B1¹⁶⁾ and interaction with the lysophospholipid receptor S1P3 (sphingosine-1-phosphate),¹⁷⁾ highlighting the importance of exercise in improving endothelial function by elevating HDL-cholesterol.

Conclusion

NO deficiency caused by superoxide production from NADH/NADPH oxidase activation and NOS uncoupling damages endothelium leading to accelerated atherosclerosis. In this process, NO deficiency lead to decreased number and function of EPC's and play an important role in the development of atherosclerosis.

Clinically, control of risk factors should be strictly performed by using statin, RAS blocker and by elevating HDL. This will be the best way to prevent and reverse CV diseases.

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