

Endothelial Function and Oxidative Stress in Cardiovascular Diseases

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The vascular endothelium is involved in the release of various vasodilators, including nitric oxide (NO), prostacyclin and endothelium-derived hyperpolarizing factor, as well as vasoconstrictors. NO plays an important role in the regulation of vascular tone, inhibition of platelet aggregation, and suppression of smooth muscle cell proliferation. Endothelial dysfunction is the initial step in the pathogenesis of atherosclerosis. Cardiovascular diseases are associated with endothelial dysfunction. It is well known that the grade of endothelial function is a predictor of cardiovascular outcomes. Oxidative stress plays an important role in the pathogenesis and development of cardiovascular diseases. Several mechanisms contribute to impairment of endothelial function. An imbalance of reduced production of NO or increased production of reactive oxygen species, mainly superoxide, may promote endothelial dysfunction. One mechanism by which endothelium-dependent vasodilation is impaired is an increase in oxidative stress that inactivates NO. This review focuses on recent findings and interaction between endothelial function and oxidative stress in cardiovascular diseases. (*Circ J* 2009; 73: 411–418)

Key Words: Cardiovascular diseases; Endothelial function; Endothelium-derived factors; Oxidative stress

Various vasodilators, including nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF), as well as vasoconstrictors, are released from the endothelium.^{1,2} NO plays an important role in the regulation of vascular tone, inhibition of platelet aggregation, and suppression of vascular smooth muscle cell proliferation.^{3,4} Impaired endothelium-dependent vasodilation has been found in the forearm, coronary, and renal vasculature of patients with hypertension,^{5–11} dyslipidemia,^{12,13} diabetes mellitus,^{14–16} and coronary artery diseases.^{17–21} Endothelial dysfunction is an early feature of both atherosclerosis and vascular diseases in humans.²² Improvement or augmentation of endothelial function will prevent the development of atherosclerosis, resulting in a reduction in cardiac events.

There are several possible mechanisms for impaired endothelial function in patients with cardiovascular diseases. Decreased NO bioavailability (decreased NO production and/or increased NO inactivation) induces endothelial dysfunction. A balance of endothelium-derived vasodilators, especially NO, and reactive oxygen species (ROS) modulates endothelial function. Therefore, an imbalance of NO and ROS, so-called oxidative stress, is involved in endothelial dysfunction through the inactivation of NO.

Oxidative Stress in Cardiovascular Diseases

ROS are produced by various oxidase enzymes, including nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase, uncoupled endothelial NO synthase (eNOS), cyclooxygenase, glucose oxidase, and lipoxygenase, and mitochondrial electron transport (**Figure 1**). ROS include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), hypochlorous acid (HOCl), NO, and peroxynitrite (ONOO⁻). O_2^- , OH, and NO are classified as free radicals that have unpaired electrons and potent oxidation ability. H_2O_2 , HOCl, and ONOO⁻ are classified as non-free radicals that also have oxidation ability. The sources of ROS are a variety of cell types, including vascular smooth muscle cells (VSMCs), endothelial cells and mononuclear cells. The antioxidant enzyme superoxide dismutase (SOD) has been identified as 3 enzymatic types: Cu/Zn SOD, Mn SOD, and extracellular SOD. SOD rapidly dismutates O_2^- to H_2O_2 , then H_2O_2 is eliminated by glutathione peroxidase (GPx) and catalase to water.

Several lines of evidence demonstrate that oxidative stress plays an important role in the pathogenesis and development of cardiovascular diseases, including hypertension, dyslipidemia, diabetes mellitus, atherosclerosis, myocardial infarction, angina pectoris, and heart failure.^{23–26} The susceptibility of vascular cells to oxidative stress is a function of the overall balance between the degree of oxidative stress and the antioxidant defense capability. Protective antioxidant mechanisms are complex and multifactorial. Antioxidant defense systems, such as SOD, GPx and catalase, scavenge ROS in the vasculature, resulting in inhibition of NO degradation. Although SOD rapidly converts O_2^- to H_2O_2 , H_2O_2 per se is involved as an intracellular second messenger in vascular remodeling, inflammation, apoptosis, and growth of VSMCs.²⁷ Oxidative stress induces cell proliferation, hypertrophy, apoptosis and inflammation through activation of various signaling cascades and redox-

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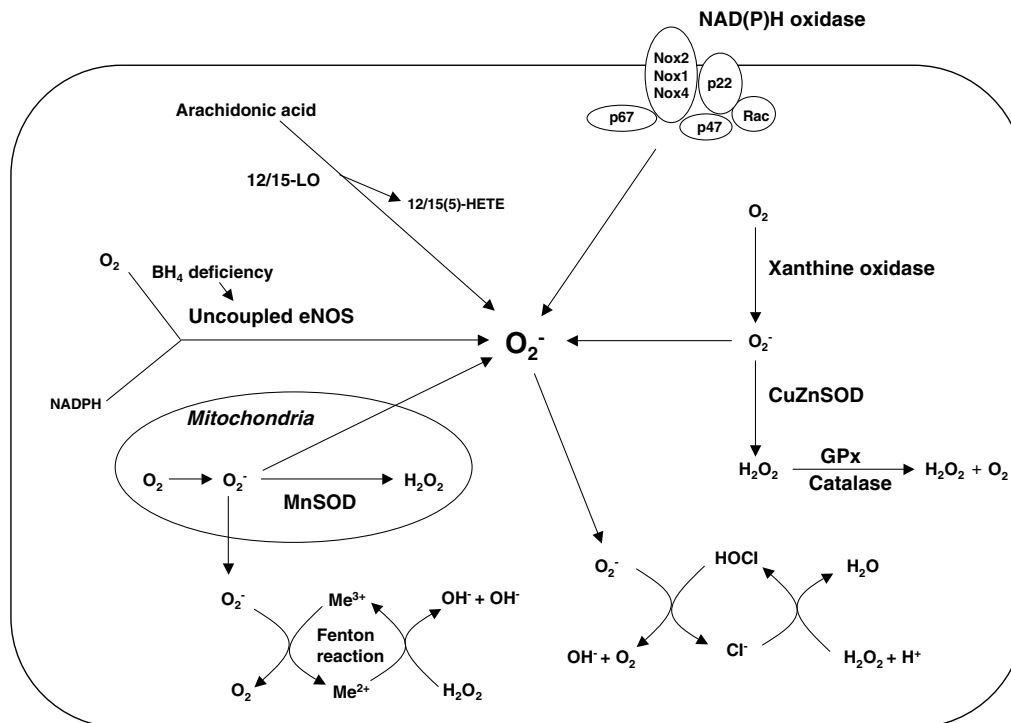


Figure 1. Activated NADH/NADPH oxidase-related ROS generation and ROS degradation system in the vasculature. NADPH, nicotinamide adenine dinucleotide phosphate; ROS, reactive oxygen species; SOD, superoxide dismutase; NO, nitric oxide; GPx, glutathione peroxidase; eNOS, endothelial NO synthase.

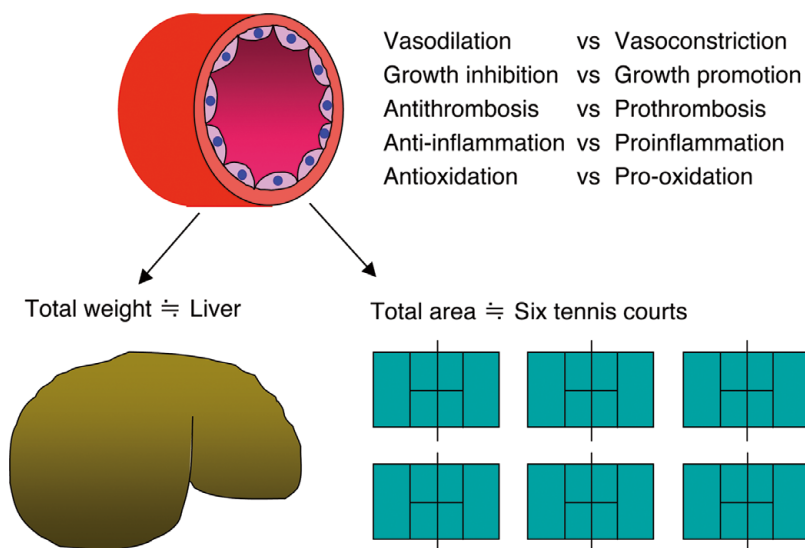


Figure 2. Structure and function of endothelial cells.

sensitive transcriptional factors. Excess ROS, especially free radicals, oxidize various molecules. Lipid peroxidation and protein oxidation induce overexpression of redox genes, intracellular calcium overload, and DNA fragmentation, resulting in damage to VSMCs, endothelial cells or myocardial cells. A vicious cycle of oxidative stress and oxidative stress-induced atherosclerosis leads to the development of atherosclerosis.

Endothelial Function in Cardiovascular Diseases

It had been thought until 1981 that the vascular endothe-

lium functioned as a wall separating the blood vessel and the inside cavity. If the endothelium of the whole body could be collected, its total weight would be equal to that of the liver, and its total area would be equal to that of 6 tennis courts. Endothelial cells secrete various vasoactive agents, such as the vasodilators NO, prostacyclin and EDHF, and the vasoconstrictors endothelin-1, angiotensin II (AngII), and thromboxane A₂¹⁻⁴. Thus, the vascular endothelium might be the biggest endocrine organ in the human body. A healthy endothelium maintains vascular tone and structure by regulating the balance between vasodilation and vasoconstriction, growth inhibition and growth promotion, antithrombosis and prothrombosis, anti-inflammation and pro-

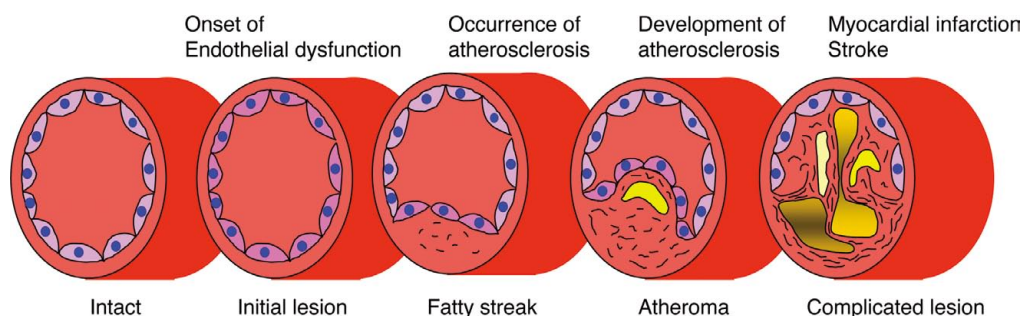


Figure 3. From endothelial dysfunction to cardiovascular complications: progression of atherosclerosis in cardiovascular diseases.

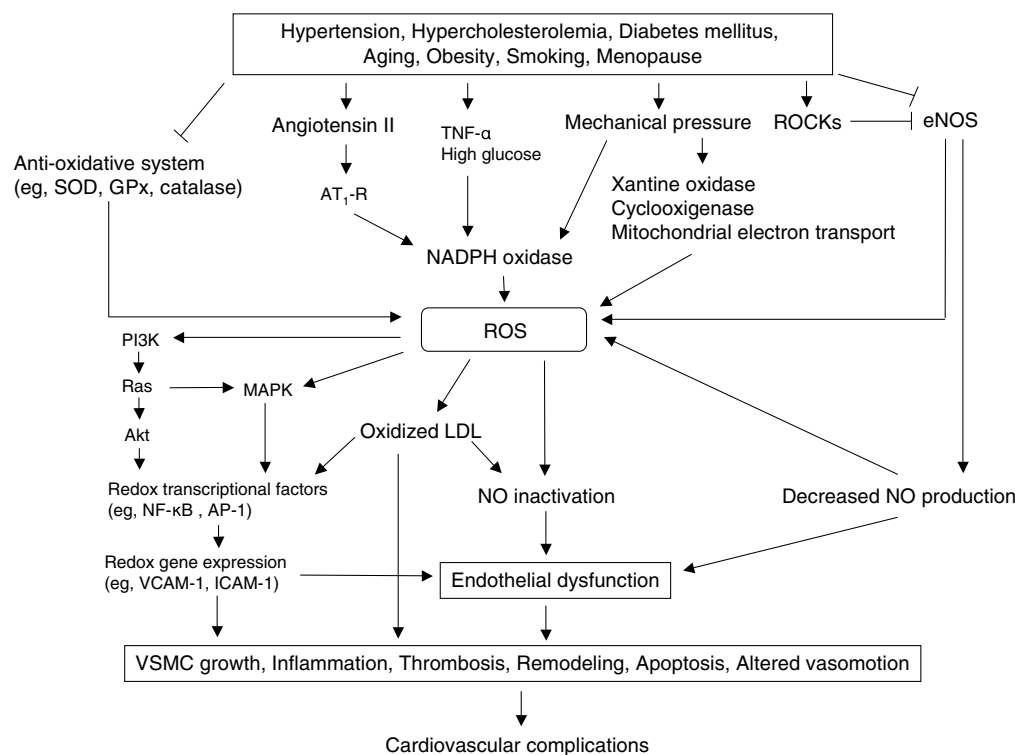


Figure 4. Mechanisms by which ROS induce endothelial dysfunction. NO, nitric oxide (NO); ROCK, Rho-associated kinase; SOD, superoxide dismutase; NADH/NADPH, nicotinamide adenine dinucleotide phosphate; ROS, reactive oxygen species; HSP, heat shock protein; HIF-1, hypoxia-induced factor-1; VEGF, vascular endothelial growth factor; eNOS, endothelial NO synthase; PI3K, phosphatidylinositol-3-kinase; MAPK, mitogen-activated protein kinase; NF- κ B, nuclear factor κ B; AP-1, plasminogen activator inhibitor-1; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intracellular adhesion molecule-1; VSMC, vascular smooth muscle cell.

inflammation, and also antioxidation and pro-oxidation (Figure 2)^{1–4}

Endothelial dysfunction is the initial step in the pathogenesis of atherosclerosis (Figure 3).²² Several investigators have shown that impaired endothelium-dependent vasodilation is found in the forearm, coronary, and renal vasculature in patients with cardiovascular diseases.^{5–21} Perticone et al evaluated cardiac outcome in patients with untreated essential hypertension characterized by 3 tertiles of acetylcholine-induced vasodilation, and found that patients with the lowest tertile of acetylcholine-induced vasodilation had a significantly higher event ratio than did the patients with a moderate or high tertile.²⁸ In patients with coronary artery diseases, severe coronary endothelial dysfunction is associated with increased cardiovascular events.²⁹ Schachinger et al

demonstrated a link between coronary endothelial dysfunction and subsequent cardiovascular events in patients with coronary artery diseases.³⁰ Acetylcholine-induced vasodilation and flow-mediated vasodilation are also useful for predicting cardiovascular events in such patients.^{23–25} Also in patients with peripheral arterial disease, conduit artery endothelial dysfunction assessed by flow-mediated vasodilation independently predicts long-term cardiac outcome.²⁶ Those clinical studies have shown that endothelial function can be an independent predictor of cardiovascular events.^{30,31}

From a clinical perspective, it is important to select an appropriate intervention that will be effective in improving endothelial function in patients with cardiovascular diseases. Indeed, several interventions, including pharmacological therapy, administration of antihypertensive agents such as

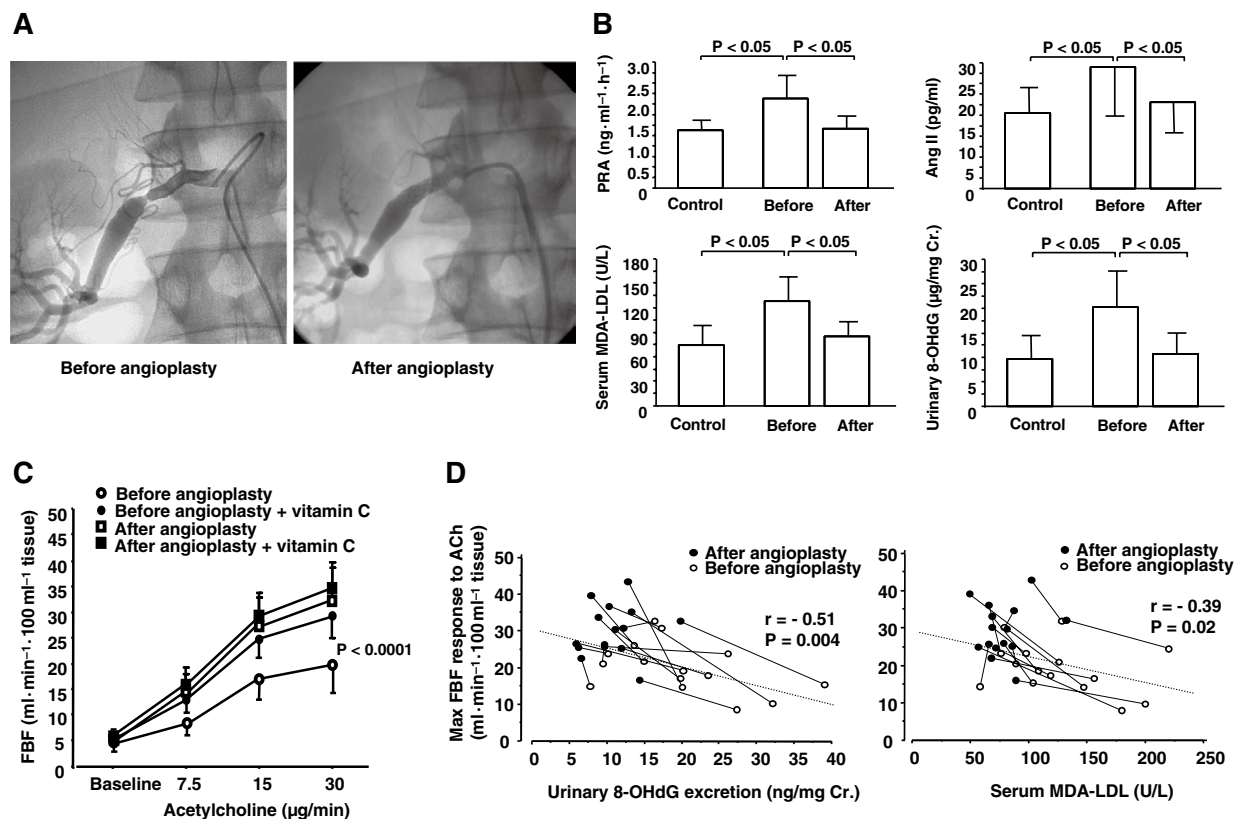


Figure 5. Endothelial function and oxidative stress in patients with renovascular hypertension. (A) Angiography before and after angioplasty. (B) Circulating renin and AngII levels and oxidative stress markers before and after angioplasty. (C) Effects of concomitant administration of the antioxidant vitamin C on the forearm blood flow response to acetylcholine administration before and after angioplasty. (D) Relationship between acetylcholine-induced vasodilation and oxidative stress. Modified from Ref. 66 with permission. Copyright ©2002 Massachusetts Medical Society. All rights reserved. AngII, angiotensin II.

angiotensin-converting enzyme inhibitors and AngII type I receptor blockers^{32–35} statins³⁶ and thiazolidinedione derivatives³⁷ supplementation therapy, such as administration of a substrate of NO L-arginine³⁸ estrogen replacement³⁹ administration of a cofactor of NO tetrahydrobiopterine⁴⁰ treatment with antioxidant vitamins such as vitamin C⁴¹ and lifestyle modifications such as aerobic exercise^{7,42,43} body weight reduction⁴⁴ and sodium restriction⁴⁵ have been shown to improve endothelial function and prevent cardiovascular complications. These findings suggest that endothelial dysfunction in patients with cardiovascular diseases is reversible.

Several investigators have reported possible mechanisms of the impairment of endothelial function in cardiovascular diseases, including abnormalities of shear stress, increased amounts of the endogenous eNOS inhibitor asymmetrical dimethylarginine, increased amounts of vasoconstrictors such as AngII, endothelin-1 and norepinephrine, and inactivation of NO by ROS^{46,47} Growing evidence reveals an interaction between oxidative stress and endothelial function (Figure 4). Enhanced production of ROS and an attenuated antioxidant system would contribute to endothelial dysfunction in cardiovascular diseases.

Decrease in NO Bioavailability

Endothelial dysfunction has been shown to be associated with an increase in ROS in atherosclerotic animal models

and human subjects with atherosclerosis.⁴⁶ The concentrations of antioxidant scavengers, such as SOD, GPx catalase, and vitamins C and E, are decreased in patients with atherosclerosis.⁴⁸ NADH/NADPH oxidase, which is a major source of production of ROS in vessel walls, is activated in experimental models of atherosclerosis.^{49,50} It has also been shown that ascorbic acid (vitamin C) restores impaired endothelium-dependent vasodilation in patients with essential hypertension, dyslipidemia, and coronary artery diseases.^{13,17,51} Enhanced NO inactivation caused by excess ROS production, rather than decreased NO production, may play an important role in the impairment of endothelium-dependent vasodilation. These findings suggest that a decrease in NO inactivation induces an improvement in the endothelial dysfunction in patients with cardiovascular diseases.

eNOS

The serine/threonine kinase Akt protooncogene is 1 of the major regulators of various cellular processes and mediates the activation of eNOS, resulting in increased NO production from endothelial cells.⁵² Fulton et al⁵³ showed that Akt activates eNOS enzyme activity by phosphorylation of eNOS, independently of an increase in intracellular free calcium concentration, leading to an increase in NO production from endothelial cells. The phosphatidylinositol-3-kinase (PI3K)/Akt pathway, which causes intracellular calcium-independent eNOS phosphorylation and activation, is involved in eNOS activation, in addition to a calcium-

dependent mechanism.

Under pathologic conditions, the PI3K/Akt pathway is diminished, resulting in decreased levels of eNOS gene expression and enzymatic activity. Andreozzi et al demonstrated that AngII enhanced Ser312 and Ser616 phosphorylation of insulin receptor substrate-1 through stimulation of c-Jun N-terminal kinase and extracellular signal-regulated kinase 1/2 activity in human umbilical vein endothelial cells, and that it impaired insulin-mediated vasodilation through inactivation of the insulin receptor substrate-1/PI3K/Akt/eNOS pathway.⁵⁴ Indeed, chronic inhibition of the renin-angiotensin system (RAS) improves endothelial function by either increasing NO production or by activating eNOS-related NO production.⁵⁵ In addition, chronic inhibition of the RAS has been shown to lead to functional and histological alterations of the vascular endothelium, resulting in enhanced vascular structure and function.⁵⁵

eNOS per se produces ROS rather than NO under the condition of eNOS uncoupling through a deficiency of tetrahydrobiopterin (BH₄), an essential cofactor for eNOS, or oxidation of BH₄. Degradation of BH₄ by ROS, including ONOO⁻, O₂⁻ and H₂O₂, is associated with downregulation of eNOS.⁵⁶ In addition, it has been demonstrated that supplementation of BH₄ improves endothelial function in vivo and in vitro, and in smokers and patients with hypertension, hypercholesterolemia or chronic heart failure.^{57–60} Recently, we also showed that the grade of oxidative stress correlates with a deficiency of BH₄, and that supplementation with BH₄ augmented endothelium-dependent vasodilation in the brachial arteries of elderly subjects.⁶¹ These findings suggest that BH₄ deficiency and decreased eNOS activity cause endothelial dysfunction in atherosclerotic patients through an increase in oxidative stress.

NADH/NADPH Oxidase

NADH/NADPH oxidase is the most important source of ROS in the vasculature.⁶² NADH/NADPH oxidase is a multi-subunit complex composed of cytosolic components, such as p47^{phox}, p67^{phox} and Rac 1, and membrane-spanning components, such as p22^{phox} and gp91^{phox} or another NOx homolog. The production of ROS by activated NADH/NADPH oxidase is mediated by several pathways.⁶³ AngII-induced NADH/NADPH oxidase activation is 1 of the major sources of ROS in atherosclerosis.^{62–65} The activated NADH/NADPH oxidase-related ROS generation and ROS degradation pathway is shown in **Figure 1**. In the aorta of spontaneously hypertensive rats, endothelial dysfunction is caused by an excess of ROS rather than a decrease in NO production and is associated with both upregulation of p22^{phox} mRNA expression and increased activity of NADH/NADPH oxidase.⁶³ Upregulation of p22^{phox} mRNA expression is a key component of AngII-induced NADH/NADPH oxidase activation, and increased expression levels of other components also play an important role in this oxidase under pathological conditions.^{64,65} Increased mRNA expression levels of p47^{phox}, p67^{phox}, p22^{phox} and NOx2 have been found in the internal mammary arteries of patients with coronary artery diseases and in those with diabetes mellitus. RAS inhibitors prevent the increase in the mRNA expression levels of p22^{phox} and NOx2 in AngII-induced hypertensive rats and reduce ROS generation, and the AT₂ receptor blockade accentuated the changes in p22^{phox} and NOx2 and increased p67^{phox}. RAS inhibition improves endothelial function in various animal models through decreased NADH/NADPH oxidase activity.⁶³ It is thought that inactivation

of NADH/NADPH oxidase may contribute to the improvement in endothelial dysfunction in patients with atherosclerosis. Recently, we also found that inactivation of the RAS, particularly AngII, by successful renal angioplasty may decrease oxidative stress, resulting in improved endothelium-dependent vasodilation in patients with renovascular hypertension, who are ideal subjects for determining how endothelial function is affected by excess AngII and AngII-related increase in oxidative stress (**Figure 5**).⁶⁶ These findings suggest that the role of the RAS in the pathogenesis of atherosclerosis may be related, at least in part, to AngII-induced production of ROS in vascular cells.

Antioxidant System

Protective antioxidant mechanisms are complex and multifactorial. The antioxidant defense system, such as SOD, GPx and catalase, scavenges ROS in the vasculature, resulting in inhibition of NO degradation (**Figure 1**). The susceptibility of vascular cells to oxidative stress is a function of the overall balance between the degree of oxidative stress and the antioxidant defense capability. The antioxidant enzyme SOD rapidly dismutates O₂⁻ to H₂O₂. SOD has been identified as 3 enzymatic types: Cu/Zn SOD, Mn SOD, and extracellular SOD. Destruction of the antioxidant system, including decreased antioxidant enzyme activity and ROS scavenging ability, may contribute to oxidative stress in patients with atherosclerosis. Various interventions, such as administration of antioxidant vitamins and antihypertensive agents and exercise training, have been shown to enhance the protein levels and enzymatic activities of SOD, such as Cu/Zn SOD and Mn SOD, in the vascular endothelium and smooth muscle cells of the aorta in experimental animal models.^{67,68} It has been reported that approximately 50% of the total SOD in the human vasculature is extracellular.⁶⁹ Fukai et al demonstrated that exercise increased eNOS and extracellular SOD protein levels in wild-type mice, but had no effect on extracellular SOD protein levels in eNOS-knockout mice and that the effect of endothelium-derived NO on extracellular SOD protein level is mediated by the cGMP/protein kinase G-dependent pathway.⁷⁰ Hornig et al have shown that extracellular SOD contributes to the improvement in endothelial function by treatment with a RAS inhibitor in patients with coronary artery diseases.⁷¹ Interestingly, extracellular SOD activity determined after its release from the endothelium by a heparin bolus injection was increased after treatment with losartan and was associated with an increase in flow-mediated vasodilation. These findings suggest that activation of extracellular SOD improves endothelial function, probably by increased NO bioavailability, in patients with coronary artery diseases.

Although SOD rapidly converts O₂⁻ to H₂O₂, H₂O₂ per se is involved as an intracellular second messenger in vascular remodeling, inflammation, apoptosis, and growth of VSMCs. Hydrogen peroxide is eliminated by GPx and catalase to H₂O. It has been shown that a physiological level of shear stress upregulates GPx mRNA levels and GPx enzymatic activity in cultured bovine aortic endothelial cells.⁷² The upregulation of Cu/Zn SOD, Mn SOD, GPx and catalase, apart from extracellular SOD induced by appropriate interventions, may improve endothelial function through the inhibition of NO degradation with a decrease in ROS.

Rho-Associated Kinases (ROCKs)

The family of ROCKs, which are small GTPase Rho effectors, mediate various cellular physiologic functions

such as cell proliferation, migration, adhesion, apoptosis and contraction, all of which may be involved in the pathogenesis of atherosclerosis.^{73–75} ROCKs consist of 2 isoforms, ROCK1 and ROCK2, and have been found to be the immediate downstream targets of RhoA.^{76,77} The RhoA/ROCK pathway has been shown to be involved in the formation of atherosclerotic lesions, vasoconstriction and myocardial hypertrophy, and to be activated in patients with hypertension and in those with coronary artery disease.^{78–83} Sauzeau et al have shown that NO also inhibits RhoA translocation from the cytosol to the membrane in VSMCs.⁸⁴ In addition, previous studies using ROCK inhibitors, such as fasudil or Y-27632, have suggested that ROCKs may play an important role in the pathogenesis of cardiovascular disease.^{74,78,80} These experimental and clinical studies have shown that ROCKs are an important therapeutic target for cardiovascular diseases.^{85,86} Previous studies have shown that activation of the RhoA/ROCK pathway impairs NO bioavailability through inhibition of eNOS mRNA stability and eNOS protein phosphorylation at Ser1177 via the PI3K/Akt pathway.^{78,87} We previously reported that intra-arterial infusion of the ROCK inhibitor fasudil improved endothelial function in smokers.⁸⁸ Several investigators have shown an interaction between the RhoA/ROCK pathway and ROS.^{89,90} Indeed, ROS induced by hyperglycemia enhance ROCK activity, leading to atherothrombogenesis through increased expression of plasminogen activator inhibitor-1 in vascular endothelial cells.⁹¹ It is well known that cigarette smoking decreases NO bioavailability through the production of ROS. Several investigators, including us, have demonstrated that there is a possible association of ROCK activity with oxidative stress and that smoking enhances the activation of ROCKs in VSMCs in vivo and in vitro.^{89,92} Taken together, the findings indicate an interaction between ROCK activity, endogenous NO, and oxidative stress.

Conclusions

Increased production of ROS impairs endothelial function in humans. One mechanism by which endothelial function is impaired is increased oxidative stress, which inactivates NO. An imbalance of reduced production of NO and increased production of ROS may be involved in impaired endothelium-dependent vasodilation in patients with cardiovascular diseases. It is thought that a vicious cycle of endothelial dysfunction and oxidative stress leads to development of atherosclerosis. In the clinical setting, it is important to select appropriate interventions for both endothelial function and oxidative stress, and it is expected such interventions will greatly improve clinical outcomes. Future study in a large clinical trial or cohort study is needed to determine the roles of endothelial function and oxidative stress in cardiovascular outcomes.

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Disclosures

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Conflict of interest is none.

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