Diagnosis and Treatment of Endothelial Dysfunction in Cardiovascular Disease

A Review

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Summary

Vascular endothelial dysfunction reflected by reduced nitric oxide (NO) availability is certainly the causative factor or promoting mechanism of arteriosclerosis. It is necessary to detect endothelial dysfunction at an early stage using appropriate methods, and to choose a treatment for the recovery of endothelial function. There are nonpharmacological and pharmacological therapies to attain endothelial repair. The former includes body weight reduction, aerobic exercise, and restriction of salt intake, while the latter includes the use of renin-angiotensin system inhibitors, calcium antagonists, some types of β blockers, statins, erythropoietin, tetrahydrobiopterin, and antioxidants. These therapies are intended to increase NO synthase activity and NO release, inhibit NO degradation, and enhance the activity of endothelial progenitor cells. (Int Heart J 2010; 51: 1-6)

Key words: Nitric oxide, Asymmetric dimethyl arginine, Endothelial protection, Endothelial progenitor cells, Angiotensin, Statins

It has been shown that vascular endothelial dysfunction precedes the development of arteriosclerosis, and thereafter plays an important role in its progression. Therefore, preservation or recovery of endothelial function is important to inhibit the development of arteriosclerosis and thereby prevent the occurrence of cardiovascular events. In this review, we discuss the diagnosis and treatment of cardiovascular disease from the viewpoint of endothelial function (Figure).

Endothelial function and NO:

1) Endothelial cells and NO synthase. NO is synthesized from L-arginine by NO synthase (NOS) in vascular endothelial cells. NOS catalyzes a guanidino nitrogen of L-arginine by oxidation, and in turn releases NO and L-citrulline. All three NOS isoforms, neuronal, inducible, and endothelial NOS, share a carboxyl terminal domain homologous to cytochrome P-450 reductase, have binding sites for NADPH, FMN, tetrahydrobiopterin (BH4), and calmodulin, and need these as coenzymes to be activated.

2) NO release mechanism. Endothelial NOS is usually activated by an increase of the intracellular concentration of Ca²⁺. The release of NO increases when endothelial cells are under shear stress caused by blood flow increase, which is the most physiological stimulus for NO release. This mechanism is the principle basis why flow-mediated dilation (FMD) at the time of reactive hyperemia is useful to clinically evaluate vascular endothelial function.

Experimentally, acetylcholine (ACh) administered into the arteries causes vasodilation because the intracellular Ca²⁺ concentration in endothelial cells is increased by ACh. Furthermore, it has been clarified that various stimuli such as insulin, estrogen, VEGF, and adrenomedullin activate the phosphatidylinositol-3 kinase (PI3K)/Akt system, and that in turn Akt phosphorylates 1177th serine of NOS.¹¹

3) Role of NO in development of arteriosclerosis. There is a large body of evidence on the role of hyperlipidemia in the onset of arteriosclerosis. However, it is also known that the...
onset of arteriosclerosis is associated with other risk factors, including hypertension, diabetes mellitus, and smoking, with a common mechanism of action. Namely, all these risk factors initially damage the endothelial cells. Such endothelial cell damage results in a reduced NO activity. NO exerts various antiparosclerotic actions other than endothelial-dependent vasodilation: NO suppresses the secretion of vasoconstrictive factors such as endothelin, and suppresses the aggregation of platelets as well as the expression of adhesive molecules, inhibiting adhesion of monocytes to the endothelial cells. Furthermore, it decreases oxidation of LDL. Through these actions NO eventually reduces the proliferation or migration of smooth muscle cells, and suppresses the development of atherosclerosis. In fact, NO synthesis inhibitors cause an elevation of blood pressure and marked vascular damage, as observed in endothelial NOS knockout (KO) mice.4

**Indicator of endothelial function:** Because it is difficult to evaluate all endothelial functions, the activity of endogenous NO is measured representatively. Among the methods available, measurement of endothelial-dependent vasodilatation in the forearm arteries using ACh is the golden standard in clinical practice. Nevertheless, NO is not the only factor involved in this reaction; but as a whole it can be said that it reflects the state of vascular endothelial function. Vascular echography or peripheral arterial tonometry (PAT) is employed to measure FMD in clinical settings in a noninvasive manner. The Framingham Heart Study found that the hyperemic response (PAT ratio) at 90-120 seconds after 5-minute forearm cuff occlusion correlated with cardiovascular risk factors in Framingham Third Generation Cohort participants; that is, 1957 subjects aged 40 years on average.5 This hyperemic response was attenuated by NOS inhibition, thus it may reflect the state of endothelial function. Recently, there have been many attempts to investigate endothelial function from viewpoints other than NO. The new potential biomarkers of endothelial function are listed in Table I.

The latest topic about endothelial function is endothelial progenitor cells (EPC). It is presumed that EPC derived from the bone marrow circulate in the peripheral blood and are involved in the repair of damaged endothelium as well as in angiogenesis. In recent years, the number of studies reporting the relevance of EPC to disease states by counting these cells and analyzing their functions has grown exponentially. According to these studies, in patients with coronary artery disease or diabetes mellitus, the numbers as well as the migrating ability of EPC are reduced, and hypertension is an independent regulator of reduced EPC function.6 Even in the general population, it was found that the larger the number of risk factors for cardiovascular disease, the smaller the number of EPC was. In addition, in patients with reduced endothelial function as assessed by measuring FMD of forearm arteries, the number of EPC was smaller than in those with preserved endothelial function. Therefore, in investigations involving the general population, the number of EPC will become an excellent alternative marker of vascular endothelial function or accumulation of cardiovascular risks.

**Which diseases affect endothelial function?:** Panza, et al5 measured FMD by infusing ACh into the forearm arteries and found that in hypertensive patients, vasodilation was decreased compared with that in normotensive individuals. Ludmer, et al6 infused ACh into the coronary arteries and demonstrated contraction of the coronary arteries in patients with coronary artery disease. Later it was clarified that in patients with coronary artery disease, endothelial-dependent vasodilation was reduced not only in the coronary arteries but also in the forearm arteries. In fact, the degree of FMD of the forearm arteries in patients with coronary artery disease was a predictor of cardiac events. Furthermore, it is known that endothelial-dependent vasodilation is reduced in patients with risk factors such as diabetes mellitus, dyslipidemia, and smoking. It is also known that in humans with or without coronary artery disease, the number of classical risk factors is inversely correlated with the state of endothelial function. Namely, the degree of reduction of endothelial-dependent vasodilation runs parallel to the number of conventional risk factors.7

**Factors that directly affect endothelial function:**

1) **Oxidative stress.** Oxidative stress by various reactive oxygen species (ROS) promotes the development of arteriosclerosis. NO is closely involved in this mechanism. The ROS react with and not only deactivate NO, but also produce a strongly oxidative substance, peroxynitrite. Oxidative stress also reduces synthesis of NOS at the protein level. Angiotensin II (AII), a known arteriosclerosis promoting hormone, produces ROS through the stimulation of NADP/NADPH oxidase in vascular smooth muscle cells. Physiologically, there is a balance between NO and AII, but in patients with arteriosclerosis NO is reduced and AII is increased, thus arteriosclerosis is worsened even further.

The evidence that oxidative stress affects endothelial function is provided by the effect of antioxidant drugs. For example, tempol reduces oxidative stress markers and exerts a marked antihypertensive effect in a number of experimental models of hypertension.8 Also in humans, the usefulness of vitamins C and E, allopurinol, flavonoid, and folic acid, which reduces the concentration of homocysteine, has been demonstrated. Vitamin C strongly inhibits the oxidation of lipids, particularly LDL. So far it has been shown that oral administration or intra-arterial infusion of vitamin C improves endothelial-dependent FMD in patients with various disease states such as hypertension, ischemic heart disease, and dyslipidemia, as well as in smokers. However, there is no evidence that the long-term administration of these antioxidants always contributes to suppress the occurrence of

### Table I. New Biomarkers of Vascular Endothelial Dysfunction

<table>
<thead>
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<th>Factor</th>
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<tr>
<td>Insulin resistance</td>
</tr>
<tr>
<td>Homocystinemia</td>
</tr>
<tr>
<td>Lipoprotein (a)</td>
</tr>
<tr>
<td>Endogenous NO synthesis inhibitors (ADMA)</td>
</tr>
<tr>
<td>Adiponectin</td>
</tr>
<tr>
<td>Inflammatory factors (CRP, IL-1, IL-6, TNF-α, MCP-1)</td>
</tr>
<tr>
<td>Endothelial progenitor cells (EPC)</td>
</tr>
<tr>
<td>Vasodilators (nitrite and nitrate, 6-keto PGF1α)</td>
</tr>
<tr>
<td>Vasoconstrictors (endothelin, thromboxan A2, ROS)</td>
</tr>
<tr>
<td>Adhesion molecules (VCAM-1, ICAM-1, P &amp; E-selectin)</td>
</tr>
<tr>
<td>Thrombotic hemostatic factors (PAI-1, TPA, von Willebrand factor, thrombomodulin)</td>
</tr>
</tbody>
</table>

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1) Ludmer, et al.
2) Panza, et al.
3) Insod.
4) Oxl.
5) Hyper.
6) NOS.
7) Art.
8) Vit.
9) Tempol.
10) Vitamin C.
11) Antioxidant.
cardiovascular events.

2) **Increase of inhibitors of endogenous NO synthesis.** Protein arginine methyltransferase acts on proteins containing methylated arginine to produce asymmetric dimethylarginine (ADMA), which inhibits the synthesis of NO similarly to L-NNMMA. Physiologically, dimethylaminohydrolase (DDAH) exists in endothelial cells, and it degrades these methyl arginines. However, in arteriosclerosis, the blood concentration of ADMA is increased. It has been shown from the analysis of genetically-engineered mice expressing increased or decreased levels of DDAH that accumulation of ADMA attenuates endothelial function, and may cause vascular disorders including coronary lesions. Thus, drugs that could reduce ADMA are expected; in fact, ACE inhibitors and ARBs are known to have that effect. ADMA promotes the expression of ACE, most likely through p38 MAP kinase, increasing Ang II and oxidative stress, thus, the effect of renin-angiotensin system inhibitors may be potentiated. Regarding statins, several studies have been conducted, but no remarkable effect has been identified. Yet, it is known that the effect of statins is decreased in the state of increased ADMD.

3) **Inflammation.** It is known that bacterial infection attenuates endothelial function. Furthermore, administration of inflammatory cytokines also attenuates endothelial function. The study by Fichtlscherer, et al, which examined the relationship between inflammatory markers and endothelial function, showed that vasodilation caused by ACh in the forearm arteries significantly correlated with CRP values in 60 patients with coronary artery disease. More directly, anti-inflammatory drugs such as COX inhibitors improve endothelial function in patients with arteriosclerosis. Whether antibacterial drugs improve vascular endothelial function is contentious, but recently comorbidity of periodontal disease and cardiovascular disease has drawn attention, as FMD is significantly improved by intensive treatment including administration of antibiotics for periodontal disease. It was shown that when porphyromonas gingivalis, which is a major bacterial pathogen of periodontal disease, was present, the endothelial thickness of the carotid artery increased or that the bacterium was found in the intimal tissues of the resected carotid artery.

4) **Adipocytokines derived from visceral fat.** Among the adipocytokines playing an important role in the pathogenesis of metabolic syndrome, TNF-α is known to reduce endothelial function by increasing insulin resistance or its inflammatory effect, and endothelial function recovers after the reduction of TNF-α. Adiponectin is a protein with antiarteriosclerotic activity whose receptor is located in vascular endothelial cells; thus, NO is released upon stimulation of endothelial cells by adiponectin. As for the releasing mechanism, adiponectin activates endothelial NOS through AMP activating kinase and the PI3K/Akt system. Ouchi, et al found a positive correlation between adiponectin and FMD in hypertensive patients. In patients with diabetes mellitus in whom adiponectin concentration is reduced, endothelial function is attenuated.

5) **Aldosterone.** The involvement of aldosterone in the onset of cardiovascular complications in hypertensive patients has recently attracted attention. Aldosterone is known to be involved in organ fibrosis independently from its action at the level of renal tubules or in hypertension, but there are many unknown issues concerning its mechanism of action. NO synthesis inhibition is associated with marked increases of aldosterone. At the same time, fibrosis of the renal interstitium is particularly increased, and these changes are all suppressed by an aldosterone receptor antagonist. FMD is reduced in hypertensive patients in the presence of a hyperaldosterone state, and it is reported that administration of spironolactone for 3 months significantly improves endothelium-dependent vasodilation. Therefore, it is possible that endothelial disorder is involved in the development of hypertension or cardiovascular disorder through an increase of aldosterone. Oxidative stress, which enhances the effect of aldosterone, may also be involved.

6) **Depletion of tetrahydrobiopterin (BH4).** BH4 is a cofactor of NOS, and plays an important role in stabilizing dimer formation of NOS. NOS starts to produce ROS when BH4 is reduced (NOS uncoupling). When the aorta of mouse GTP cyclohydrolase 1 (GTPCH1), which is the rate-limiting enzyme for BH4 synthesis, is inhibited, endothelial-dependent vasodilation is decreased, and concurrently blood pressure, ROS, or adhesive molecules increase. In the state of hypertension, arteriosclerosis and ischemia, which are states of insufficient utilization of BH4, endothelial function is improved by supplying BH4.

**Usefulness of endothelial-dependent FMD measurement as a predictor of prognosis and cardiovascular events:** Vascular changes that become the primary cause of cardiovascular events start early in childhood, and it is important to detect them at an early stage. Even in the Framingham study, prediction of a cardiovascular event was possible only in 60-70% of the cases. If it were possible to detect asymptomatic patients who have such risk factors at the earliest possible stage by other methods, the advantages of such methods would be remarkable. As one of such methods, direct measurement of vascular endothelial function has gained significance.

Schachinger, et al investigated endothelial-dependent vasodilation of the coronary arteries in 147 patients with coronary artery disease, and followed them up for 7.7 years. In patients with decreased vasodilation, cardiovascular events occurred with significant frequency, and decreased endothelial function was an additional predictor, independent of the other risk factors for coronary lesions. On the other hand, Perticone, et al investigated the vascular reactivity of the forearm arteries induced by ACh in 225 hypertensive patients, and followed them for a mean of 31.5 months. They found that even in those with essential hypertension, the endothelial function of forearm arteries was a predictor of cardiovascular events because the incidence of cardiovascular events was the highest in the group with reduced endothelial-dependent FMD.

The relation between the number of EPC, which is correlated with endothelial-dependent FMD and prognosis of cardiovascular diseases, has also been reported. Werner, et al measured the number of EPC in patients with ischemic heart disease, and analyzed its relation with the frequency of cardiovascular events one year later. They showed that the frequency of cardiovascular death, onset of primary major cardiovascular events, revascularization, and hospitalization increased in relation to the decrease in the number of
circulating EPC.

The incidence of cardiovascular events in our country is particularly low, therefore, to evaluate endothelial function as a surrogate marker of cardiovascular disorder may be extremely useful to diagnose severity, determine therapeutic strategy, or estimate the prognosis.

Can endothelial function be improved by treatment?: It is known that in a number of arteriosclerotic diseases endothelial function is improved by appropriate treatment. However, the treatment effect and the improvement of endothelial function do not always run in parallel, and the effect of drugs per se on endothelial function cannot be disregarded.

1) Nonpharmacological therapy

a) Reduction of body weight: It has been shown that endothelial function recovers when obese patients with coronary disease lose weight. Improvement of a variety of risk factors may be involved, but decreases in circulating cytokines, especially inflammatory cytokines released from visceral fat, may contribute to this.

b) Restriction of salt intake: Intake of sodium elevates blood pressure, and in case of salt-dependent hypertension, endothelial dysfunction is remarkable. It was recently clarified in vitro that in the presence of aldosterone, stiffness of cultured endothelial cells increased when the extracellular sodium concentration was increased from 135mM to 145mM. This is, NO release decreases because of the reduced capacity of endothelial cells to transform. The vascular protective effects of NO are observed independent of the fall in blood pressure by limiting salt intake.

c) Exercise: It has been confirmed that even in human coronary arteries, endothelial function is improved by exercise.

### Table II. Reports on the Effect of Irbesartan on Human Vascular Endothelial Function

<table>
<thead>
<tr>
<th>Authors</th>
<th>Subjects</th>
<th>Number of cases</th>
<th>Administered drug (mg)</th>
<th>Administration period</th>
<th>Endothelial-dependent vasodilation (evaluation method)</th>
<th>Other effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warnholtz A</td>
<td>Coronary artery disease</td>
<td>72</td>
<td>Irbesartan 300</td>
<td>6 months</td>
<td>Brachial arteries FMD ↑</td>
<td>ADMA →</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Intracoronary acetylcholine →</td>
<td></td>
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</tbody>
</table>
| Morawietz H      | CABG patients                   | 49              | Irbesartan 150         | 4 weeks               | Removed internal mammary artery acetylcholine ↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑∪
An increase in blood flow during exercise increases shear stress on the vascular walls, which increases NO release. Exercise has also been reported to reduce oxidative stress and increase the number of EPC.

2) Pharmacological therapy

a) Calcium antagonists: The vasodilatory effect of calcium antagonists decreases after removal of endothelial cells or administration of NO donors. Guanylate cyclase inhibitors, or bradykinin (BK) receptor antagonists. A number of reports have found that NO production is increased by calcium antagonists. In clinical cases, endothelial-dependent FMD is improved by their long-term administration. Reduction in blood pressure, increase in blood flow, antioxidant effect, increase in VEGF, increase in superoxide dismutase (SOD), and BK increasing effect have been suggested as possible mechanisms of action.

b) β blockers: In general, the endothelial protective effect of β blockers is not very strong. However, the involvement of NO in the vasodilatory mechanism of action of some β blockers has been pointed out. An increase of intracelular Ca2+ by activation of phospholipase C and an increase of NO release by acting on the estrogon receptor of the endothelial cells have been postulated as the mechanism of NO release by nebivolol. Concerning celiprolol, NO release was reported to take place through an increase in intracelular calcium by stimulation of serotonergic receptors. Furthermore, celiprolol contributed to NO release by activating the PI3K/Akt pathway system. On the other hand, some β blockers such as carvediol have been shown to have an endothelial protective effect based on their antioxidant effect.

c) ACE inhibitors: There have been several reports describing a strong improvement of endothelial function with ACE inhibitor administration. Initially, the mechanism by which ACE inhibitors protect the endothelium was explained as follows: They exclusively inhibit the degradation of BK, increase BK, and stimulate BK type 2 receptors of endothelial cells to release NO. However, in several cardiovascular diseases, production of ROS by AII is increased. Therefore, it is believed that when AII is reduced by ACE inhibitors, the production of ROS decreases and NO activity increases.

d) ARBs: ARBs improve endothelial function in a number of arteriosclerotic diseases. The effects of irbesartan, which are frequently reported in humans, are summarized in Table II. Endothelial-dependent FMD improved in coronary disorder, diabetes mellitus, and essential hypertension after long-term administration of irbesartan. The primary mechanism of action of ARBs is an AII-receptor antagonistic action; thus, blood and local concentrations of AII increase stimulating AII type 2 receptors. As a result, vasodilation and an antiapoptotic effect occur; this is considered to be an NO release mechanism of action. Similarly, an active metabolite of AII, AII (1-7), which is known to have an NO release effect, increases. In various arteriosclerotic diseases, ARBs improve endothelial function in a relatively short period of time, and concurrently exert anti-inflammatory and antioxidant effects. Furthermore, ARBs were reported to increase the number of EPC in patients with type 2 diabetes.

e) Renin inhibitors: Similarly to the above 2 drugs, a renin inhibitor improves endothelial function in experimental animals. In Watanabe hyperlipidemic rabbits, the renin inhibitor aliskiren enhanced both the increase in blood NO concentration induced by ACh and the decrease in the NO release after treatment with L-NMMA to a degree similar to that obtained with valsartan; furthermore, it concurrently reduced the area of plaques in the aorta. These effects were additively enhanced by combination use of aliskiren and valsartan, suggesting an AII-independent action of aliskiren. Reports of their effects in humans are expected soon.

f) Statins: Statins (HMG CoA reductase inhibitors) are cholesterol lowering drugs that inhibit the onset of ischemic heart disease and cerebral stroke, as demonstrated by numerous large-scale clinical studies. It is known that administration of statins for 6 months improves endothelial-dependent vasodilation in coronary arteries or forearm arteries of patients with hypercholesterolemia. The endothelium-dependent vasodilation in the forearm arteries improves in 2-12 weeks, and this effect does not run parallel to the lowering effect on LDL cholesterol, thus this is considered to be a pleiotropic effect. However, LDL apheresis or cholestyramine also improves endothelial function, thus the improvement of endothelial function must also be due to the cholesterol-lowering effect of the drug. Activation of the PI3-K/Akt pathway, a decrease in caveolin-1, an increase of eNOS and Hsp90 interaction, stabilization of eNOS mRNA by inhibition of the Rho/Rho kinase pathway, and a decrease of adhesion molecules are involved in the mechanism of direct enhancement of NO release by statins. In addition, statins increase the number of EPC in patients with coronary disease or cardiac failure. Nevertheless, this action on EPC is not observed when NO does not work, thus this may also occur due to an NO increasing effect of statins.

g) Insulin-resistance improving drugs: Patients with hypertension, diabetes, obesity, and dyslipidemia are not only in an insulin resistance state, but also have endothelial damage. This may be explained by a reduction of endothelial-dependent vasodilation by insulin. Insulin-induced NO release due to activation of NOS through the PI3-K/Akt pathway. Thiazolidinediones, which increase insulin sensitivity, lower CRP and ADM values and improve endothelial-dependent vasodilation in diabetic patients. These drugs also prevented the occurrence of cardiovascular events in a large-scale clinical study.

h) Erythropoietin (EPO): EPO activates the PI3-K/Akt pathway and promotes NO release. Furthermore, there is a report indicating that blood EPO concentration is directly proportional to the number of EPC in patients with coronary artery disease. Also, administration of EPO increased the number of EPC. These findings suggest that endogenous EPO may play an important role in the production of EPC.

i) Treatments that affect the number of EPC: The existence of risk factors leads to endothelial dysfunction, which in turn becomes the stimulus to enhance the mobilization of EPC, their accumulation at the damaged site, and angiogenesis. Exercise increases the number and function of EPC in humans. The number of EPC decreases in the presence of high levels of ADMA and LDL cholesterol, low HDL cholesterol, hypertension, diabetes mellitus, smoking, and hyperhomocysteinemia. On the other hand, the number of
EPC is increased by drugs such as statins, ARBs, ACE inhibitors, PDE5 inhibitors, rosiglitazone, or erythropoietin. The population of EPC is proportional to the plasma concentration of estrogen. Rapamycin decreases the number of EPC. Circulating EPC may contribute to the repair of the endothelium.

**Conclusion:** There is no doubt that in the treatment of arteriosclerotic disease, it is essential to consider the improvement and repair of endothelial function. However, it is desirable to establish a simple method to measure endothelial function and to develop a more specific treatment to improve it.

**References**