Endothelium-Dependent Effects of Statins
Sebastian Wolfrum, Kristin S. Jensen, James K. Liao

Abstract—The vascular endothelium is a dynamic endocrine organ that regulates contractile, secretory, and mitogenic activities in the vessel wall and hemostatic processes within the vascular lumen. Risk factors for cardiovascular disease, such as cigarette smoking, hypertension, and elevated serum lipid levels, impair endothelial function and lead to the development of atherosclerotic vessels. Recent studies suggest that statins reduce cardiovascular events in part by improving endothelial function. Statins reduce plasma cholesterol levels, thereby decreasing the uptake of modified lipoproteins by vascular wall cells. There is increasing evidence, however, that statins may also exert effects beyond cholesterol lowering. Indeed, many of these cholesterol-independent or “pleiotropic” vascular effects of statins appear to involve restoring or improving endothelial function through increasing the bioavailability of nitric oxide, promoting re-endothelialization, reducing oxidative stress, and inhibiting inflammatory responses. Thus, the endothelium-dependent effects of statins are thought to contribute to many of the beneficial effects of statin therapy in cardiovascular disease. (Arterioscler Thromb Vasc Biol. 2003;23:729-736.)

Key Words: 3-hydroxy-3-methyl-glutaryl coenzyme A reductase inhibitor ■ endothelium ■ atherosclerosis ■ cholesterol ■ protein kinase Akt

The vascular endothelium is the inner lining of blood vessels and serves as an important autocrine/paracrine organ that regulates vascular wall contractile state and cellular composition. Because of its strategic location between the circulation and the vascular wall, the endothelium interacts with both cellular and hormonal mediators from these two compartments. There is growing evidence that endothelial dysfunction, which is often defined as the decreased synthesis, release, and/or activity of endothelial-derived nitric oxide (NO), is an important factor leading to atherosclerosis and acute coronary syndromes.1,2 This realization, in part, is based on the fact that the lack of NO in atherosclerotic vessels contributes to impaired vascular relaxation,3 platelet aggregation,4 increased vascular smooth muscle proliferation,5 and enhanced leukocyte adhesion to the endothelium.6 Indeed, vasoconstriction, platelet activation, and thrombosis caused by the rupture of atherosclerotic plaques are primary features of acute coronary syndromes and other cardiovascular events.7 In addition to endothelial dysfunction, another important feature of atherosclerotic vessels is endothelial cell activation.8 The activated endothelium expresses cell–surface adhesion molecules, such as vascular cell adhesion molecule-1, intercellular adhesion molecule-1, and endothelial-leukocyte adhesion molecule, which facilitate the attachment of circulating leukocytes to the endothelium.9 Monocyte adhesion to the vessel wall and its subsequent differentiation into macrophages are crucial events leading to the development of macrophage-derived foam cells in atherosclerotic plaques. Cytokines, oxidized LDLs (ox-LDLs), and infectious agents, such as cytomegalovirus and Chlamydia pneumoniae, promote vascular oxidation and inflammation, which lead to endothelial cell activation.10,11 Thus, endothelial dysfunction and activation caused by coronary risk factors and vascular inflammation are the basis for the development of atherosclerotic lesions.

Endothelial Dysfunction and Atherosclerosis
The endothelium produces vasoactive substances in response to environmental factors, such as blood flow, oxygen tension, and receptor-mediated stimulants.12–14 Endothelium-
dependent vascular relaxation is predominantly mediated by NO and to lesser degrees by prostacyclin and activators of ATP- and calcium-sensitive potassium channels (ie, hyperpolarizing factor). Endothelium-derived vasoconstrictive substances include endothelin (ET)-1 and thromboxane A2. The balance between these opposing endothelium-derived vasoactive substances under normal and pathological conditions ultimately determines the contractile and perhaps the mitogenic state of the underlying vascular smooth muscle. Endothelium-derived NO plays an important physiological role in the regulation of blood pressure, and blood flow and has been widely used as a clinical marker of endothelial function. Indeed, endothelial dysfunction or decreased NO bioavailability is often associated with cardiovascular diseases, such as atherosclerosis.1

Endothelial dysfunction may also be attributed to abnormal or excessive release of vasoconstricting substances, such as ET-1. Circulating levels and tissue immunoreactivity of ET-1 are elevated in patients with advanced atherosclerosis and acute coronary syndromes.16,17 Whereas NO is vasodilatory and antiproliferative in its effects on the underlying vascular smooth muscle, ET-1 is vasoconstrictive and mitogenic. Exposure to cardiovascular risk factors, such as ox-LDL, enhances the production and release of ET-1.18 Increased ET-1 levels in combination with platelet-derived growth factors promote vascular smooth muscle proliferation in the neointima of atherosclerotic lesions.19 Although definitive proof that ET-1 is a primary inducer of atherosclerosis is still elusive, it is highly likely that ET-1 is at least an important contributor to the atherogenic process.

Numerous studies demonstrate that endothelial dysfunction is one of the earliest manifestations of atherosclerosis, even in the absence of angiographic evidence of disease.20 Conversely, improved endothelial function is one of the earliest clinical markers after atherogenic risk factor modification. There is a strong association between atherogenic risk factors and endothelial dysfunction. For example, LDL, especially ox-LDL, is a potent inhibitor of endothelial function.21 The mechanisms by which LDL inhibits endothelial-derived NO activity include downregulation of endothelial NOS expression,22 decreased receptor-mediated NO release,23 and NO inactivation via increases in superoxide anion production.24 Furthermore, LDL facilitates the development of atherosclerosis by enhancing monocyte adhesion to endothelial cells in vitro,25 a process that might be mediated by an increased expression of adhesion molecules, such as intercellular adhesion molecule-1 (Figure 1).26

Statins and Endothelial Function

Hypercholesterolemia impairs endothelial function and by blocking the conversion of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) to mevalonate, statins inhibit an early rate-limiting step in cholesterol biosynthesis. This leads to increased hepatic LDL receptors and enhanced uptake of cholesterol by the liver. Indeed, therapeutic doses of statins potently reduce serum cholesterol levels in humans,27 and several large clinical trials have demonstrated that inhibition of HMG-CoA reductase by statins markedly decreases the incidence of cardiovascular events in hypercholesterolemic individuals.27–30 Because of the strong association between elevated serum cholesterol levels and coronary atherosclerotic disease, reduction of serum cholesterol levels by statins has been proposed to be the predominant mechanism underlying the beneficial effects of statins. Indeed, acute plasma LDL apheresis improves endothelium-dependent vasodilatation,31 suggesting that statins could restore endothelial function, in part, by lowering serum cholesterol levels.

In some studies with statins, however, restoration of endothelial function occurs before significant reduction in serum cholesterol levels,32,33 suggesting that there are additional effects on endothelial function beyond that of cholesterol reduction. In a recent study in normocholesteremic patients, improvement of endothelial function was observed within 3 hours after a single bolus of cerivastatin.34 Indeed, in patients, statin therapy has been found to rapidly improve vasomotor response to endothelium-dependent agonists.35
enhance coronary blood flow, and reduce the levels of adhesion molecules. The mechanism is caused, in part, by statin’s ability to increase endothelial NO production by stimulating and upregulating endothelial nitric oxide synthase (eNOS). Furthermore, statins have been shown to restore eNOS activity in the presence of hypoxia and ox-LDL, conditions that lead to endothelial dysfunction. Statins also increase the expression of tissue-type plasminogen activator (t-PA) and inhibit the expression of endothelin-1, a potent vasoconstrictor and mitogen. Statins, therefore, exert many favorable effects on the endothelium and attenuate endothelial dysfunction in the presence of atherosclerotic risk factors.

Statin Pleiotropism and Endothelial Function

A number of recent studies have focused on the pleiotropic effects of statins. It has been reported that statins decrease the extent of cerebral and myocardial ischemia/reperfusion injury in rodents without changes in serum cholesterol levels. The vascular protective effects of statins were associated with increased blood flow, attenuated P-selectin expression, and leukocyte adherence. By using genetic and pharmacological approaches, the role of eNOS in cholesterol independent protection by statins was also delineated in vivo models. Pretreatment with statins increased eNOS mRNA expression as well as eNOS activity, and protection by statins was completely abolished in eNOS knockout mice or after inhibition of eNOS with L-NAME.

The realization that inhibition of HMG-CoA reductase by statins not only reduces cholesterol production but also prevents the formation of various isoprenoid intermediates has given rise to statin pleiotropism on the vascular wall, in particular, the endothelium (Figure 2). Farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP), for example, serve as important lipid attachments for the post-translational modification of a variety of proteins, including the subunit of heterotrimeric G-proteins and small GTP-binding protein Ras, and Ras-like proteins, such as Rho, Rab, Rac, and Rap. Protein isoprenylation allows the covalent attachment, subcellular localization, and intracellular trafficking of several membrane-associated proteins. Although the effects of statins on Ras and Rho isoprenylation are reversed in the presence of FPP and GGPP, respectively, the effect of statins on eNOS expression is only reversed with GGPP and not by FPP or oxLDL. Indeed, direct inhibition of geranylgeranyltransferase or Rho leads to increases in eNOS expression. These findings are consistent with a noncholesterol-lowering effect of statins and suggest that inhibition of Rho by statins mediates the increase in eNOS expression. Indeed, statins upregulate eNOS expression by prolonging eNOS mRNA half-life but not eNOS gene transcription. Because hypoxia, ox-LDL, and cytokines, such as tumor necrosis factor-α, decrease eNOS expression by reducing eNOS mRNA stability, the ability of statins to prolong eNOS half-life may make them effective agents in counteracting conditions which downregulate eNOS expression.

Furthermore, it has been recently reported by Kureishi et al. that statins can activate protein kinase Akt. The serine-threonine kinase Akt is an important regulator of various cellular processes, including cell metabolism and apoptosis. Stimulation of receptor tyrosine kinases and G-protein-coupled receptors lead to activation phosphatidylinositol 3-(PI3) kinase, the products of which, namely 3’ phospholipids, provoke the phosphorylation and activation of Akt. Indeed, inhibitors of PI3 kinase, such as wortmannin, block the effects of statins on Akt activation. Akt has shown to modulate several targets, such as caspase-9 and eNOS, by phosphorylation. Consequently, activation of Akt by statins inhibits apoptosis and increases NO production in cultured endothelial cells. Therefore, in addition to stabilizing eNOS mRNA by inhibition of Rho, there is increasing evidence that activation of the PI3 kinase/Akt pathway may also contribute to the endothelium-dependent effects of statins although the precise mechanisms how PI3 kinase is activated by statins are not yet identified (Figure 2).

Because several vasoconstricting agents counteract the vasodilating effect of NO, endothelial dysfunction and development of atherosclerosis may also be attributed to the
release of potent vasoconstrictors like ET-1 or angiotensin II (Ang II). Indeed, circulating concentrations and tissue immunoreactivity of ET-1 are increased in patients with severe atherosclerosis.16 ET-1 acts as a vasoconstrictive and mitogenic agent. Exposure to ox-LDL leads to an increased production and release of ET-1,16 which promotes neointima proliferation of atherosclerotic lesions.19 Statins have been shown to inhibit preproET-1 mRNA expression in a concentration-dependent manner and to reduce immunoreactive ET-1 in bovine endothelial cells, a phenomenon that has been suggested to be mediated by Rho proteins (Figure 1).42-56 Furthermore, statins attenuate the increased expression of endothelin receptors achieved by basic fibroblastic growth factor.53 Ichiki et al58 recently reported that statins also modulate the renin–angiotensin system. In the study, statins downregulated the expression of angiotensin receptor subtype 1 (AT1) in a Rho A–dependent manner and attenuated the biological function of Ang II.

Another potential mechanism by which statins may improve endothelial function is through their antioxidant effects. For example, statins attenuate Ang II–induced free radical production in vascular smooth muscle cells by inhibiting Rac1-mediated NAD(P)H oxidase activity and downregulating angiotensin AT1 receptor expression.59 More recently, Wassmann et al60 reported, that atorvastatin reduced vascular mRNA expression of essential NAD(P)H oxidase subunits p22phox and nox1 by a mechanism that might involve the translocation of Rac1 from the cytosol to the cell membrane. Because NO is scavenged by ROS, these findings indicate that the antioxidant properties of statins may also contribute to their ability to improve endothelial function (Figure 1). Furthermore, withdrawal of statin treatment in mice has been shown to impair endothelium-dependent relaxation by increasing vascular superoxide anion generation via a pathway involving the Rac-dependent activation of the gp91phox-containing vascular NAD(P)H oxidase.61 ROS directly affects the endothelial function, and the endothelium itself has also been shown to generate ROS.62 Because the amount of ROS generated in the endothelium is relatively low, most studies focus on other sources of ROS, such as smooth muscle cells and leukocytes.60,63

Statins and Blood Pressure Control
Because statins can increase eNOS activity and inhibit the expression of vasoconstrictive substances, such as ET-1, it is likely that statins, either alone or given with another agent, will have some effect on systemic blood pressure. Indeed, in several animal models of hypertension, such as Dahl salt-sensitive rats and spontaneously hypertensive rats, statins reduce blood pressure and prevent hypertension-induced glomerular injury.64-67 In contrast, Yamashita et al68 did not find a decrease in blood pressure in stroke-prone spontaneously hypertensive rats despite a marked reduction in proteinuria and renal fibrosis. Thus, it is not entirely clear whether statins alone can decrease systemic blood pressure. Nevertheless, a reduction in cholesterol levels is correlated with a lower diastolic blood pressure.69 Indeed, a small crossover study with 26 hypertensive and diabetic patients revealed that statin therapy reduced diastolic blood pressure, whereas another cholesterol lowering agent, cholestryamine, had no effect, despite similar reduction in cholesterol level as that of statins.70 Finally, patients receiving statin therapy in addition to antihypertensive drugs have a more pronounced reduction in blood pressure, an effect that was independent from cholesterol lowering.11 It is interesting to speculate that a reduction in blood pressure, and not cholesterol, by statins may explain some of their protective effects in ischemic stroke, a disease that is not generally associated with elevated cholesterol levels.

Statins and Endothelial Inflammatory Response
Atherosclerosis is a complex inflammatory process that is characterized by the presence of monocytes or macrophages and T lymphocytes in the atheroma.72 Inflammatory cytokines secreted by these macrophages and T lymphocytes can modify endothelial function, smooth muscle cell proliferation, collagen degradation, and thrombosis.73 An early step in atherogenesis involves monocyte adhesion to the endothelium and penetration into the subendothelial space.72 Statins have been shown to reduce the number of inflammatory cells in atherosclerotic plaques and therefore possess anti-inflammatory properties. The mechanisms have yet to be fully elucidated but may involve inhibition of adhesion molecules such as intercellular adhesion molecule-1 and cytokines as interleukins 6 and 8, which are involved in the recruitment of inflammatory cells.74,75 In addition, a recent study has shown that statins can suppress the inflammatory response independent of HMG-CoA reductase inhibition by binding directly to a novel regulatory site of the β2 integrin, leukocyte function antigen-1. This regulatory site serves as a major counterreceptor for intercellular adhesion molecule-1 on leukocytes.76 The mechanism of the anti-inflammatory properties of statins was further elucidated by Yoshida et al,77 who recently demonstrated that cerivastatin reduced monocyte adhesion to vascular endothelium by decreasing expression of integrins and actin polymerization through the inactivation of RhoA.

A clinical marker of inflammation is high-sensitivity C-reactive protein (hs-CRP).78 hs-CRP is an acute phase reactant that is produced by the liver in response to proinflammatory cytokines, such as interleukin 6, and reflects low-grade systemic inflammation.79 Elevated levels of hs-CRP have been shown to be predictive of increased risk for coronary artery disease in apparently healthy men and women.80,81 hs-CRP is elevated in patients with coronary artery disease, coronary ischemia, and myocardial infarction compared with normal subjects.82-84 It has been suggested that CRP could contribute to the development of atherosclerosis by binding to modified LDL within atherosclerotic plaques.85 Once CRP becomes bound, it activates complement, which has been shown to play a role in promoting atherosclerotic lesion progression.86 In two recent studies CRP has also been shown to impair endothelial function by decreasing eNOS expression in cultured endothelial cells.87,88 However, further studies are needed to fully elucidate the role CRP plays in atherosclerosis.

Statin therapy lowers hs-CRP levels in hypercholesterolemic patients.78,89 In the CARE trial, statins significantly
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<td>Decrease in the expression of AT₁-receptor</td>
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decreased plasma hs-CRP levels over a 5-year period in patients who did not experience recurrent coronary events. Similarly, an analysis of baseline and 1-year follow-up from the AFCAPS/TexCAPS study demonstrated that hs-CRP levels were reduced in statin-treated patients who were free of acute major coronary events. Furthermore, preliminary data from the PRINCE study confirm that statin therapy can significantly reduce serum hs-CRP levels in primary and secondary prevention populations. After 24 weeks of therapy with a statin, the hs-CRP level was reduced by approximately 13% in primary and secondary prevention populations, whereas placebo treatment of subjects in the primary prevention arm of the study had no effect. These studies, therefore, indicate that statins are effective in decreasing systemic and vascular inflammation. However, any potential clinical benefits conferred by the lowering of hs-CRP are difficult to separate from that of the lipid-lowering effects of statins without performing further clinical studies.

Statins and Re-endothelialization

Stimulation of re-endothelialization or neovascularization is a therapeutic aim to reduce ischemia-induced tissue injury. Postnatal neovascularization was mainly attributed to angiogenesis, for example, proliferation, migration, and remodeling of preexisting endothelial cells. However, some studies recently demonstrated that bone marrow–derived circulating endothelial cells are also involved in this process. Circulating endothelial cells can be grown out of isolated CD133+ or CD34+ cells. Transplantation of these cells leads to postnatal neovascularization in the ischemic hindlimb, augments ischemia-induced neovascularization in vivo, and even improves postschismic cardiac function.

Recent studies revealed that statins also promote vasculogenesis. Llevadot et al. demonstrated in vitro that simvastatin evokes proliferation, migration, and cell survival of circulating endothelial cells. The signal pathway for this effect includes activation of protein kinase Akt, which was confirmed by functional blocking with dominant-negative Akt overexpression. Dimmeler et al. showed in vitro and in vivo that statins do not only increase the number of circulating endothelial cells but also induce their differentiation. This might be of clinical relevance because it has been recently reported by Walter et al. that induction of these cells under statin treatment is associated with an accelerated re-endothelialization after carotid balloon injury.

In contrast, some studies report an antiangiogenic effect of statins that might be mediated by RhoA. It has been suggested that these conflicting results are dose related. Low doses of a statin may activate endothelial Ras and promote Akt and eNOS phosphorylation leading to an angiogenic effect, whereas higher statin doses are antiangiogenic although they promote an increase in eNOS protein expression. This suggestion remains controversial because high doses of statins have also been shown to be angiogenic and further studies are necessary to clarify this topic.

Statins and Endothelial Fibrinolysis

Plasminogen activator inhibitor type-1 (PAI-1) is the major endogenous inhibitor of t-PA, and it also plays a pivotal role in the regulation of fibrinolysis. High PAI-1 plasma levels and decreased levels of t-PA activity have been shown to be associated with coronary heart disease. PAI-1 mRNA has also been found in human atherosclerotic lesions, underlining its role in the development of these disorders. There is increasing evidence from in vitro studies that statins positively affect the fibrinolytic system of cultured smooth muscle cells as well as endothelial cells. In these studies a decrease in PAI-1 and an increase in t-PA was observed after co-treatment with statins in endothelial cells. Several in vivo studies have also investigated the effect of statin therapy on the regulation of the fibrinolytic system. Although the results are inconsistent, in some studies statins decrease PAI-1 plasma levels. Therefore, statins may interfere with the progression of the atherosclerotic plaque as well as with thrombotic events in hyperlipidemic patients, independently of their ability to reduce plasma cholesterol, but further studies have to delineate its physiological significance.

Summary

It is well established that the reduction of plasma cholesterol by statins improves endothelial function and limits atherosclerosis. In addition, statins may exert other cholesterol-independent effects on the endothelium, which lead to further improvements in vascular function (Table). Most of these effects of statins are mediated by inhibition of isoprenoid synthesis and increase in NO release or bioavailability. Because statins also reduce plasma cholesterol levels in
normocholesterolemic subjects, it is often difficult, if not impossible, to separate the cholesterol lowering from the pleiotropic effects. Indeed, in the CARE and Heart Protection Study, statins were effective in reducing cholesterol levels in so-called “normocholesterolemic” subjects.\textsuperscript{29,110} Perhaps future clinical trials comparing the effects of statins alone versus statins with ezetimibe may help address the question regarding the relative contribution of lipid lowering and pleiotropy by statins to cardiovascular protection. Thus, it remains to be determined, however, which of these effects are more predominant, in terms of clinical outcome, in patients with low or average cholesterol levels. Nevertheless, both direct and indirect endothelium-dependent effects of statins play important roles in limiting the development of atherosclerosis and vascular inflammation.

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