Brief Review

Pleiotropic Effects of 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Inhibitors

Masao Takemoto, James K. Liao

Abstract—The 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors or statins are potent inhibitors of cholesterol biosynthesis. Several large clinical trials have demonstrated the beneficial effects of statins in the primary and secondary prevention of coronary heart disease. However, the overall clinical benefits observed with statin therapy appear to be greater than what might be expected from changes in lipid profile alone, suggesting that the beneficial effects of statins may extend beyond their effects on serum cholesterol levels. Indeed, recent experimental and clinical evidence indicates that some of the cholesterol-independent or “pleiotropic” effects of statins involve improving or restoring endothelial function, enhancing the stability of atherosclerotic plaques, and decreasing oxidative stress and vascular inflammation. Many of these pleiotropic effects of statins are mediated by their ability to block the synthesis of important isoprenoid intermediates, which serve as lipid attachments for a variety of intracellular signaling molecules. In particular, the inhibition of small GTP-binding proteins, Rho, Ras, and Rac, whose proper membrane localization and function are dependent on isoprenylation, may play an important role in mediating the direct cellular effects of statins on the vascular wall. (Arterioscler Thromb Vasc Biol. 2001;21:1712-1719.)

Key Words: endothelium ■ vascular smooth muscle ■ platelets ■ atherosclerosis ■ inflammation

Atherosclerosis is the underlying disorder in the majority of patients with cardiovascular disease.1 Although the development of atherosclerosis is dependent on many factors and processes, a clear association has been established between elevated serum cholesterol levels and increased atherosclerotic disease.2-5 Recent large clinical trials have demonstrated that statins decrease the incidence of coronary heart disease in patients with hypercholesterolemia and atherosclerosis.6-10 Depending on the dose and the type of the statin used, LDL was decreased anywhere from 19% to 60% in response to therapy. In addition, HDL cholesterol levels were increased, and triglycerides were decreased as a result of statin therapy. These lipid effects of statins are believed to slow the progression of atherosclerosis, because atherosclerosis is mediated, in part, by the uptake of modified LDL, which eventually constitutes the lipid core of atherosclerotic lesions.11

Because serum cholesterol levels are strongly associated with coronary atherosclerotic disease,12 it has been generally assumed that cholesterol reduction by statins is the predominant, if not the only, mechanism underlying their beneficial effects in cardiovascular diseases. However, subgroup analyses of large clinical trials have challenged this notion and have suggested that the beneficial effects of statins may extend to mechanisms beyond cholesterol reduction. For example, subgroup analysis of the West of Scotland Coronary Prevention (WOSCOP) and Cholesterol and Recurrent Events (CARE) studies indicates that despite comparable serum cholesterol levels among the statin-treated and placebo groups, statin-treated individuals have a significantly lower risk of coronary heart disease than do age-matched placebo-controlled individuals.8,9,13,14 Furthermore, meta-analyses of cholesterol-lowering trials suggest that the risk of myocardial infarction in individuals treated with statins is significantly lower than that in individuals treated with other cholesterol-lowering agents or modalities despite comparable reduction in serum cholesterol levels in both groups.15,16 These findings suggest that statins may have beneficial effects beyond cholesterol lowering.

Further evidence in support of the noncholesterol benefits of statin therapy is provided by angiographic trials, which have demonstrated clinical improvements with statins that far exceed changes in the size of atherosclerotic lesions. For example, in the Familial Atherosclerosis Treatment Study (FATS) trial, statin therapy with bile acid resin decreased the incidence of coronary events by 70% despite producing only a 0.7% change in lesion regression.15,17 Indeed, many of the beneficial effects of statins in the FATS trial were attributed to plaque stabilization and remodeling. However, in the recent Myocardial Ischemia Reduction With Aggressive Cholesterol Lowering (MIRACL) trial, statins were found to be effective in reducing recurrent ischemic events as early as 16 weeks after acute coronary ischemia.18 Although the serum LDL cholesterol was reduced by 40%, this time frame was
Statins and Isoprenylated Proteins

By inhibiting l-mevalonic acid synthesis, statins also prevent the synthesis of other important isoprenoid intermediates of the cholesterol biosynthetic pathway, such as farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP); see Figure 1. These intermediates serve as important lipid attachments for the posttranslational modification of a variety of proteins, including the γ subunit of heterotrimeric G proteins, Heme-a, nuclear lamins, and the small GTP-binding proteins Ras and Rho-like proteins, such as Rho, Rab, Rac, Ral, and Rap. Thus, protein isoprenylation permits the covalent attachment, subcellular localization, and intracellular trafficking of membrane-associated proteins. Members of the Ras and Rho GTPase family are major substrates for posttranslational modification by prenylation. Ras and Rho are small GTP-binding proteins that cycle between the inactive GDP-bound state and active GTP-bound state. In endothelial cells, Ras translocation from the cytoplasm to the plasma membrane is dependent on farnesylation, whereas Rho translocation is dependent on geranylgeranylation. Statins inhibit Ras and Rho isoprenylation, leading to the accumulation of inactive Ras and Rho in the cytoplasm.

Because Rho is a major target of geranylgeranylation, inhibition of Rho and its downstream target, Rho kinase, is a likely mechanism mediating some of the pleiotropic effects of statins on the vascular wall. Each member of the Rho family serves specific functions in terms of cell shape, motility, secretion, and proliferation, although overlapping functions between the members could be observed in overexpressed systems. The activation of Rho in Swiss 3T3 fibroblasts by extracellular ligands, such as platelet-derived lysophosphatidic acid, leads to myosin light chain phosphorylation and the formation of focal adhesion complexes. Indeed, Rho-associated protein kinase increases the sensitivity of vascular smooth muscle to calcium in hypertension and coronary spasm. In contrast, activation of Rac leads to the formation of lamellipodia and membrane ruffles, whereas activation of Cdc42 induces actin-rich surface protrusions called filopodia. Thus, changes in Rho-induced actin cytoskeleton can affect intracellular transport, membrane trafficking, mRNA stability, and gene transcription. Indeed, evidence suggests that inhibition of Rho isoprenylation mediates many of the cholesterol-independent effects of statins not only in vascular wall cells but also in leukocytes and bone.

Statins and Endothelial Function

The vascular endothelium serves as an important autocrine and paracrine organ that regulates vascular wall contractile state and cellular composition. Hypercholesterolemia impairs endothelial function, and endothelial dysfunction is one of the earliest manifestations of atherosclerosis, occurring even in the absence of angiographic evidence of disease. An important characteristic of endothelial dysfunction is the impaired synthesis, release, and activity of endothelium-derived NO. Endothelial NO has been shown to inhibit several components of the atherogenic process. For example, endothelium-derived NO mediates vascular relaxation and inhibits platelet aggregation, vascular smooth muscle proliferation, and endothelium-leukocyte interactions. Inactivation of NO by superoxide anion (O2−) limits the bioavailability of NO and leads to nitrate tolerance, vasoconstriction, and hypertension.

Acute plasma LDL apheresis improves endothelium-dependent vasodilatation, suggesting that statins could re-
Statins and SMC Proliferation

The proliferation of vascular SMCs is a central event in the pathogenesis of vascular lesions, including postangioplasty restenosis, transplant arteriosclerosis, and venous graft occlusion.44 Recent studies have shown that statins attenuate vascular proliferative disease, such as transplant-associated arteriosclerosis.44 In contrast to atherosclerosis, transplant-associated arteriosclerosis is more of an immunologic than a lipid disorder, although hypercholesterolemia exacerbates the immunologic process.55 Inhibition of isoprenoid but not cholesterol synthesis by statins decreased platelet-derived growth factor (PDGF)-induced DNA synthesis in vascular SMCs.50,56 Treatment with statins decreased PDGF-induced retinoblastoma gene product (Rb) hyperphosphorylation and cyclin-dependent kinase (cdk)-2, cdk-4, and cdk-6 activities. This was correlated with increases in the level of the cdk inhibitor p27Kip1, without concomitant changes in p16INK4, p21Waf1, or p53 levels. These findings indicate that statins inhibit vascular SMC proliferation by arresting the cell cycle between the G1 phase–to–S phase transition. It remains to be determined whether the upregulation of p27Kip1 is responsible for the cell cycle arrest and whether there are differences between the various statins in terms of p27Kip1 upregulation.

Because the small GTP-binding proteins, Ras and Rho, require posttranslational modification for membrane localization and activity and are implicated in cell cycle regulation, they are likely targets for the direct antiproliferative vascular effects of statins. Ras can promote cell cycle progression via activation of the mitogen-activated protein kinase pathway,57 whereas Rho causes cellular proliferation possibly through destabilizing p27Kip1 protein.58 Interestingly, inhibition of vascular SMC proliferation by statins was reversed by GGPP, but not FPP or LDL cholesterol.59 Indeed, direct inhibition of Rho by Clostridium botulinum C3 transferase, which ADP-ribosylates and inactivates Rho, or by a dominant-negative Rho mutant increased p27Kip1 and inhibited Rb hyperphosphorylation and SMC proliferation after PDGF stimulation.30 Taken together, these findings indicate that Rho mediates PDGF-induced SMC proliferation and that inhibition of Rho by statins is the predominant mechanism by which statins inhibit vascular SMC proliferation.

Statins and Platelet Function

Platelets play a critical role in the development of acute coronary syndromes.59 Acute thrombus formation at the site of plaque rupture and vascular injury accounts for most episodes of acute coronary syndromes.60–62 Hypercholesterolemia is associated with increases in platelet reactivity.63,64 These abnormalities are linked to increases in the cholesterol/phospholipid ratio in platelets. Other potential mechanisms include increases in thromboxane A2 biosynthesis,65 platelet α2-adenoreceptor density,66 and platelet cytosolic calcium.67

Statins have been shown to inhibit platelet function.68–70 Potential mechanisms include a reduction in the production of thromboxane A2, and modifications in the cholesterol content of platelet membranes.71,72 The cholesterol content of platelet and erythrocyte membranes is reduced in patients undergoing statin therapy. This may lead to a decrease in the thrombogenic potential of these cells. Indeed, animal studies suggest

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Figure 2. Regulation of eNOS expression by statins. Statins inhibit HMG-CoA reductase and block the synthesis of isoprenoids and cholesterol. The isoprenoid, geranylgeranyl (GG), is an important lipid attachment for Rho, which permits the subsequent membrane translocation and activation of Rho. Inhibition of Rho geranylgeranylation by geranylgeranyl transferase inhibitor (GGT1), Rho activity by Clostridium botulinum C3 transferase, or Rho kinase activity by Rho kinase inhibitors leads to increases in eNOS expression.

store endothelial function, in part, by lowering serum cholesterol levels. However, in some studies with statins, restoration of endothelial function occurs before significant reduction in serum cholesterol levels,43–45 suggesting that there may be additional effects on endothelial function beyond that of cholesterol reduction. Indeed, statins increase NO bioavailability by stimulating and upregulating endothelial NO synthase (eNOS)24,46 or by decreasing oxidative stress.47 Further effects on endothelial function beyond that of cholesterol reduction. Indeed, statins increase NO bioavailability by stimulating and upregulating endothelial NO synthase (eNOS).24,46 or by decreasing oxidative stress.47 Further findings indicate that the antioxidant properties of statins may prove endothelial function is through their antioxidant effects. Hypoxia48 and oxidized LDL,24 conditions that downregulate eNOS expression. These findings are consistent with a non-endothelial origin of the antioxidant effects of statins. Indeed, statins upregulate eNOS expression by prolonging eNOS mRNA half-life but not eNOS gene transcription.25

Because hypoxia, oxidized 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 that downregulate eNOS expression.

Another potential mechanism by which statins may improve endothelial function is through their antioxidant effects. For example, statins enhance endothelium-dependent relaxation by inhibiting the production of reactive oxygen species (ROS), such as such as superoxide and hydroxy radicals, from aortas of cholesterol-fed rabbits.57 Although lipid lowering by itself can lower vascular oxidative stress,51,52 some of these antioxidant effects of statins appear to be cholesterol independent. For example, statins attenuate angiotensin II–induced free radical production in vascular smooth muscle cells (SMCs) by inhibiting Rac1-mediated NADH oxidase activity and downregulating angiotensin type 1 receptor expression.53 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.40,41

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that statin therapy inhibits platelet deposition on damaged vessels and reduces platelet thrombus formation.\textsuperscript{61,73,74} Furthermore, in vitro experiments have demonstrated that statins inhibit tissue factor expression by macrophages, thereby potentially reducing the thrombotic potential of the vascular wall.\textsuperscript{75}

\textbf{Statins and Plaque Stability}

Plaque rupture is a major cause of acute coronary syndromes.\textsuperscript{34,76–79} The atherosclerotic lesion contains highly thrombogenic materials in the lipid core that are separated from the bloodstream by a fibrous cap.\textsuperscript{80} Fissuring, erosion, and ulceration of the fibrous cap eventually leads to plaque rupture and ensuing thrombosis.\textsuperscript{78} Collagen is the main component of fibrous caps and is responsible for their tensile strength. Because macrophages are capable of degrading the collagen-containing fibrous cap, they play an important role in the development and subsequent stability of atherosclerotic plaques.\textsuperscript{81,82} Indeed, degradation of the plaque matrix appears to be most active in macrophage-rich regions.\textsuperscript{76,77} Secretion of proteolytic enzymes, such as matrix metalloproteinases (MMPs), by activated macrophages may weaken the fibrous cap, particularly at the “vulnerable” shoulder region, where the fibrous cap joins the arterial wall.\textsuperscript{83,84} Weakened fibrous caps lead to plaque instability, rupture, and ensuing thrombosis, which ultimately present as acute coronary syndromes.\textsuperscript{79,85}

Lipid lowering by statins may contribute to plaque stability by reducing plaque size or by modifying the physiochemical properties of the lipid core.\textsuperscript{86,87} However, as mentioned previously, changes in plaque size by lipid lowering tend to occur over extended time and are quite minimal, as assessed by angiography. Rather, the clinical benefits from lipid lowering are probably due to decreases in macrophage accumulation in atherosclerotic lesions and inhibition of MMP production by activated macrophages.\textsuperscript{75} Indeed, statins inhibit the expression of MMPs and tissue factor by cholesterol-dependent and -independent mechanisms,\textsuperscript{75,86,88} with the cholesterol-independent or direct macrophage effects occurring at a much earlier time frame. Therefore, the plaque-stabilizing properties of statins are mediated through a combined reduction in lipids, macrophages, and MMPs.\textsuperscript{89} These effects of statins may reduce the incidence of acute coronary syndromes by lessening the propensity for plaque to rupture.

\textbf{Statins and Vascular Inflammation}

Atherosclerosis is a complex inflammatory process that is characterized by the presence of monocytes or macrophages and T lymphocytes in the atheroma.\textsuperscript{11,90} Inflammatory cytokines secreted by these macrophages and T lymphocytes can modify endothelial function, SMC proliferation, collagen degradation, and thrombosis.\textsuperscript{79} An early step in atherogenesis involves monocyte adhesion to the endothelium and penetration into the subendothelial space.\textsuperscript{83} Recent studies suggest that statins possess anti-inflammatory properties by their ability to reduce the number of inflammatory cells in atherosclerotic plaques.\textsuperscript{71} The mechanisms have yet to be fully elucidated but may involve inhibition of adhesion molecules such as intercellular adhesion molecule-1, which are involved in the recruitment of inflammatory cells.\textsuperscript{91} Indeed, a recent study has shown that statins can suppress the inflammatory response independent of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibition by binding directly to a novel regulatory site of the β\textsubscript{2} integrin, leukocyte function antigen-1, which serves as a major counterreceptor for intercellular adhesion molecule-1 on leukocytes.\textsuperscript{92} Furthermore, statins protect the ischemic myocardium by attenuating P-selectin expression and leukocyte adhesion in normocholesterolemic and diabetic animals.\textsuperscript{93–97} These cholesterol-independent effects of statins were absent in eNOS-deficient or N\textsuperscript{G}-nitro-L-arginine methyl ester–treated mice, suggesting that eNOS mediated the vascular protective effects of statins.

A clinical marker of inflammation is high-sensitivity C-reactive protein (hs-CRP).\textsuperscript{98} 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.\textsuperscript{99} Elevated levels of hs-CRP have been shown to be predictive of increased risk of coronary artery disease in apparently healthy men and women.\textsuperscript{36,100–103} hs-CRP is elevated in patients with coronary artery disease, coronary ischemia, and myocardial infarction compared with normal subjects.\textsuperscript{43,104,105} Statin therapy lowers hs-CRP levels in hypercholesterolemic patients.\textsuperscript{98,106,107} In the CARE trial, statins significantly decreased plasma hs-CRP levels over a 5-year period in patients who did not experience recurrent coronary events.\textsuperscript{108,109} Similarly, an analysis of baseline and 1-year follow-up from the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) demonstrated that hs-CRP levels were reduced in statin-treated patients who were free of acute major coronary events.\textsuperscript{98} Therefore, these studies 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 the benefits of the lipid-lowering effects of statins without performing further clinical studies.

\textbf{Statins and Ischemic Stroke}

An intriguing result of large clinical trials with statins is the reduction in ischemic stroke.\textsuperscript{110} Although myocardial infarction is closely associated with serum cholesterol levels, neither the Framingham Heart Study nor the Multiple Risk Factor Intervention Trial (MRFIT) demonstrated significant correlation between ischemic stroke and serum cholesterol levels.\textsuperscript{5,111} Thus, the findings of these large statin trials raise the interesting question of how a class of cholesterol-lowering agents can reduce ischemic stroke when ischemic stroke is not related to cholesterol levels. It appears likely that there are pleiotropic effects of statins that are beneficial in ischemic stroke. Some of these beneficial effects of statins in ischemic stroke may be due, in part, to their ability to upregulate eNOS expression and activity.\textsuperscript{24,46} For example, mice that were prophylactically treated with statins for up to 2 weeks had 25% to 30% higher cerebral blood flow and 50% smaller cerebral infarct sizes after cerebrovascular occlusion.\textsuperscript{26,112} No increase in cerebral blood flow or neuroprotection was observed in eNOS-deficient mice treated with statins, indicating that the upregulation of eNOS accounts for most, if not all, of the neuroprotective effects of these agents. Interestingly, treatment with statins did not affect blood pressure or heart rate before, during, or after cerebrovascular ischemia and did not alter serum cholesterol levels in mice,
consistent with the cholesterol-independent neuroprotective effects of statins.

In addition to increases in cerebral blood flow, other beneficial effects of statins that have an impact on the severity of ischemic stroke are likely to occur. For example, statins attenuate P-selectin expression and leukocyte adhesion via increases in NO production in a model of cardiac ischemia and reperfusion.93,96 Others have reported that statins upregulate tissue-type plasminogen activator and downregulate plasminogen activator inhibitor-1 expression through a similar mechanism involving the inhibition of Rho geranylgeranylation.49 Thus, the absence of neuroprotection in eNOS-deficient mice emphasizes the importance of endothelium-derived NO not only in augmenting cerebral blood flow but also, potentially, in limiting the impact of platelet and white blood cell accumulation on tissue viability after ischemia. It is possible that statins may have contributed to the decrease in the incidence of ischemic strokes in clinical trials, in part, by reducing cerebral infarcts size to levels that are clinically unappreciated.

Summary
Statin exert many pleiotropic effects on the vascular wall. These include beneficial effects on endothelial function and blood flow, decreasing LDL oxidation, enhancing the stability of atherosclerotic plaques, inhibiting vascular SMC proliferation and platelet aggregation, and reducing vascular inflammation (Figure 3). Recent evidence suggests that most of these effects are mediated by the inhibitory effect of statins on isoprenoid synthesis. In particular, inhibition of Rho GTPases in vascular wall cells by statins leads to increased expression of atheroprotective genes and inhibition of vascular SMC proliferation. Although the list of cellular effects of statins on the vascular wall continues to grow, it remains to be determined which, if any, of these effects accounts for the clinical benefits of statin therapy in cardiovascular disease.

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