Effects of Statins on Endothelium and Signaling Mechanisms

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Abstract—Endothelium dysfunction may result from increased production of reactive oxygen species and decreased availability of nitric oxide. Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (ie, statins) exert cholesterol-independent vasoprotective effects that are mediated, in part, through the inhibition of small G-proteins Rho and Rac. Rho negatively regulates endothelial nitric oxide synthase and Rac contributes to NAD(P)H-oxidase activation and superoxide production. Statins inhibit both Rho and Rac GTPase activity via inhibition of geranylgeranylation, which confers endothelial nitric oxide synthase upregulation and decreases superoxide production, respectively. Sudden discontinuation of statin therapy may have negative effects. Withdrawal of statin treatment leads to an overshoot activation of Rho and Rac with dramatic effects on nitric oxide bioavailability, NAD(P)H-oxidase activity, and superoxide production. (Stroke. 2004;35[suppl I]:2708-2711.)

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Endothelial dysfunction may result from increased production of reactive oxygen species (ROS) and decreased synthesis, release, or activity of endothelial-derived nitric oxide (NO). Lack of NO contributes to impaired endothelium-dependent vasorelaxation, platelet aggregation, enhanced leukocytes adhesion to the endothelium, and increased blood pressure. Increased production of superoxide anions, on the other hand, can lead to further decreases in ambient levels of NO.

3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are potent cholesterol-lowering drugs and reduce the risk of myocardial infarction and stroke. The latter is somewhat unexpected because cholesterol is not an established risk factor for ischemic stroke. Indeed, increasing evidence suggests that protection conferred by statins relates not only to cholesterol-lowering but rather to direct effects on endothelium function as well as antithrombotic and antiinflammatory effects. In humans, improvement of endothelium function is one of the earliest clinical effects after initiation of statin treatment.

Inhibition of HMG-CoA reductase not only reduces cholesterol levels but also prevents the formation of isoprenoid intermediates with important biological functions. In particular, farnesylpyrophosphate and geranylgeranylpyrophosphate serve as important lipid attachments for the posttranslational modification of a variety of proteins, including heterotrimeric G-proteins and small GTP binding proteins. Isoprenylation converts small GTPases from a cytosolic (inactive) state to a membrane-bound (active) state.

Here, we review evidence that statins (1) inhibit NAD(P)H oxidase and superoxide production and (2) upregulate the expression and activity of endothelial nitric oxide synthase (eNOS) via inhibition of geranylgeranylation of RhoA and Rac1 GTPases (Figure 1).

Antioxidative Effects of Statins via Inhibition of Rac1 and NAD(P)H-Oxidase

There are several sources of reactive oxygen species (ROS) within vascular cells, including xanthine oxidase, NOS, and membrane-bound NAD(P)H-oxidase. Statins inhibit angiotensin II-induced ROS production via 2 important mechanisms. First, statins downregulate AT-1 receptor gene expression mediated by destabilization of AT-1 messenger ribonucleic acid (mRNA). Second, statins inhibit the activation of Rac1 which is critically involved in the activation of NAD(P)H-oxidase by preventing the geranylgeranyl-dependent translocation of Rac1 from the cytosol to the cell membrane. In fact, statins reduce vascular ROS production also in spontaneously hypertensive rats in vivo.

Statins Upregulate eNOS via Inhibition of RhoA and the Actin Cytoskeleton

A well-characterized pleiotropic effect of statins is the upregulation and activation of eNOS. First, statins activate protein kinase Akt, which is an important regulator of a number of cellular processes including metabolism and apoptosis. Activation of phosphatidylinositol 3-kinase provokes the phosphorylation and activation of Akt, and phosphatidylinositol 3-kinase inhibitors block the effects of statins on
Akt. Akt activation by statins then inhibits apoptosis and acutely increases NO production.

In addition, statins upregulate eNOS via inhibition of geranylgeranylation of the small G-protein Rho. Translocation of inactive Rho from the cytosol to the membrane depends on geranylgeranylation (Figure 2). Activated Rho binds and activates Rho-associated kinases, which in turn leads to phosphorylation of myosin light chains required for the formation of actin stress fibers and focal adhesion complexes. Anchoring of mRNAs to the actin cytoskeleton is necessary for their stability and translational expression. Consequently, Rho-mediated reorganization of the actin cytoskeleton may regulate the trafficking and subcellular localization of specific mRNAs. Together, disruption of Rho-mediated endothelial actin cytoskeleton leads to eNOS upregulation via prolongation of eNOS mRNA half-life (Figure 2).

In line with this evidence, eNOS can be upregulated both in vitro and in vivo by (1) inhibition of geranylgeranylation through statins, (2) direct pharmacologic inhibitors of Rho such as the Clostridium botulinum C3 transferase, or (3) the actin cytoskeleton disrupter cytochalasin D. In mouse models of cerebral ischemia, chronic treatment with statins elevates cerebral blood flow by eNOS upregulation and NO generation. Accordingly, treatment with statins, C3 transferase, or cytochalasin D results in smaller cerebral infarctions following MCA occlusion. Interestingly, no neuroprotection was observed with these agents in eNOS knockout mice. Together, these results demonstrate that eNOS gene expression is regulated by changes in the endothelial actin cytoskeleton, which may account for some of the noncholesterol effects of statins.

**Statin Withdrawal**

It is well-known that withdrawal of some cardiovascular drugs, in particular β-blockers and nitrates, can exert pronounced rebound symptoms, requiring a “stealing out” of the therapy. Discontinuation of statin treatment may have negative effects on the vasculature. In cultured endothelial cells, statins block Rho membrane translocation and GTPase binding activity. Rho expression however is regulated by a negative feedback mechanism mediated by the actin cytoskeleton. In fact, statins dramatically increase Rho gene transcription, as do direct disrupters of actin filaments such as cytochalasin D. Therefore chronic statin treatment leads to the accumulation of nonisoprenylated Rho in the cytosol. Abrupt withdrawal of statin treatment then restores the availability of isoprenoids and results in an “overshoot” translocation and activation of Rho, causing downregulation of endothelial NO production below baseline levels (see Figure 3).

These findings can be transferred to an in vivo scenario: Mice were treated for 14 days with atorvastatin, which expectedly conferred the upregulation of eNOS expression and activity. Withdrawal of statins however resulted in a dramatic 90% decrease of eNOS expression and NO production after 2 days. Accordingly, statin treatment decreased RhoA membrane expression by ~50% while statin withdrawal resulted in a 4-fold increase of RhoA in the membrane. In addition, withdrawal of statin treatment induced a dramatic increase in platelet activation markers, and platelet hyperactivity was also demonstrated after discontinuation of statin treatment in man. Indeed, statin withdrawal has an impact on outcome in animal models of vascular injury: stroke protection after chronic statin treatment was rapidly and completely abrogated after acute withdrawal of treatment. While 14 days of atorvastatin treatment reduced lesion size following focal cerebral ischemia by as much as 40%, protection was lost only 2 days after withdrawal of treatment and lesion size reached control levels 4 days after treatment was stopped. Of note, atorvastatin has a plasma half-life of ~14 hours (and even longer for active metabolites) which is
oxidase may contribute to the vascular effects of statin.

Rac is anchored in the membrane and activates NAD(P)H oxidase after statin withdrawal. After statin withdrawal, increased superoxide production could lead to increased vascular superoxide anion generation. However, in wild-type mice, withdrawal of atorvastatin attenuated NO bioavailability and significantly improved, but withdrawal of treatment, however, did not attenuate endothelial superoxide anion formation.

These results are supported by independent findings by Vecchione and Brandes. Mice were treated with cerivastatin, atorvastatin, or placebo. Vascular reactivity was examined in isolated aortic rings after 10 days of treatment and after cessation of therapy. Indeed, statin treatment significantly improved, but withdrawal of treatment, however, attenuated acetylcholine-induced endothelium-dependent vascular reactivity. This was attributed to an increase of NO scavenging by superoxide radicals. In wild-type mice, withdrawal of atorvastatin attenuated NO bioavailability and increased vascular superoxide anion generation. However, in gp91phox knockout mice, which do not express functional NAD(P)H-oxidase, statin withdrawal was not associated with attenuation of NO production or increased vascular superoxide anion generation. Together, NAD(P)H-oxidase plays a central role in mediating the statin withdrawal phenomenon. Moreover, statin withdrawal was associated with Rac translocation to the membrane and increased superoxide production could be blocked with a Rac inhibitor. After statin withdrawal, geranylgeranylation promotes Rac activation, induces Rac relocalization in the membrane and activates NAD(P)H oxidase. Hence, Rac-dependent activation of NAD(P)H oxidase may contribute to the vascular effects of statin withdrawal.

Clinical Evidence

There is also clinical evidence that withdrawal of statin medication acutely impairs vascular function and negatively affects outcome. One study in patients with stable coronary heart disease showed a more than 3-fold increase in thrombotic vascular events after simvastatin treatment was stopped and continued with relatively lower doses of fluvastatin. A subgroup analysis of the Platelet Receptor Inhibition in Ischemic Syndrome Management (PRISM) Study demonstrated that statin pretreatment in patients with acute coronary syndromes was associated with improved clinical outcome, but discontinuation of statins after onset of symptoms completely abrogates this beneficial effect. It should be noted, however, that some patients were excluded from the analysis and, after re-evaluation of the data, the effects trended in the same direction but were more modest. Differences were apparent during the initial 72 hours despite a lack of change in the cholesterol levels, indicating a cholesterol-independent mechanism. Indeed, in another study 80-mg atorvastatin improved endothelium-dependent function in healthy, normocholesterolemic people within 24 hour and this effect sustained for one month. Withdrawal of treatment, however, acutely impaired vascular function independent of cholesterol levels and the inflammation state. In a retrospective analysis, Spencer and colleagues demonstrated that early withdrawal of statin therapy in patients with acute coronary syndromes contributes to hospital morbidity and mortality.

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References


