Neuroprotective Properties of Statins in Cerebral Ischemia and Stroke

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**Background**—The atheroma-retarding properties of \( \beta \)-hydroxy-\( \beta \)-methylglutaryl coenzyme A reductase (HMG-CoA) inhibitors, or “statins,” in both the coronary and carotid arterial beds are well established. However, a growing body of recent data suggests that statins possess important adjunctive properties that may confer additional benefit beyond the retardation of atherosclerosis. In this article, we review the emerging evidence that statins have beneficial effects within the cerebral circulation and brain parenchyma during ischemic stroke and reperfusion.

**Summary of Review**—Clinical studies show that statins reduce the incidence of ischemic stroke through probable effects on precerebral atherosclerotic plaque and through antithrombotic mechanisms. Additionally, statins have been shown to reduce infarct size in experimental animal models of stroke. Statins both upregulate endothelial nitric oxide synthase (eNOS) and inhibit inducible nitric oxide synthase (iNOS), effects that are potentially neuroprotective. The preservation of eNOS activity in cerebral vasculature, particularly in the ischemic penumbra, may be especially important in preserving blood flow and limiting neurological loss. Statins may also attenuate the inflammatory cytokine responses that accompany cerebral ischemia, and they possess antioxidant properties that likely ameliorate ischemic oxidative stress in the brain.

**Conclusions**—In addition to reducing stroke, the statin class of drugs exhibits a number of important neuroprotective properties that likely attenuate the effects of ischemia on the brain vasculature and parenchyma. Further investigation of the role of statins in human neuroprotection by use of neuroimaging and cognitive studies is warranted to explore these preliminary observations. In addition to reducing ischemic stroke, early evidence indicates that statins may also be neuroprotective. *(Stroke. 1999;30:1969-1973.)*

**Key Words:** endothelium ■ HMG-CoA reductase inhibitors ■ inflammation ■ nitric oxide ■ neuroprotection

Recent clinical trials and meta-analyses of \( \beta \)-hydroxy-\( \beta \)-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) have demonstrated a significant reduction in ischemic stroke in patients with a history of coronary artery disease, both with and without elevations of serum cholesterol. Statins have been shown to attenuate the development of atherosclerosis in both the coronary and carotid arterial beds (“downstream effects”). Recent data suggest that statins have other beneficial properties in addition to the retardation of atherosclerosis. In this article, we review the emerging evidence that statins have additional beneficial “upstream effects” in cerebrovascular disease. We review anti-inflammatory, antioxidant, and endothelial protective effects of statins and discuss the putative neuroprotective properties of these compounds in cerebral ischemic syndromes.

**Downstream Effects**

Despite the established role of cholesterol in the pathogenesis of coronary artery disease, current epidemiological evidence does not demonstrate a clear relationship between the risk of stroke and serum cholesterol level.\(^1\)\(^,\)\(^2\) However, recent studies indicate that statins significantly reduce ischemic stroke. In the CARE study,\(^3\) pravastatin significantly reduced the specified end point of stroke by 31%, without increased hemorrhagic stroke. Post hoc analysis of the 4S trial\(^4\) showed a similar significant reduction in stroke. This clinical benefit seen in secondary prevention trials is corroborated by 2 meta-analyses\(^5\)\(^,\)\(^6\) that demonstrate that statin therapy lowers stroke risk by \(\approx 30\%\). The clinical benefit of statins is also supported by the observation that statin treatment reduces progression of carotid intima-media thickening.\(^7\) The majority of nonlacunar ischemic strokes are caused by thromboemboli arising from atheromatous disease outside the brain, such as the carotid artery or the aortic arch, vascular sites in which hypercholesterolemia is an important risk factor for the development of atherosclerosis. The downstream benefit of statins is therefore likely due to the stabilization of atherosclerosis at these sites, in addition to favorable hemorheological and antithrombotic properties of statins, which decrease plaque disruption and reduce artery-to-artery thromboembolism.\(^8\)\(^,\)\(^9\)
Upstream Effects

In addition to the above effects in the precerebral macrovasculature, emerging evidence indicates that statins may have important upstream effects that ameliorate a number of pathophysiological processes that occur within the cerebral vasculature and brain parenchyma during cerebral ischemia and reperfusion. The data suggest that statins can ameliorate ischemic damage by improving blood flow to the ischemic brain and by making the brain parenchyma intrinsically more resistant to the effects of ischemia. The clinical importance of protecting cerebral microvascular integrity is highlighted by recent observations indicating that silent strokes are much more prevalent than previously suspected. This has been elegantly demonstrated with MRI in the Cardiovascular Health Study, in which the incidence of infarct-like lesions (ILLs) in subjects aged >65 years was 31%. In this study, the presence of ILLs correlated with both cognitive decline and motor deficit. Although the impact of statins on ILLs has not been studied to date, it is possible that statin therapy may become an important means of reducing silent stroke and preventing vascular neurological decline. This effect may be accentuated by concomitant antiplatelet therapy, although this has not been investigated with prospective neuroimaging studies.

Endothelium

The different isoforms of nitric oxide synthase (NOS) play important but opposing roles in cerebral ischemia. The inducible form of NOS (iNOS) has been implicated as an important mediator of inflammatory responses during ischemia and reperfusion. Astrocytes elaborate iNOS in response to a series of proinflammatory mediators, including cytokines such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6). Expression of iNOS has been demonstrated in neutrophils infiltrating the ischemic brain and in blood vessels within the ischemic territory in human ischemic stroke. Nitric oxide (NO) derived from iNOS in both astrocytes and macrophages and its oxidative by-product peroxynitrite are thought to contribute to neuronal death due to oxidation of structural neuronal proteins during ischemia (Figure 1). Additionally, neuronal NO (produced by neuronal NOS) may contribute to neurological damage by promoting glutamate-mediated neurotoxicity. In contrast, NO produced by endothelial NOS (eNOS) has a protective physiological role and orchestrates the paracrine homeostatic functions of the endothelium, which include inhibition of leukocyte and platelet adhesion, control of vascular tone, and maintenance of a thromboresistant interface between the bloodstream and the vessel wall (Figure 1). Consistent with the concept that eNOS plays a protective role in focal cerebral
ischemia is the observation that eNOS knockout animals experience larger infarcts after middle cerebral artery occlusion.\textsuperscript{14} In contrast, mice lacking the gene for iNOS have significantly reduced infarct volumes compared with wild-type controls.\textsuperscript{15} Together, these fascinating observations suggest a relative compartmentalization of NOS isoform activity in the brain, with contrasting roles for eNOS and iNOS in the setting of ischemia. Preliminary studies with statins have demonstrated that these compounds may be able to modulate brain NOS isoform activity in a neuroprotective manner.

Statin therapy favorably modifies endothelial control of vasomotor function in both the coronary and forearm circulations.\textsuperscript{16,17} Similarly, statin therapy may be beneficial during cerebral ischemia through the modulation of brain eNOS. Recent experimental data from a murine model of ischemic stroke demonstrate that prophylactic statin therapy augments cerebral blood flow, reduces infarct size by $\sim30\%$, and improves neurological outcome in normocholesterolemic animals.\textsuperscript{18} In this intriguing investigation, statin therapy directly upregulated eNOS activity in the brain without altering expression of nNOS. These effects occurred independently of change in cholesterol level and were reversible by cotreatment with mevalonate or geranylgeranyl pyrophosphate. This suggests that intermediates in cholesterol biosynthesis independently modulate eNOS. Although untested in humans, this observation suggests that statins may protect the cerebral endothelium and attenuate ischemic burden.

Astrocytes exhibit both constitutive NOS and iNOS activity under various conditions, and activated microglia also express iNOS.\textsuperscript{19} The induction of iNOS in glial cells may occur in response to ischemia or proinflammatory signals. Excessive glial cell–derived production of NO can be toxic to neurons in the surrounding brain, thus contributing to further neuronal loss. Recent observations suggest that statin therapy modulates the activity of iNOS. Lovastatin has been shown to inhibit cytokine-mediated upregulation of iNOS and production of NO in rat astrocytes and macrophages.\textsuperscript{20} Given the putative deleterious effects of this NOS isoform in the central nervous system, its inhibition by statins may suppress inflammatory responses that accompany acute ischemia. Moreover, in aggregate, these observations suggest a dual role for statins in cerebral ischemia, whereby they may simultaneously upregulate eNOS and inhibit iNOS in a synergistically neuroprotective manner.

### Inflammation

In addition to biochemically remodeling the endothelium, HMG-CoA reductase inhibitors have been shown to inhibit a number of inflammatory processes known to be important during cerebral ischemia and reperfusion. Upregulation of adhesion molecule expression has been documented in animal and human cerebral ischemia and reperfusion.\textsuperscript{21} It has been postulated that the enhanced expression of adhesion molecules on both endothelium and glial cells within the infarct and in the surrounding ischemic penumbra facilitates postischemic migration of leukocytes through the brain parenchyma. Statin therapy has been shown to reduce enhanced leukocyte-endothelium interactions in hypercholesterolemic animals\textsuperscript{22} and to inhibit neutrophil adhesion to coronary endothelium.\textsuperscript{23} In humans, both simvastatin and lovastatin reduce monocyte CD11b expression and ex vivo CD11b-dependent monocyte adhesion to endothelium in subjects with hypercholesterolemia.\textsuperscript{24} It has been speculated that this effect may be mediated through reduced isoprenylation of leukocyte G-proteins\textsuperscript{23} or reduced isoprenoid-dependent anchoring or dimerization of adhesion molecules such as CD11b/CD18 on monocytes (Figure 2). Because statin therapy has been shown to reduce monocyte adhesion molecule expression, and anti-CD11b/CD18 monoclonal antibodies have been shown to reduce ischemic cell damage after transient middle cerebral artery occlusion,\textsuperscript{25} statin therapy may also reduce neurological injury through effects on adhesion molecule expression or behavior.

In addition to these potential salutary effects on adhesion molecules, statin therapy may modulate central nervous system cytokine production. Cytokines are prominent mediators of inflammatory and immunologic responses in the brain and are produced by neurons, glial cells, and endothelium (Figure 1). Although the precise role of different cytokines in cerebral ischemic syndromes remains to be elucidated, cytokines appear to modulate adhesion molecule expression on cerebral endothelium and inflammatory cells, promote cell migration, enhance thrombogenesis through tissue factor expression, and augment elaboration of platelet activating factor.\textsuperscript{26} IL-1β, a proinflammatory cytokine, is overexpressed in the brains of experimental animals after stroke and appears to contribute to neuronal damage, perhaps through induction of neuronal apoptosis.\textsuperscript{28} Although it has been suggested that TNF-α is neuroprotective,\textsuperscript{29,30} TNF-α and IL-6 are elevated in experimental models of cerebral ischemia and may contribute to neuronal loss.\textsuperscript{21} TNF-α not

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**Figure 2.** Statins and isoprenoids. Isoprenoids are derivatives of intermediates in cholesterol biosynthesis and have a number of actions, including effects on G-proteins, adhesion molecules, and cell proliferation. Some of the anti-inflammatory effects of statins may be mediated by reduced isoprenoid bioavailability. IPP indicates isopentyl pyrophosphate; DPP, 3,3-dimethylallyl pyrophosphate; GPP, geranyl pyrophosphate; and FPP, farnesyl pyrophosphate.
only upregulates adhesion molecule expression by glial and endothelial cells but also alters the blood-brain barrier and mediates a prothrombotic transformation of the cerebral endothelium.\textsuperscript{21} Although the precise role of different cytokines in cerebral ischemia needs further clarification, the importance of cytokines in ischemia is highlighted by experimental studies demonstrating a reduction in cerebral infarct size in animals treated with cytokine receptor antagonists.\textsuperscript{31} Thus, statin therapy may represent a novel means of suppressing cytokine responses that occur during ischemia and reperfusion by directly reducing the in vivo induction of inflammatory mediators such as iNOS, IL-1β, and TNF-α in astrocytes and macrophages. The demonstration that these effects of statins are reversible with coadministration of mevalonate or farnesyl pyrophosphate suggests that statins may be anti-inflammatory because they decrease isoprenylation (and hence activity) of proteins involved in intracellular signaling and inflammation\textsuperscript{20} (Figure 2). In summary, these preliminary observations support the concept that statins represent a novel means of attenuating inflammatory neuronal loss occurring during cerebral ischemia.

**Antioxidant Effects**

Finally, HMG-CoA reductase inhibitors may be neuroprotective through potential antioxidant effects. Oxidative injury appears to be a fundamental mechanism of many neurological disorders, including cerebrovascular disease.\textsuperscript{12,32} Chronic oxidant injury may play a pathophysiological role in precerebral atherogenesis, and the enhanced liberation of free radical species after acute stroke and during both spontaneous and therapeutic reperfusion may accentuate tissue injury in the ischemic penumbra. The generation of free radicals causes neuronal and endothelial damage through the induction of lipid peroxidation, protein oxidation, and direct damage to nucleic acids (Figure 1). The elaboration of reactive oxygen species has been reported to induce apoptosis of endothelial cells through activation of CPP32-like proteases.\textsuperscript{34} Moreover, during ischemia and reperfusion, the protective endogenous antioxidant systems (such as the enzymes superoxide dismutase and catalase) may be overwhelmed.

Several studies indicate that therapy with statins may reduce lipoprotein oxidation and ameliorate free radical injury. As well as having favorable antioxidant effects as measured by several ex vivo systems, such as increased lag time of copper-induced LDL oxidation\textsuperscript{35} and reduced leukocyte-induced LDL oxidation,\textsuperscript{36} statins may have broader antioxidant effects. Hydroxy metabolites of atorvastatin have been shown in an in vitro model to inhibit oxidation in a concentration-dependent manner,\textsuperscript{37} and in a study of hypercholesterolemic patients, treatment with simvastatin increased the α-tocopherol/total cholesterol ratio,\textsuperscript{38} thus possibly boosting membrane-specific antioxidant defenses. Most studies have explored the antioxidant properties of statins in relation to LDL; however, statins may exert broader antioxidant effects through preservation of superoxide dismutase activity.\textsuperscript{36}

In addition to antioxidant properties, it has been shown that statins may reduce the biosynthesis of the endogenous lipophilic mitochondrial antioxidant coenzyme Q\textsubscript{10}, or ubiquinone.\textsuperscript{39} Although this effect could negate any potential free radical–scavenging actions of statins, the combined exogenous administration of a statin with coenzyme Q\textsubscript{10} could exert potent synergistic neuroprotective and antioxidant effects, because coenzyme Q\textsubscript{10} itself appears to have important neuroprotective effects.\textsuperscript{40} To the best of our knowledge, this approach has yet to be tested either in animal models or in humans.

**Future Directions**

In addition to reducing ischemic stroke, there is an emerging body of evidence indicating that statins are also neuroprotective. Statins reduce the incidence of ischemic stroke through downstream effects by pacifying precerebral atherosclerotic plaque and through their antithrombotic actions. Statins have a number of additional upstream effects within the cerebral vasculature and brain parenchyma that are potentially neuroprotective in the setting of cerebral ischemia and reperfusion. These emerging neuroprotective properties of statins may confer significant additional clinical benefit (Figure 3). There is also growing evidence indicating that some of these effects are cholesterol independent and are mediated by interruption of isoprenoid biosynthesis. Therapy with HMG-CoA reductase inhibitors may remodel endothelium in a manner that may become clinically important in the face of a proximate ischemic insult. In particular, the preservation of eNOS activity in cerebral vasculature, and especially in the ischemic penumbra, may limit neurological deficit. Moreover, putative anti-inflammatory and antioxidant properties of statins may confer additional neuroprotection.

Given the already widespread indications for statin usage, it is interesting to speculate that these drugs possess additional important neuroprotective properties within the central nervous system. Further investigation with a number of modalities, including neuroimaging studies and cognitive studies, are warranted to explore these preliminary observations. If these potential cholesterol-independent neuroprotective effects of statins are proven to be clinically important in human neuroprotection, this class of drugs will find wide-ranging utility in the management of a variety of cerebrovascular disease entities in patients with and without hypercholesterolemia.
References


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