Statins: Multiple Mechanisms of Action in the Ischemic Brain

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Statins: Multiple Mechanisms of Action in the Ischemic Brain

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Although substantial epidemiological studies have failed to find a correlation between cholesterol levels and stroke, clinical trials have shown that HMG-CoA reductase inhibitors (or statins, the most potent hypocholesterolemic drugs available) greatly reduce the incidence of stroke. These clinical observations have opened the way to a number of studies of the non–cholesterol-dependent (or pleiotropic) effects in animal models of stroke, indicating that the neuroprotection is attributable to multiple activities. One of the main protective mechanisms elicited by statin administration is the increase in nitric oxide bioavailability that regulates cerebral perfusion and improves endothelial function, but others include antioxidant properties, the inhibition of inflammatory responses, immunomodulatory actions, the regulation of progenitor cells, and the stabilization of atherosclerotic plaques. Many of these effects are due to the inhibited synthesis of isoprenoid intermediates, which serve as lipid attachments for a variety of intracellular signaling molecules. This article describes the mechanisms involved in the neuroprotective effects of statins. NEUROSCIENTIST 13(3):208–213, 2007.
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Stroke is the third most common cause of death in Western countries and a leading cause of permanent disability, but despite the intensive basic and clinical research carried out over the past few decades, many clinical trials of the most promising therapeutic tools have only led to failure, and cerebral ischemia remains an unmet medical need awaiting new and selective drugs capable of counteracting the ischemia-induced progression of neurodegeneration.

The development of animal models of brain ischemia has recently led to an exponential increase in the number of preclinical studies aimed at elucidating the molecular mechanisms underlying the pathophysiology of stroke and the efficacy of putative neuroprotective drugs (Lo and others 2003; Turley and others 2005).

Pathophysiology of Ischemic Brain Injury

It has been demonstrated that both transient and permanent blood vessel occlusion can induce an energy failure in the neuronal cells present in the territory irrorated by the occluded vessel. This process is characterized by a marked depletion in the phosphate reserves used by cells to maintain ion homeostasis, and the severe energy depletion leads to the collapse of transcellular ion pumping, with sustained neuronal and glial depolarization, and release of glutamate into the extracellular space. Depolarization induces the influx of sodium and the efflux of potassium, as well as passive chloride entry that leads to intracellular water accumulation, severe swelling, and cell death. Another key process responsible for neurodegeneration in ischemia is the massive stimulation of glutamate receptor subtypes, including *N*-methyl-D-aspartate (NMDA), AMPA, and metabotropic receptors. Excessive stimulation of these receptors by excitatory amino acids massively increases intracellular calcium concentration, which plays a pivotal role in the neurodegenerative process because it is responsible for the calcium-dependent overactivation of degradative enzymes such as endonucleases, proteases, and phospholipases (Dirnagl and others 1999; Mergenthaler and others 2004).

The increased formation of reactive oxygen species generated during reperfusion or ischemia-induced altered metabolic processes also contributes to the onset and progression of ischemic brain damage. In particular, increased phospholipase A2 and cyclooxygenase activities generate free-radical species that are responsible for membrane lipid peroxidation, the disruption of structural cell components, and ultimate cell death (Orrenius and others 2003).

The sustained increase in intracellular calcium levels in neurons due to the activation of a specific isoform of nitric oxide synthase generates toxic levels of nitric oxide (NO), which give rise to the production of free radicals that bind the cytochromes of the electronic transport chain in mitochondria and block the oxidative phosphorylation process. Under these and other pathological conditions, NO can also react with the superoxide anion to form peroxynitrite, a highly reactive species that promotes tissue damage. A further source of large amounts of NO comes from the delayed activation by

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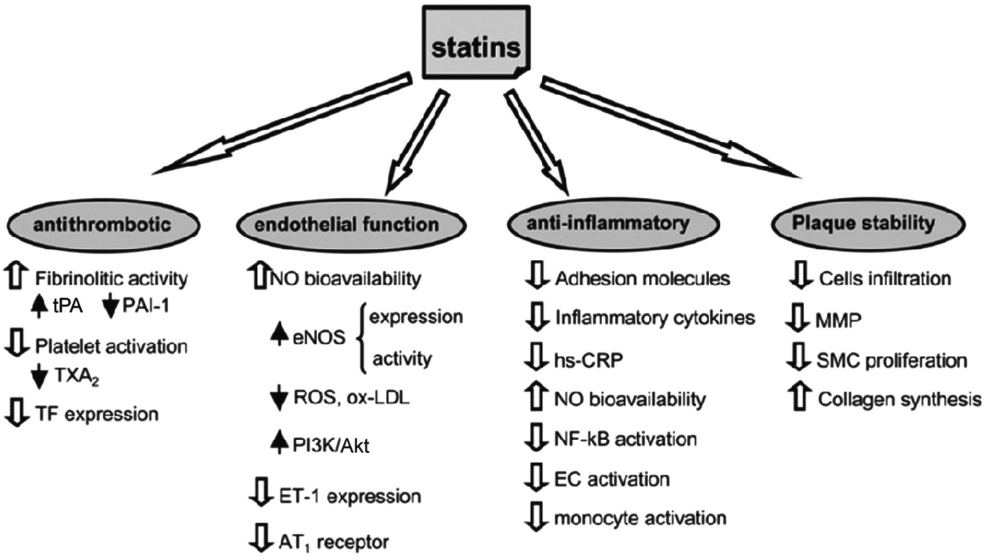

Fig. 1. Pleiotropic effects of statins on the vasculature. TXA_2, thromboxane A_2; PAI-1, plasminogen activator inhibitor–1; tPA, tissue plasminogen activator; TF, tissue factor; NO, nitric oxide; ROS, reactive oxygen species; LDL, low-density lipoprotein; PI3K, phosphatidylinositol-3 kinase; ET-1, endothelin–1; MMP, metalloproteinase; SMC, smooth muscle cell; CRP, C-reactive protein.

inflammatory mediators of the inducible isoform of nitric oxide synthase (NOS) in the macrophages that reach the region of the infarct from the circulation (Lo and others 2003; Moro and others 2004).

Inflammation is another detrimental factor in the evolution of ischemic brain injury. A number of studies have demonstrated an increase in inflammation-dependent biochemical and cellular events following ischemia. One of the early events occurring in response to an ischemic insult is the increased synthesis by neurons and glia of proinflammatory cytokines, including interleukin-1β (IL-1β), tumor necrosis factor–α (TNF-α), IL-6, and IL-18. These molecules stimulate the expression and synthesis of other important proteins—such as transcription factor NF-κB and endothelial adhesion molecules, intercellular adhesion molecule–1 (ICAM-1), E-selectins, and P-selectins—that are directly involved in the ischemia-induced inflammatory process: they trigger the expression of specific genes and allow neutrophils and monocytes to cross the vascular wall and migrate to the injured tissue, where they exert their pathogenic action (Iadecola and Alexander 2001; Danton and Dietrich 2003).

Apoptosis is a very active process of cell death triggered by an ischemic insult that deserves particular attention because apoptotic cells are abundant in the penumbra, the less severely damaged brain region that can be rescued by selective pharmacological treatments. The major players in the apoptotic program are a family of cysteine protease enzymes known as caspases. Activated caspases initiate the apoptopic pathway by cleaving key components of the cellular infrastructure and activating the factors responsible for cell damage. A large body of evidence demonstrates that apoptosis plays a critical role in ischemic brain injury because the activity of these enzymes (particularly caspase-3) is increased after an ischemic insult, and the inhibition of this activated pathway has a protective effect on the progression of cerebral damage and on functional outcome (Zhang and others 2004; Chan 2004).

Statins as Neuroprotective Agents in Cerebral Ischemia

Statins, the most widely used lipid-lowering drugs, share a common mechanism of action as they all are competitive inhibitors of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme of the mevalonate pathway for cholesterol biosynthesis. It has been demonstrated that a statin-induced reduction in total serum cholesterol levels is beneficial in patients with cardiovascular diseases, and they also can reduce the occurrence of cardiovascular events regardless of serum cholesterol levels. Furthermore, over the past 10 years, large-scale clinical trials have shown that statins reduce the incidence of cerebrovascular events, even though cholesterol is not an established risk factor for stroke (Endres 2005). It has since been demonstrated that statins have a number of cholesterol-independent (or pleiotropic) effects that often occur before they induce a significant reduction in serum cholesterol levels (Fig. 1). These non–lipid-dependent effects include improving endothelial function, inhibiting inflammatory responses, antioxidant activities, increasing endothelial NO levels, immunomodulatory actions, stabilizing atherosclerotic plaque, and reducing cell apoptosis, and they can be explained by the fact that, in addition to inhibiting cholesterol synthesis, statins also decrease the production of isoprenoid intermediates such as farnesylpyrophosphate

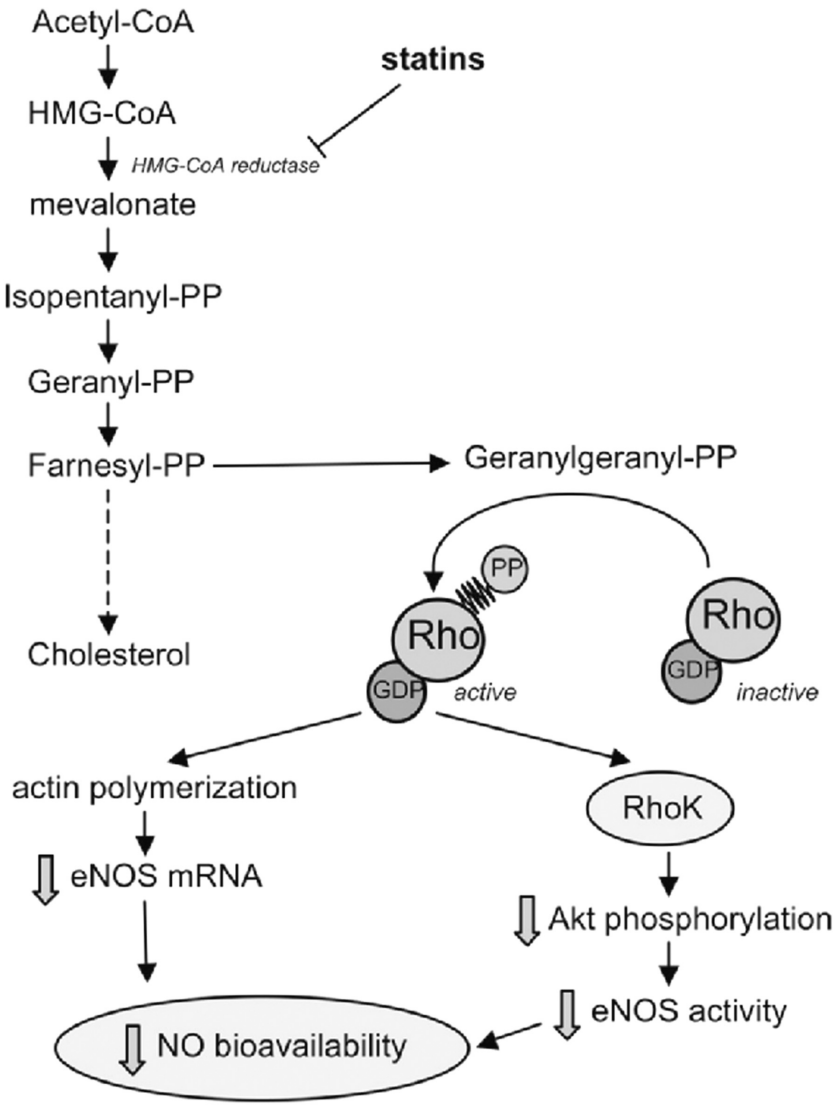

Fig. 2. Statins inhibit the conversion of hydroxymethylglutaryl coenzyme A (HMG-CoA) to mevalonate, thus preventing the synthesis of geranylgeranyl-PP, which is involved in the activation of Rho. NO, nitric oxide.

(FPP) and geranylgeranylpyrophosphate (GGPP) (Fig. 2). Both FPP and GGPP induce posttranslational modifications of a variety of cytosolic proteins, including the small GTP-binding proteins such as Ras, Rab, Rap, and Rho GTPases, thus facilitating their cell membrane anchorage and enhancing their activity (Liao and Laufs 2005).

Targeting Ischemic Injury with Statins: Mechanisms of Action

On the basis of clinical evidence indicating that long-term statin treatment reduces the incidence of stroke, experimental studies using animal models of cerebral ischemia were carried out to prove the protective effect of the different statins and reveal the molecular mechanisms underlying their benefits on stroke outcome. It has been demonstrated in different animal models that statin pre- and posttreatment increases absolute cerebral blood flow, reduces lesion volume by slowing or inhibiting the progression of tissue damage, and improves behavioral performance and neurological function, thus indicating that statin treatment can ameliorate the functional consequences of stroke (Endres and others 1998; Balduini and others 2003; Sironi and others 2003).

Investigations into the mechanism(s) involved in the beneficial effects of HMG-CoA reductase inhibitors on cerebral ischemia started from the observation that statin administration increases eNOS expression thus increasing circulating NO bioavailability and had no stroke-protective effect in eNOS knockout animals. These findings, as well as results showing an increase in cerebral blood flow following statin treatment or an intravenous infusion of the eNOS substrate L-arginine, indicated that at least some of the statin-dependent protective effects were due to increased eNOS expression leading to vasodilatation and improved perfusion in the damaged region (Endres and

others 1998; Yamada and others 2000). These experimental data raised the question as to what molecular mechanism or mechanisms underlie this statin-induced increase in eNOS expression.

Statins and eNOS Activity

Studies over the past 10 years have demonstrated that statins increase eNOS expression via both cholesterol-dependent and cholesterol-independent mechanisms. Endothelial NOS expression is negatively regulated by oxidized low-density lipoprotein (LDL) cholesterol, which suggests that reducing cholesterol levels indirectly increases eNOS activity. Among the cholesterol-independent mechanisms, particular attention was given to the pleiotropic effect of statins because it was shown that the small GTPase protein, Rho, modulates eNOS expression (Laufs 2003; Endres and others 2004). In particular, one of the mechanisms responsible for the direct up-regulation of eNOS by statins involves the inhibition of GGPP synthesis. The reduced isoprenylation of Rho inhibits its translocation to the cell membrane and increases its inactive cytosolic state, which positively regulates eNOS mRNA stability by prolonging its half-life. This effect on eNOS expression is due to remodeling of the actin cytoskeleton because the inhibition of Rho and Rho kinase (ROCK) by statins reduces the formation of the actin stress fibers that inversely correlate with eNOS mRNA levels (Fig. 2). The direct involvement of Rho and ROCK in the up-regulation of eNOS expression and activity has been highlighted in studies demonstrating that the selective inhibition of the small GTPase or its kinase leads to an increase in eNOS mRNA half-life and that this effect is reversed when GGPP is supplemented (Rikitake and Liao 2005).

In addition, statins can also induce the phosphorylation and activation of eNOS via the phosphatidylinositol-3 kinase (PI3K)/protein kinase Akt pathway. In endothelial cells, statins increase the phosphorylation and activation of Akt, which, in turn, phosphorylate eNOS and increase NO production. The ability of statins to activate this pathway is blocked by PI3K inhibitors, thus suggesting that activation of the PI3K/Akt pathway is directly involved in the rapid increase in eNOS activity induced by statins (Kureishi 2000). Interestingly, the inhibition of Rho or ROCK leads to rapid phosphorylation and activation of Akt and eNOS, which indicates that the Rho/ROCK pathway negatively regulates eNOS expression and activity by means of two distinct mechanisms (Rikitake and Liao 2005). Taken together, the statin-dependent increase in eNOS expression and activity that enhances NO production and improves endothelial function may partially explain the stroke-protective effects of HMG-CoA reductase inhibitors.

Statins and Oxidative Stress

However, other mechanisms may also be involved in the beneficial effect of statins on stroke. The activation of various enzymatic systems after ischemia and particularly reperfusion leads to the massive production of reactive oxygen species (ROS) that directly damage the main constituents of cells such as lipids, proteins, nucleic acids, and proteins and thus cause ischemic cell death. Another important feature of statins is the antioxidative effects that may act on the vascular district and brain parenchyma. In the vascular wall, NADPH oxidase, eNOS, and xanthine oxidase provide most superoxide radicals (Gorlach and others 2000). NADPH oxidase, the major source of ROS in the vascular district, is activated by the GTP binding protein rac1, which is under the positive control of angiotensin II. The angiotensin II/rac1-dependent increase in free radicals generated by the enhanced activity of NADPH oxidase is inhibited by statins via a dual mechanism that involves the reduced expression of AT-1 receptors and decreased isoprenylation of rac1, thus preventing its translocation to the plasma membrane (Wagner and others 2000; Wassmann and others 2001). eNOS can also generate superoxide radicals in situations that reduce the availability of its cofactor tetrahydrobiopterin. In vitro studies have shown that statins increase tetrahy drobiopterin synthesis by facilitating coupling between the enzyme and its cofactor, thus restoring the normal production of NO.

In brain parenchyma, the constitutive (nNOS) and the inducible (iNOS) forms of NOS expressed in neurons or inflammation-activated astrocytes, microglia, and macrophages are respectively stimulated within a few minutes and many hours of ischemia. The enhancement of both nNOS and iNOS plays a detrimental role in ischemia, as demonstrated by the neuroprotective effects of specific antagonists. In contrast with their effect on eNOS, statins inhibit the ischemia-induced activation of nNOS and iNOS and thus provide a synergistic protective action on ischemic infarct (Moro and others 2004; Vaughan and Delanty 1999).

Statins and Inflammation

The role of inflammation in ischemic brain damage has been documented in humans and animal models. The proinflammatory cascade of vascular events in stroke often starts with an increased expression of cell adhesion molecules such as ICAM-1, P-selectin, and E-selectin, which are responsible for neutrophil and platelet accumulation in the vessel wall (Frijns and Kappelle 2002). After ischemia, inflammation is also observed in the parenchyma and further amplifies the progression of tissue damage. Activated microglia, astrocytes, macrophages, and leukocytes migrate to the ischemic region, where, in addition to increasing the production of ROS, they up-regulate transcription factors such as NF-κB and generate the overproduction of inflammatory mediators, including iNOS, cyclooxygenase-2, cytokines (IL1, IL6, IL8, TNFα), and chemokynes (monocyte chemoattractant protein 1 [MCP1]). The crucial role of these proinflammatory molecules in stroke is based on the evidence that their targeted disruption reduces ischemic damage (Barone and Feuerstein 1999; Zheng and Yenari 2004; Iadecola and Alexander 2001; Boutin and others 2001).

It has been demonstrated that statins have anti-inflammatory and immunomodulatory effects, and recent

clinical studies have shown that inflammatory markers such as C-reactive protein (CRP) and lipoprotein-associated phospholipase A2, both of which are associated with an increased risk for stroke, are reduced by statin treatment (Elkind 2006).

There is accumulating evidence of the anti-inflammatory effect of statins on ischemic injury in various animal models. In particular, it has been shown that statin administration reduces the levels of a number of molecular markers of inflammation such as NF-κB, ICAM-1, iNOS, interleukins, and cytokines (Pahan and others 1997; Carloni and others 2006; Sironi and others 2006). Taken together, these findings suggest that the protective effect of statins is also due to their inhibiting the production of detrimental proinflammatory molecules and reducing the recruitment of neutrophils and monocytes to ischemic brain parenchyma from the vascular bed.

Statins and Thrombosis

Among the pleiotropic effects of statins, their influence on platelet function and plaque stability plays a crucial role in the onset of an acute ischemic insult. Vascular injury and plaque rupture are associated with increased platelet reactivity, as shown by the increase in the cholesterol/phospholipid ratio, thromboxane A_2 (TXA_2) biosynthesis, and cytosolic calcium levels (Le Quan Sang and others 1995). Statins improve platelet function insofar as increased eNOS expression and activity, which enhances endothelial NO availability, inhibits platelet aggregation. Further effects of statins on platelets involve reducing the production of TXA_2 and decreasing membrane cholesterol content (Vaughan and others 2000). The statin-induced changes in these markers of platelet reactivity suggest a reduced thrombogenic potential.

The well-known beneficial effect of statins on acute coronary syndromes is at least partially attributable to their ability to stabilize the plaque of atherosclerotic lesions because damage to the fibrous cap can lead to plaque rupture and the potential risk of thrombosis. In the atherosclerotic process, activated macrophages may secrete proteolytic enzymes such as metalloproteinases (MMPs), which are capable of degrading the collagen-containing fibrous cap and thus allowing the release of thrombogenic material into the bloodstream. The beneficial effects of statin-dependent lipid lowering are probably due to a decrease in plaque macrophage accumulation and the consequent inhibition of MMP production by activated macrophages rather than the reduction of plaque size (which is minimal and occurs over an extended period of drug treatment) (Liao and Laufs 2005).

It has recently been demonstrated in a mouse model of embolic focal ischemia that both simvastatin and atorvastatin increase the expression of tissue plasminogen activator (tPA) while having no effect on its endogenous inhibitor (Asahi and others 2005). The enhanced clot lysis induced by HMG-CoA reductase inhibitors in this study provides an additional mechanism of action related to the protective effects of statins in cerebral ischemia.

Statins and Progenitor Cells

Cell replacement therapies involving the implantation or systemic administration of stem cells in models of cerebral ischemia have emerged as a promising means of improving the functional consequences of stroke. However, this may not be easy because the administered stem cells have to reach the ischemic area, differentiate in neuronal cells, and integrate with the damaged tissue. Over recent years, it has been suggested that a complementary (and perhaps more "physiological") approach may be the pharmacological stimulation of progenitor cells. In this regard, it has been shown that statins can induce the mobilization of bone marrow–derived cells and thus increase their number in the bloodstream. In particular, statins increase the number of circulating endothelial progenitor cells (EPCs) via a mechanism that involves the PI3K/Akt pathway and promote the proliferation of neuronal progenitor cells (NPCs) in the brain (Dimmeler and others 2001; Llevadot and others 2001). The increase in EPCs and NPCs contributes to cerebral neovascularization and neurogenesis after ischemia, thus suggesting that statins may induce angiogenesis and synaptogenesis, as well as lead to the formation of new blood vessels and a new neuronal network in the damaged brain by promoting this drug-dependent neuroprotective effect (Werner and others 2002; Zhang and others 2005).

Conclusions

Despite our acquired knowledge of the molecular mechanisms involved in the pathophysiology of stroke, as well as the growing body of evidence showing that putative neuroprotective drugs appear to work in animals, our therapeutic armamentarium for counteracting the deleterious consequences of an ischemic attack is still limited, and thrombolysis with recombinant tPA remains the only therapy for acute stroke approved for clinical use.

The evolution of ischemic brain damage seems to be a multifactorial process involving various biochemical pathways that are often interlinked. The existence of this "network" leading to cell death and ischemic damage may be one of the reasons why drugs selecting a single target that appear to be neuroprotective in animal models have failed in clinical trials. It is therefore possible that only therapies capable of interfering with multiple targets may be efficacious in ischemic brain injury.

Clinical and experimental data have shown that statins decrease the risk of stroke, reduce the volume of ischemic damage, and improve functional outcomes. The search for the mechanisms underlying these beneficial effects has shown that statins meet the criteria of a multifactorial drug because they have a broad range of protective pharmacological actions (also known as pleiotropic effects) that are distinct from their main lipid-lowering activity.

The results of animal studies and ongoing clinical trials may open up the road to the use of statins in individuals at high risk for stroke, as well as answer the question as to whether statins may be beneficial in patients with previous stroke.

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