New Insights into the Pleiotropic Effects of Statins for Stroke Prevention

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Abstract: There is compelling evidence that treatment with 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors - "statins" - the most important class of lipid lowering agents, reduces ischemic stroke incidence independent on their effect on serum cholesterol levels. In this review, the non-lipid-mediated - "pleiotropic" - effects of statins as well as their potential implication in developing new treatment strategies for stroke prevention will be discussed.

Key Words: HMG-CoA reductase inhibitors, stroke, pleiotropic effects.

INTRODUCTION

Over the last decades, both observational studies and clinical trials have clearly demonstrated that a linear relation exists between serum cholesterol levels and cardiovascular disease and that lowering plasma total cholesterol decreases the risk of coronary heart disease (CHD). As opposed to CHD, the epidemiologic link between raised plasma concentration of cholesterol and ischemic stroke remains much less clear, except in patients aged <45 years [1]. Thus, the evidence of any beneficial effect of plasma cholesterol reduction on the incidence of ischemic stroke is less definitive. This appears somewhat surprising when taking into account the critical role of dyslipidemia in the pathogenesis of atherosclerosis. One plausible reason for this might be that most large epidemiologic studies have not analyzed stroke in terms of various causative subtypes but have lumped heterogenous mechanisms (including large-vessel atherosclerosis, lacunar stroke, and cardioembolic stroke) into a single category, likely weakening the ability to find a clear association [2]. However, even assuming that some methodological shortcomings might have influenced the results of these studies, leading to speculation that an effect of increased plasma levels of cholesterol on stroke risk is likely to exist, it appears obvious that lowering plasma levels of this molecule might have only a modest influence on disease risk.

In the recent years, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (HMGRI, or "statins") have emerged as the most important class of lipid lowering agents. Although numerous clinical trials have been reported demonstrating the beneficial effect of statins in reducing the burden of cardiovascular disorders, even a cursory review of these data suggests that statin-associated benefits may occur more rapidly, and to a greater degree, than that which would be expected from the changes in cholesterol levels alone. Furthermore, clinical and experimental data have recently shown that these compounds may also decrease the risk of ischemic stroke, reduce the volume of ischemic damage, and improve functional outcomes [3], while statins withdrawal after an acute ischemic stroke is associated with increased risk of death or dependency at 90 days [4] and increased mortality during the first year [5].

Based on the results of the recent Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) trial [6], an updated version of the AHA/ASA Recommendations for the Prevention of Stroke in Patients With Stroke and Transient Ischemic Attack has been reported, including the new recommendation to use statin therapy with intensive lipid-lowering effects for patients with atherosclerotic ischemic stroke or TIA and without known CHD to reduce the risk of stroke and cardiovascular events [7].

Because of the weak, if any, relation between cholesterol plasma levels and stroke, cholesterol reduction is not assumed to be the predominant mechanism underlying the beneficial effects of statins on brain ischemia. These observations, in addition to emerging data sets demonstrating that statins may also alter the course of many non-cardiovascular disease states, such as sepsis, Alzheimer's disease, osteoporosis, and ankylosing spondylitis [8-11], indicate that statins may have several cholesterol-independent effects.

This is, essentially, the concept of statins pleiotropy.

PHARMACOKINETIC OF STATINS

The rate-limiting enzyme in cholesterol biosynthesis in the liver is HMG-CoA reductase, which catalyzes a four-electron reductive deacylation of HMG-CoA to CoA and mevalonate. The primary consequence of HMG CoA reductase inhibition is the lowering of serum cholesterol levels and subsequent upregulation of low-density lipoprotein (LDL)-receptors in the liver [12].

One of the first natural inhibitors of HMG-CoA reductase which was isolated from *Penicillium citrinium* in 1976 was *mevastatin* (compactin, ML-236B). In spite of its clear effect in inhibiting cholesterol biosynthesis, it also caused unacceptable hepatocellular toxicity and further clinical devel-

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opment was discontinued. Subsequently, a more active fungal metabolite, mevinolin or *lovastatin*, which did not cause hepatocellular toxicity when given to rats, was isolated. Lovastatin, therefore, became the first of this class of cholesterol-lowering agents to be approved for clinical use in humans. Since then, several new statins, both natural and chemically modified, have become commercially available, including *pravastatin*, *simvastatin*, *fluvastatin*, *atorvastatin*, *cerivastatin*, and, more recently, *pitavastatin* and *rosuvastatin*.

Statins work by reversibly inhibiting HMG-CoA reductase through side chains that bind to the enzyme's active site and block the substrate-product transition state of the enzyme. Thus, all statins share an HMG-like moiety and inhibit the reductase by similar mechanism (Fig. **1**).

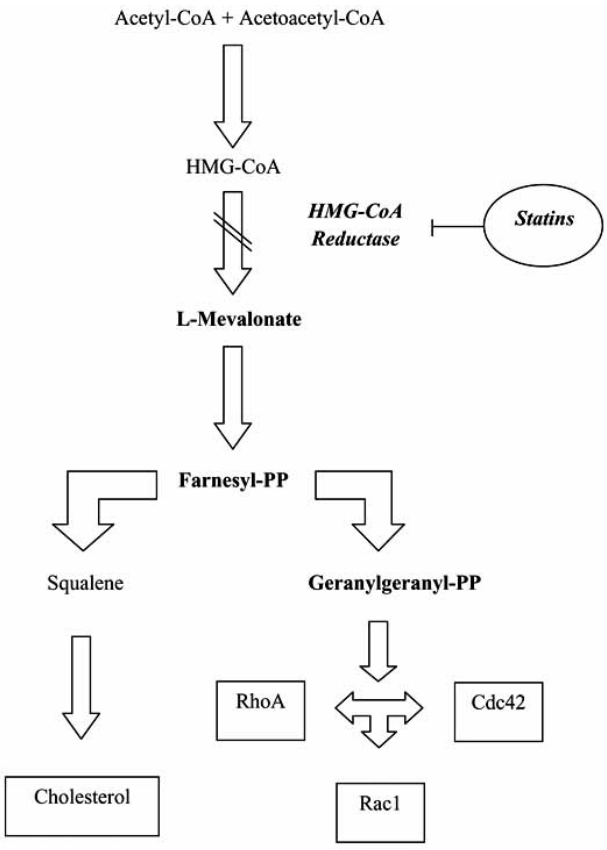

Fig. (1). Cholesterol biosynthesis pathway and the effects of HMG-CoA-reductase inhibition by statins.

Until recently, all cholesterol-independent effects of statins were believed to be mediated by inhibition of mevalonate synthesis. However, statins can bind to a novel allosteric site within the ß2 integrin function-associated antigen-1 (LFA-1), independent on mevalonate production. LFA-1 belongs to the integrin family and plays an important role in leukocyte trafficking and in T cell activation. Furthermore, 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). These intermediates serve as important lipid attachments for the post-translational modification of a variety of proteins. Protein isoprenylation permits the covalent attachment, subcellular localization, and intracellular trafficking of membrane-associated proteins. Members of the Ras and Rho GTPase family (also including other nuclear lamins, such as Rac and Rap) are major substrates for posttranslational modification by prenylation [13,14]. Both Ras and Rho are small GTP-binding proteins, which cycle between the inactive GDP-bound state and the active GTP-bound state (Fig. **2**). In endothelial cells, Ras translocation from the cytoplasm to the plasma membrane is dependent on farnesylation, whereas Rho translocation is dependent on geranylgeranylation [15,16]. Statins inhibit both Ras and Rho isoprenylation, leading to the accumulation of inactive Ras and Rho in the cytoplasm. Because Rho is 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 [17].

The biological effects of Rho are mediated by its downstream effectors, including ROCK, protein kinase N-related kinases, citron kinase, rhotekin, mDia and the myosin-binding subunit of myosin light-chain (MLC) phosphatase [18]. Although the precise roles of these many effectors remain to be determined, the best characterized is the effect of ROCK on the actin cytoskeleton. ROCK phosphorylates and inhibits the myosin-binding subunit of MLC phosphatase. Inhibition of MLC phosphatase increases MLC phosphorylation and myosin contractility, which drive the formation of stress fibers and focal adhesions [19]. ROCK activity is often elevated in disorders of the cardiovascular system [20]. Thus, statins could affect vascular smooth-muscle contraction at least partially through effects on Rho/ROCK [21,19]. Through inhibition of isoprenylation of Rho, translocation of Rho to the cell membrane is inhibited and the downstream activation of ROCK is reduced [22]. Indeed, ROCK inhibitors prevent cerebral vasospasm after subarachnoid hemorrhage [23], inhibit the development of atherosclerosis [24] and prevent arterial remodeling after vascular injury [25]. The Rho/ROCK pathway could also regulate cellular functions other than the actin cytoskeleton. For example, ROCK can phosphorylate insulin receptor substrate-1 (IRS-1) and modulate the insulin/PI3K/Akt pathway [26]. The Rho/ROCK pathway is involved in oxidative stress, aortic stiffness and changes in blood pressure [27]. Furthermore, ROCK regulates cell survival through phosphorylation of the protein kinase B/Akt and FOXO [28]. ROCK can also regulate adipogenesis and myogenesis. In p190-B Rho GAP-deficient mice, the Rho/ROCK pathway is activated chronically and there is a defect in adipogenesis with a predilection towards myogenesis [29]. Other processes or conditions involving the RhoA/ROCK pathway include angiogenesis [30], hypertension [21], cardiac hypertrophy [31], perivasclar fibrosis [32] and pulmonary hypertension [30]. Fasudil, a selective ROCK inhibitor, improves endothelial function in patients with coronary artery disease [33]. Experimental evidence suggests that inhibition of Rho isoprenylation mediates many of the cholesterol-independent effects of statins not only in vascular wall cells [15,34], but also in leukocytes [35] and bone [36].

Rac inhibition might also contribute to some of the pleiotropic effects of statins. Two important effector-response

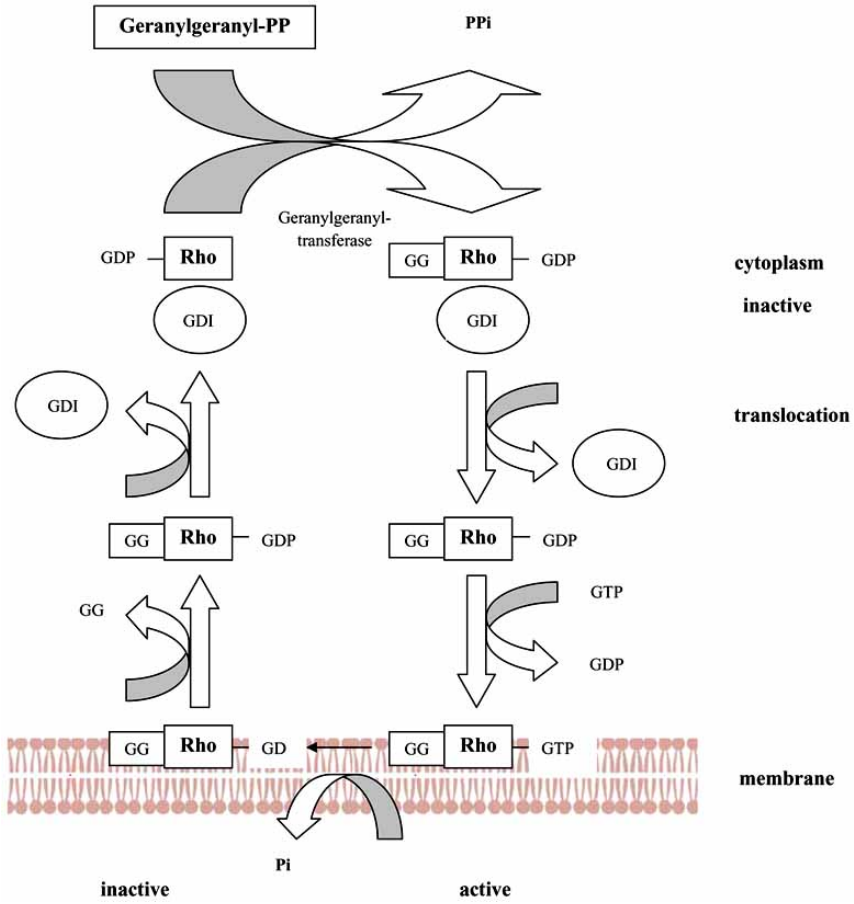

Fig. (2). Regulation of the Rho GTPase cycle.

pathways lie downstream of Rac: cytoskeletal remodeling and reactive oxygen species (ROS) generation. Rac1 influences multiple cytoskeletal remodeling proteins, such as Wiskott-Aldrich syndrome protein, calmodulin-binding GTPase-activating proteins and p21-activated kinase. Rac1 also binds to p67phox and leads to activation of the NADPH oxidase system and subsequent generation of ROS. Indeed, Rac activity is closely related to ROS production and ROS generated by NADPH oxidase in response to growth factors and inflammatory cytokines is mediated by Rac [37]. Importantly, statins inhibit Rac1-mediated NADPH oxidase activity and thereby reduce angiotensin II-induced ROS production and hypertrophy in smooth muscle and heart [38,39]. The activation of Rac1 in the vascular wall has been associated with atherosclerosis, neointimal proliferation, cardiac hypertrophy and endothelial dysfunction [40]. Rac1 has multiple roles in diverse cellular processes and cardiovascular physiology [41]. Thus, Rac1 inhibition might also contribute to some of the pleiotropic effects of statins. Taken together, these findings suggest that protein isoprenylation might be a crucial step in the cascade of events leading to the pleiotropic effects of statin therapy.

PLEIOTROPIC EFFECTS OF STATINS

Recent *in vivo*, *ex vivo*, and *in vitro* studies have begun to unravel the physiology underlying each of the pleiotropic effects of statins. These non-lipid-dependent effects include 1) improving endothelial function (at least in part, mediated by increased endothelial nitric oxide (NO) levels), 2) inhibiting inflammatory responses, 3) antioxidant activities, 4) immunomodulatory actions, 5) stabilizing atherosclerotic plaque, and 6) modulating platelet function (Fig. **3**). Furthermore, a role of statins in poststroke neurogenesis and synaptogenesis has been hypothesized.

STATINS AND ENDOTHELIAL FUNCTION

The influence on endothelium is the most widely described of the pleiotropic effects of HMG-CoA reductase inhibitors and, since endothelial dysfunction is one of the earliest manifestations of atherosclerosis [42], it is also strongly related to stroke occurrence. The endothelium produces vasoactive substances in response to environmental factors and serves as an important autocrine and paracrine organ that regulates the vascular-wall contractile state and cellular composition. Endothelium-derived nitric oxide (NO) mediates vasodilation, inhibits platelet aggregation and leukocyte adhesion and decreases vascular smooth-muscle proliferation [43]. Endothelium-derived NO, therefore, is protective for the vasculature and decreased NO bioavailability is often associated with increased risk of cardiovascular disease [44]. Elevated serum cholesterol levels lead to endothelial dysfunction [45]. The mechanism by which LDL-

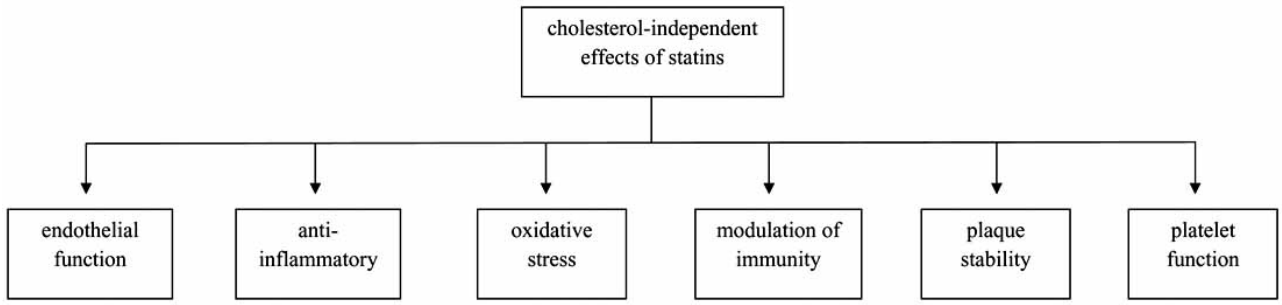

Fig. (3). Pleiotropic effects of statins.

cholesterol causes endothelial dysfunction and decreases NO bioactivity involves downregulation of endothelial NOS expression, decreased receptor-mediated NO release [46] and decreased NO bioavailability owing to increase in ROS production [47]. Furthermore, oxLDL can also recruit leukocytes to the arterial wall and activates NF-kB, a major proinflammatory transcription factor that is crucial for the induction of vascular cell adhesion molecule (VCAM)-1 and monocyte chemotactic protein (MCP)-1 [48]. Statins improve endothelial function by cholesterol-dependent and -independent mechanisms. Clinical trials have shown that LDL apheresis, which removes plasma LDL particles physically, can improve endothelium-dependent vasomotion through acute reduction in serum cholesterol levels [49]. Cholesterol lowering modifies atherosclerotic-plaque biology, thereby decreasing vascular inflammation and leukocyte activation [50]. Thus, statins can improve endothelial function through reduction in serum cholesterol levels. However, in some studies, statins improve endothelial function before significant reduction in serum cholesterol levels occurs [51,52]. This, in part, is mediated by the upregulation of endothelial nitric oxide synthase (eNOS) in the presence of hypoxia [53] and oxLDL [54]. Statins affect eNOS expression and activity mainly through three mechanisms [15]. First, statins increase eNOS expression by prolonging eNOS mRNA half-life rather than by inducing eNOS gene transcription. The mechanism is owing to inhibition of RhoA geranylgeranylation, alteration of the cytoskeleton and localization of the eNOS mRNA [16]. Second, statins reduce caveolin-1 abundance. Caveolin-1 is an integral membrane protein and binds to eNOS in caveolae, thereby inhibiting NO production directly [55]. Third, statins can activate the phosphatidylinositol 3-kinase (PI3K)/protein kinase Akt pathway [56]. Akt is a serine/threonine kinase that regulates various cellular functions, such as survival, growth and proliferation. Because Akt, in turn, phosphorylates and activates eNOS, statins can also increase eNOS activity through the PI3K/Akt pathway [57]. Several vasoconstricting agents, such as endothelin-1 (ET-1) or angiotensin II, counteract the vasodilating effect of NO and might contribute to the development of atherosclerosis. ET-1 acts as a potent mitogenic agent, which promotes neointima formation and proliferation of smooth muscle cells [58]. ET-1 is found to be elevated in patients with severe atherosclerosis [59]. Statins inhibit the expression of preproET-1 [60] and downregulate endothelin and angiogensin subtype 1 receptors [61,62] in a RhoA-dependent manner. Statins also affect the fibrinolytic system of vascular smooth muscle and endothelial cells [63]. Plasminogen activator inhibitor type-1 (PAI-1) is the major endogenous inhibitor of tissue plasminogen activator. Elevated PAI-1 level is an independent cardiovascular risk factor and is associated with atherothrombotic disease [64,65]. Statins increase the expression of tissue-type plasminogen activator and inhibit the expression of PAI-1 [54]. The inhibitory effect of statins on PAI-1 expression is mediated, in part, through the PI3K/Akt pathway [66]. Statins also induce the expression of heme oxygenase-1 (HO-1) [67]. HO-1 is a stress-response protein, which is induced in response to ultraviolet radiation, cytokines and free radicals. Induction of HO-1 leads to the degradation of heme to carbon monoxide and biliverdin. Biliverdin is then converted to the antioxidant bilirubin [68]. Interestingly, HO-1 prevents the development of atherosclerosis in mice [69]. Further studies are needed to determine whether some of the anti-atherosclerotic effects of statins are mediated by HO-1 induction.

Effects of Statins on Re-Endothelialization

Accelerated re-endothelialization after angioplasty/deendothelialization is known to inhibit neointimal hyperplasia, which leads to luminal narrowing or restenosis at the injured site. Re-endothelialization has been shown to be promoted by vascular endothelial growth factor (VEGF) [70], hepatocyte growth factor (HGF) [71], estrogen [72], prostacyclin [73], blockade of TNFα [74], and now statins, which can promote endothelial cell migration and proliferation, in part by enhancing VEGF production [75]. Recent evidence has shown that bone marrow-derived endothelial progenitor cells (EPCs) contribute to the accelerated re-endothelialization induced by estrogen [76], granulocyte-colony stimulating factor (G-CSF), granulocyte/macrophage-colony stimulating factor (GM-CSF), monocyte chemoattractant protein-1 (MCP-1), and peroxisome proliferator-activated receptor-gamma (PPAR-gamma). Statins have been shown to enhance re-endothelialization both by stimulating pre-existing endothelial cells and by mobilizing EPCs from bone marrow, altering the profile of expression of certain adhesion molecule e.g., integrin α5β1 and αVβ5. Although the mechanisms for EPC mobilization from bone marrow by statins have not been fully clarified, eNOS and metalloproteinase-9 (MMP-9) activation by statins may be critical [77].

Effect of Statins on Angiogenesis

Angiogenesis is characterized by endothelial cell proliferation, migration, and remodeling . The activation of angiogenesis by low-dose statins has been reported in numerous animal models [78], and the mechanism is considered to be

due to the activation of PI3 kinase/Akt signaling pathway, which results in eNOS phosphorylation/NO production [79]. On the other hand, bone marrow-derived endothelial progenitor cells (EPCs) have been demonstrated to be involved in neovascularization [80]. Statins also promote the survival, migration and differentiation of adult bone marrow-derived EPCs *via* an Akt-dependent mechanism, and enhance EPC recruitment to sites of neovascularization [81]. The administration of statins reduces senescence and increases EPC proliferation [82], and mobilizes circulating CD34+ EPCs in patients with stable coronary artery disease. eNOS is required to induce EPC mobilization thus resulting in neovascularization.

In contrast to the pro-angiogenic effects of statins described above, other studies have suggested that statins inhibit endothelial cell migration [83], proliferation *in vitro* and angiogenesis in *in vivo* models [84]. The anti-angiogenic effects of statins were attributed to cholesterol-independent effects that involved the Rho/focal adhesion kinase/Akt signaling pathway inhibition [84], while additional data suggest that the statin-dependent inhibition of angiogenesis could be related to an increase of cell cycle inhibitors, e.g., p19, p21 and Wnt5a and the downregulation of angiogenesis-related gene such as PAI-1, vitronectin, HoxD3 and Notch4 gene. These findings are consistent with other data in which statins interfere with angiogenesis by inhibiting the geranylgeranylation of the small guanosine triphosphate-binding protein RhoA [85]. It has been suggested that the above conflicting results are dose related. Low doses of statins may activate endothelial Ras and promote Akt and eNOS phosphorylation leading to proangiogenic effects, while high doses result in anti-angiogenic effects by inhibiting Ras and RhoA without changing eNOS upregulation [79]. These biphasic activities of statins on endothelial cell biology can be explained by the properties of the biosynthetic pathways that originate from mevalonic acid. In addition to cholesterol, mevalonic acid plays an essential role as a precursor for several cellular components including ubiquinone, isopentenylated transfer RNAs, and prenylated proteins. Mevalonate-derived intermediates, especially farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP), have a higher affinity for the enzymes that catalyze non-sterol product formation than for the cholesterol biosynthetic enzymes. Low doses of statins, therefore, will mainly affect cholesterol synthesis and will promote the biosynthesis of FPP/Ras and GGPP/RhoA that are required for cellular proliferation and migration, respectively. In contrast, high dose statins will cause a significant inhibition of FPP and GGPP synthesis resulting in the inhibition of cell proliferation and migration, although eNOS activation will be further activated by inhibition of RhoA and activation of PI3K/Akt signaling. The regulation of proliferation, migration, and eNOS activation in endothelial cells has been further confirmed with the reversal effects by replenishment of FPP or GGPP.

Furthermore, because the positive effects of statins on vascular outcomes have now been noted in a variety of acute settings as well as following chronic administration [86,87], while a detrimental effect has been noted after withdrawal statins [4,5], it is likely that beyond the biphasic dose-dependent response noted with respect to statin effects on angiogenesis a differential effect might depend on acute *vs* chronic administration *in vivo*.

STATINS AND INFLAMMATION

The role of inflammation in ischemic brain damage has been documented in both 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 [88]. 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 chemokines (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 [89]. 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. There is accumulating evidence of the anti-inflammatory effect of statins on ischemic injury in various animal models. In particular, statin administration reduces the levels of a number of molecular markers of inflammation such as NF-κB, ICAM-1, iNOS, interleukins, and cytokines [90,91]. 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 OXIDATIVE STRESS

Statins may also improve endothelial function through their antioxidant effects. Lipid lowering by itself lowers vascular oxidative stress [92]. However, other antioxidant effects of statins appear to be cholesterol-independent. The major source of ROS in the vascular wall is the NAD(P)H oxidase complex. Rac1 is important for the assembly of the NAD(P)H oxidase enzyme complex. Inhibition of Rac1 isoprenylation by statin treatment prevents the activation of NAD(P)H oxidase and ROS release [93]. These enzymes catalyse electron transfer from NADPH to molecular oxygen, resulting in the formation of O_2^-. Interestingly, ROS produced by NADPH oxidases can promote ROS generation by other sources, thereby amplifying total levels of ROS. For example, O_2^- from NADPH oxidase may oxidize and degrade BH4, thereby leading to NOS uncoupling [94]. Similarly, NADPH oxidase derived ROS may also activate xanthine oxidase [95]. After treatment of spontaneously hypertensive rats with atorvastatin, carbachol-induced vasorelaxation in aortic segments was significantly improved. Furthermore, vascular production of ROS was reduced. Interestingly, statin therapy reduced blood pressure in this rat model and downregulated the angiotensin II type 1 (AT1) receptor ex-

pression. Moreover, the expression of the eNOS expression and activity was enhanced.

Besides ROS generating enzymes, antioxidative defense systems are important for the oxidative stress that ultimately results. The SOD isoforms, GPX and CAT are enzymes residing within the vasculature that finally lead to the elimination of free radicals by the generation of water and oxygen [96,97]. The ultimate oxidative stress within vascular cells is determined by ROS production and corresponding elimination processes. The latter are realized by the radical scavenging enzymes GPX, the SOD isoforms and CAT. Whereas atorvastatin had no influence on the expression of GPX and SODs, CAT expression and activity were profoundly upregulated *in vitro* and *in vivo*. Physiologically, the upregulation of CAT can be observed after an increase of hydrogen peroxide concentrations. However, reduced superoxide production by NAD(P)H oxidase after decreased expression of essential subunits leads to reduced concentrations of hydrogen peroxide when SOD levels are not altered [98]. Because CAT is used in the elimination of hydrogen peroxide, increased levels of CAT further reduce the concentrations of this radical, thereby accelerating the turnover of superoxide to hydrogen peroxide. Finally, this leads to a decrease of the overall intracellular free radical load in VSMCs. Therefore, upregulation of CAT may represent another antioxidative action of statins.

STATINS AND IMMUNOMODULATION

Inflammatory cells have an important role in the pathogenesis of atherosclerosis. Immunomodulation by statins, therefore, might contribute to some of the cholesterol-independent effects of statins. For example, simvastatin inhibits MHC II expression, which is upregulated in several inflammatory diseases. Antigen presentation requires endocytosis of antigen, internal processing and presentation of MHC II molecules at the cell surface. These processes all involve changes in the actin cytoskeleton, which are controlled by small GTPases. Indeed, the inhibitory effect of statins on MHC II expression is reversed by mevalonate and GGPP but not squalene (Fig. **1**), suggesting the involvement of small GTPases as the underlying mechanism [99].

Statins decrease macrophage expression of tumor necrosis factor (TNF) and interleukin (IL)-1b and also inhibit the proliferation of peripheral blood mononuclear cells [100]. Statins also regulate T-cell phenotype. Statins prevent experimental T helper 1 (Th1)-mediated autoimmune diseases [101] and induce IL-4-dependent Th2-cell differentiation [102] and the secretion of anti-inflammatory Th2-type cytokines [103]. Finally, statins can also bind to a novel allosteric site within the b2-integrin function-associated antigen-1 protein, independent of mevalonate production [104]. Inhibition of b2-integrin function-associated antigen-1 can decrease lymphocyte adhesion to ICAM-1 and impair T-cell costimulation [104]. This is one of the few reported pleiotropic effects of statins that does not involve the inhibition of small GTPases.

STATINS AND PLAQUE STABILITY

The atherosclerotic lesion contains highly thrombogenic materials in the lipid core that are separated from the bloodstream by a fibrous cap. Fissuring, erosion, and ulceration of the fibrous cap eventually lead to plaque rupture and ensuing thrombosis [105]. 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. Indeed, degradation of the plaque matrix appears to be most active in macrophage-rich regions. Secretion of proteolytic enzymes, such as metalloproteinases (MMPs), by activated macrophages may weaken the fibrous cap, particularly at the "vulnerable" shoulder region where the fibrous cap joins the arterial wall. Weakened fibrous caps lead to plaque instability, rupture, and ensuing thrombosis, which ultimately present as acute ischemic disease.

Lipid lowering by statins may contribute to plaque stability by reducing plaque size or by modifying the physiochemical properties of the lipid core [106,107]. However, 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 [108]. Indeed, statins inhibit the expression of MMPs and tissue factor by cholesterol-dependent and -independent mechanisms [108], with the cholesterol-independent or direct macrophage effects occurring at a much earlier time point. The plaque-stabilizing properties of statins, therefore, are mediated through a combined reduction in lipids, macrophages, and MMPs [109]. These effects of statins may reduce the incidence of acute ischemic stroke by lessening the propensity for plaque to rupture.

STATINS AND PLATELET FUNCTION

Circulating platelets are associated with mural thrombus formation at the site of plaque rupture and vascular injury [110,111]. Hypercholesterolemia is associated with increases in platelet reactivity [112]. These abnormalities are linked to increases in the cholesterol/phospholipid ratio in platelets. Other potential mechanisms include increases in thromboxane A2 (TXA2) biosynthesis [113], platelet α2-adrenergic receptor density [114], and platelet cytosolic calcium [115]. Statins have been shown to influence platelet function, although the precise mechanisms involved are not fully understood [116,117]. One of the well-characterized effects of endothelial NO is the inhibition of platelet aggregation. Statin-mediated upregulation of eNOS has been shown to be associated with downregulation of markers of platelet reactivity [118]. Potential additional mechanisms include a reduction in the production of TXA2 and modifications in the cholesterol content of platelet membranes [119]. The cholesterol content of platelet and erythrocyte membranes is reduced in patients taking statin therapy. This may lead to a decrease in the thrombogenic potential of these cells. Indeed, animal studies suggest statin therapy inhibits platelet deposition on damaged vessels and reduces platelet thrombus formation. Furthermore, *in vitro* experiments have demonstrated that statins inhibit tissue factor expression by macrophages, thereby potentially reducing thrombotic events in the vascular wall.

STATINS AND NEUROGENESIS-SYNAPTOGENESIS AFTER STROKE

Many patients improve in the weeks to months following stroke, implying an innate capacity for brain repair. One means by which repair might be achieved is neurogenesis, which occurs in the adult human brain *in situ*, is increased in animal models of brain injury including stroke [120-123], and is associated with migration of newborn neurons to injured brain regions [124-127].

Neurogenesis is a process primarily occurring in two regions, the subgranular zone (SGZ) and subventricular zone (SVZ), which contain specialized arrangements or niches of progenitor, glial, and endothelial cells leading to both self-renewal of the progenitor cell population and orderly differentiation of progenitor cells along committed glial and neuronal lineages [128]. Adult neurogenesis normally supplies newly born neurons from the SGZ to the dentate gyrus of the hippocampus and from the SVZ to the olfactory bulb [129].

Stroke alters this normal pattern of adult neurogenesis to stimulate cell proliferation within the SVZ and SGZ and migration of newly born, immature neurons into areas of damage. In the normal animal and after cortical injury, total cell number in the SVZ is a balance between increases in cell number through proliferation, losses, and migration [130, 131]. Stroke increases cell proliferation [127] and reduces migration from the SVZ to the olfactory bulb. After proliferation in the SVZ, immature neurons migrate to areas of ischemic damage in the striatum [124,125] and cortex [126]. Migration occurs over the first two weeks but can be sustained for several months. Within the normal neuroblast migration, neuroblasts closely associate with blood vessels and astrocyte processes, in a migration pattern termed *vasophilic migration* [132]. In the ischemic striatum, neuroblasts also migrate in close association with blood vessels [133]. Thus, in both the normal and poststroke brain, neuroblast migration involves cellular interactions between migrating immature neurons, blood vessels, and astrocytic processes. Angiogenesis is associated with normal (nonstroke) neurogenesis in the germinal zones of the adult brain in a cellular arrangement termed the *neurovascular niche*. This indicates that the two processes are causally linked in the peri-infarct cortex after stroke.

Several lines of evidence support the assumption that statins have neuroprotective effects in neurological injury and disease, as well as on restoration of function after neural injury. In particular, treatment of acute experimental stroke with statins has been shown to significantly improve functional outcome. This benefit appears to be mediated by statin-induced neurogenesis and synaptogenesis. As Chen and co-workers recently suggested, administration of atorvastatin may promote neurogenesis in the adult human brain after stroke and, thus, may play a role in neurological functional recovery [134]. Low-dose atorvastatin administered 24 hours after middle cerebral artery occlusion (MCAo) significantly promotes synaptophysin expression in the ischemic boundary, leading to extensive synaptic and functional reorganization [135]. Activation of PI3K/Akt and Erk signaling in neurons, which plays a central role in controlling synaptic plasticity and memory [136], may be implicated in atorvastatin induction of neurogenesis and synaptogenesis. Activated Akt differentiates PC12 cells, accelerates motor axon regeneration *in vivo* [137] and increases axon caliber and branching in sensory neurons, while p-Erk-2 fusion protein enters the nucleus and promotes axon elongation [138]. Atorvastatin promotes p-Akt and p-Erk in primary cortical neurons. Phosphorylation of PI3K/Akt leads to posttranscriptional activation of endothelial NOS, which promotes NO production [139]. NO activates soluble guanylyl cyclase leading to the formation of cGMP. In the central nervous system, NO/cGMP signaling promotes synaptic plasticity, axonal outgrowth and neurogenesis [140-143]. NO/cGMP regulates expression of transcriptional genes and some of these genes, such as the cAMP response element–binding protein, are involved in cellular survival, neurogenesis [141,142], differentiation, synaptogenesis, neurotransmitter release, and synaptic plasticity [143,144].

Therefore, statins provide a strategy of targeting multiple events of neuroprotection and neurorestoration after brain ischemic injury.

CONCLUSION

Indications for statin use continue to expand. Statins demonstrate considerable benefit in reducing cerebrovascular events and death, and we now are beginning to recognize their utility in stroke prevention. Although the clinical importance of the pleiotropic effects of statins in addition to cholesterol-lowering remains to be clarified in more detail, the epidemiological and clinical evidence is strong enough to support the recommendation that patients with stroke or TIA be treated with statin therapy, unless a specific contraindication is present.

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