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Review

Neuroprotective properties of statins

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Abstract:
Treatment with statins (3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors) reduces the risk of ischemic stroke among patients with increased risk of vascular disease. Recent experimental data point to neuroprotective properties of statins in acute cerebral ischemia. There is a proven link between bioavailability of nitric oxide and the activity of statins and ischemic stroke. Due to their ability to up-regulate nitric oxide synthase, statins have been considered in the therapy of a number of the central nervous system disorders, including cerebral ischemia, Alzheimer's disease, Parkinson's disease, tumors, and trauma. It has been claimed that they suppress inflammatory response and secondary injury after acute ischemia.

Key words:
statins, Alzheimer's disease, stroke, multiple sclerosis, neuroprotection

Abbreviations: Aβ – amyloid beta, AD – Alzheimer's disease, APP – amyloid precursor protein, CNS – central nervous system, eNOS, NOS3, NOSIII – endothelial nitric oxide synthase, HMG-CoA – 3-hydroxy-3-methylglutaryl-coenzyme A, LDL – low density lipoprotein, NO – nitric oxide, SM – multiple sclerosis

Introduction

The main mechanism of action of statins is based on the inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase and thus the limitation of the enzyme in the biosynthesis of cholesterol. That results in the increase in a number and activity of low density lipoprotein (LDL) receptors, and consequently in the decrease in this cholesterol fraction in the plasma [74]. Apart from cholesterol-dependent mechanisms of action, statins also directly up-regulate endothelial nitric oxide synthase (eNOS, also termed NOS3 or NOS III) expression, independent of cholesterol levels [22, 39, 57, 69]. By inhibiting mevalonate synthesis, statins prevent the formation of several isoprenoids (including farnesylpyrophosphate and geranylgeranylpyrophosphate), which posttranslationally modify small G proteins (GTPases) that cycle between membrane-bound (active) and cytosolic (inactive) states [34]. Among G proteins, small GTPases are an important target for pleiotropic effects of statins. These GTPases, among which are *Rho, Rac* and *Cdc42*, act as molecular switches, capable of regulating cell function, polarity, protrusion and adhesion (cytoskeletal response), the synthesis and migration of DNA, phospholipase D activation, sensitivity of cell responses to Ca^{2+}, and myocyte hypertrophy [8]. *Rho* proteins, in particular, have a role in accelerating eNOS mRNA degradation [8]. An inhibition of *Rho* occurs by reduced prenylation, as a consequence of HMG-CoA reductase inhibition by statins, and therefore, leads to the reduced eNOS mRNA degradation and higher levels of eNOS protein and activity

[8]. Inhibiting geranylgeranylation of *RhoA* GTPase increases the stability of eNOS mRNA through the remodeling of endothelial actin microfilaments [36]. Moreover, statins directly increase eNOS activity within minutes by activating the pathway involving phosphoinositide 3-kinase (PI3K) [10, 33]. As stated above, pravastatin immediately improves endothelium-dependent vasodilation in isolated aortic rings [2, 13].

Several modalities like HMG-CoA reductase inhibitors (statins), steroid hormones, nutrients and physical activity up-regulate eNOS expression and its activity. They all increase NO bioavailability, leading to enhanced cerebral blood flow and protection from ischemic stroke. Thus, therapeutic modalities that target eNOS not only serve as preventative measures to reduce stroke risk but could also represent novel treatment strategies for reducing brain injury during cerebral ischemia [13]. Also Di Napoli et al. [8] have accumulated data which indicate that statins may be neuroprotective during cerebral ischemia through the modulation of brain eNOS. When formed by the vascular endothelium, NO diffuses to the adjacent cells and activates soluble guanylate cyclase, which mediates many of the beneficial effects of NO. In the vascular smooth muscle, NO is a potent vasodilator and regulator of regional blood flow [26, 41, 54, 55].

In addition, NO is antithrombotic, anti-inflammatory and antiproliferative. By contrast, its insufficient concentration contributes to impaired vascular relaxation, platelet aggregation, increased proliferation of vascular smooth muscle, enhanced leukocyte adhesion to the endothelium and increased blood pressure [26]. Taken together, endothelium-derived NO serves as a protectant in the vascular wall. Thus, statins are of importance for their vasoprotective effects [71]. NO production by eNOS is under complex and tight control and is regulated by multiple mechanisms, including transcription factors, regulation of mRNA stability, protein-protein interaction and cellular localization [71].

Activation of inducible NOS (iNOS), an enzyme that produces toxic amounts of NO, has been implicated in a number of central nervous system (CNS) disorders, including cerebral ischemia, Alzheimer's disease (AD), Parkinson's disease, tumors, and trauma [68]. Astrocytes produce iNOS in response to a number of proinflammatory mediators, including cytokines and tumor necrosis factor [23]. Astrocyte- and macrophage-derived-iNOS, along with its oxidative byproduct**,** peroxynitrate, probably contribute to neuronal death due to oxidation of structural neuronal proteins during ischemia [66]. The induction of iNOS and subsequent NO production in rat astrocytes, microglia, and macrophages may be reduced by lovastatin [18, 31, 50], supporting the possibility that statins may suppress this component of the inflammatory response and secondary injury after acute ischemia.

As Laufs [34] has proven, oxidized LDL-cholesterol negatively regulates eNOS expression through caveolin-1, which is the major constituent of the cell-membrane invaginations known as caveolae. Moreover, oxidized LDL up-regulates lectin-like oxidized LDL receptor 1, which also negatively affects eNOS expression. Thus, reduction of cholesterol levels by any intervention indirectly increases eNOS expression [34].

Isoprenoids**,** formed as the cellular consequences of depletion of intermediates in the cholesterol biosynthetic pathway, play fundamental roles in cell growth, signal transduction, and mitogenesis. In addition to reducing stroke risk, emerging data suggest that statins may reduce dementia. Further studies are needed to fully address the role of statins in the prevention of stroke in patients without established vascular disease and the role of cholesterol modulation in the treatment of dementia [64].

It was found that some isoprenoids inhibit L-type Ca^{2+} channels in vascular smooth muscle cells that could offer an explanation for HMG-CoA reductase-dependent mechanism of statin-induced $[Ca^{2+}]_i$ increase [40]. However, endothelial cells are devoid of L-type calcium channels [40]. According to Lorkowska et al. [40], atorvastatin, simvastatin, cerivastatin, lovastatin, with the exception of pravastatin, induce an immediate increase in $[Ca^{2+}]_i$ in endothelium. This pleiotropic activity of statins in endothelium, which most likely is not related to the inhibition of HMG-CoA reductase, may correlate with the immediate intracellular release of NO and PGI_2 by these drugs [40].

Statins and stroke

Statins reduce strokes of different etiology by a variety of mechanisms, including modulation of precerebral atherothrombosis in the aorta and the carotid artery, thus preventing plaque disruption and artery-to-artery thromboembolism by inhibiting coagulation at its different stages, decreasing tissue factor, conversion of prothrombin to thrombin activity [49]. Statins exert favorable effects on hemostatic parameters, in-

cluding those being risk factors for cardiovascular diseases [49]. They are proven to inhibit fibrinolysis and also improve endothelial homeostasis by increasing the bioavailability of NO, which is responsible for the paracrine antiatherosclerotic functions of the endothelium. Vaughan et al. [65] have shown in experimental models of ischemic stroke that statin therapy reduces the size of brain infarct and improves neurologic outcome by direct up-regulation of brain endothelial NO synthase. Further, the anti-inflammatory actions of statins are also likely to contribute to neuroprotection and stroke prevention.

Apart from experimental studies, many clinical trials have been performed so far. The Asymptomatic Carotid Artery Progression Study (ACAPS)24 used carotid ultrasound to demonstrate retardation (or even reversal) of intimal-medial thickening in the carotid artery in men and women with elevated cholesterol treated with lovastatin for 3 years. The Pravastatin Lipids and Atherosclerosis in the Carotids II (PLAC-II) study also demonstrated a significant reduction in carotid intimal-medial thickness in pravastatin-treated subjects. The impact of statin therapy on aortic atherosclerosis has not been widely explored [65]. The reduction of stroke incidence by statins like pravastatin, simvastatin [21] and lovastatin were also confirmed in short-term clinical studies [8].

In patients with diabetes, the effect of statins on stroke severity was very essential. Greisenegger et al. [19] have indicated that these patients may be better protected by statins than those without this disease. None of them had an unfavorable outcome. On the other hand, in patients without diabetes the risk of severe stroke was not significantly reduced by statins [19].

In the experiment conducted by The Large Prospective Studies Collaboration Group, there was no correlation between cholesterol and stroke. However, they did not distinguish between ischemic and hemorrhagic stroke [6]. Cucchiara et al. [6] have shown that the opposing effects of cholesterol on ischemic versus hemorrhagic stroke risk may confound attempts to correlate cholesterol levels with stroke unless stroke type (i.e. hemorrhagic *vs*. ischemic) is taken into account.

Data from all the major statin trials indicate convincingly that these drugs reduce the incidence of stroke. In the Scandinavian Simvastatin Survival Study (4S, a secondary prevention trial), there was a significant reduction in the total number of fatal and non-fatal strokes (70 *vs*. 98) in the simvastatin compared to the placebo group, although the numbers of deaths due to cerebrovascular accident were similar. Ischemic, non-embolic strokes and transient ischemic attacks were reduced by 51% and 35%, respectively [56]. In the Cholesterol and Recurrent Events (CARE) Trial, the pravastatin group had a 31% lower incidence of all strokes, although again the incidence of fatal strokes was about the same. Summarizing, there was no increase in the rate of hemorrhagic stroke [52].

As fibrinogen is one of cardiovascular risk factors, its increase due to simvastatin may be unfavorable especially in case of patients with stroke history [49]. In their clinical study, Okopień et al. [49] have indicated no differences in the effects of simvastatin and fluvastatin on fibrinogen level between patients with bacterial infections. Although it was expected, the results have suggested that the *Helicobacter pylori* or *Chlamydia pneumoniae* infections do not modulate the statins activity on the hemostatic parameter [49]. However, in case of simvastatin, a 20% increase in fibrinogen in plasma levels was found after 12 weeks of therapy with the drug, which has indicated that time course effect is noticeable in case of some statins.

Recent reports suggest a possible withdrawal effect after discontinuation of statin therapy. Patients with stable coronary heart disease showed a more than threefold increase in vascular events after simvastatin treatment was stopped and continued with relatively lower doses of another statin [64]. Increased event rates were also reported in patients with acute coronary syndromes after withdrawal of statins [21]. In the animal model of cerebral ischemia, stroke protection disappeared only days after discontinuation of statin treatment [12].

Comparing statins, Laufs et al. [37] have demonstrated the results that suggest that rosuvastatin is at least as effective as simvastatin and atorvastatin and provided better protection than lovastatin and mevastatin in the mouse middle cerebral artery (MCA) stroke model. Cerebral ischemia was induced by occlusion of the MCA for 2 h and infarct size was determined after 22 h of reperfusion [37].

Statins and Alzheimer's disease

The pathophysiology of Alzheimer's disease (AD) is thought to be attributable to the effects of amyloid beta (Aβ), a peptide that accumulates in the brain, causing neurotoxicity and neurodegeneration. Experimental and clinical studies suggest that there is a pa-

thophysiological relation between Aβ and cholesterol levels. Elevated Aβ 42 levels and the ε4 allele of the apolipoprotein E (apoE4) are risk factors for AD [5, 11, 16, 51, 62]. In addition, apoE4 has been correlated with increased risk of atherosclerosis and amyloid plaque formation [3, 72]. In transgenic mice overexpressing amyloid precursor protein (APP), cholesterol levels inversely regulated Aβ production and AD pathology in the mouse brain. Epidemiological studies also show that patients with elevated serum cholesterol have an increased incidence of AD [27, 47]. ApoE4 plays an important role in the central nervous system as a cholesterol transport protein [5] and functions as a "chaperone" to promote conversion of Aβ to an insoluble form. The detailed molecular mechanisms through which cholesterol may regulate neuronal Aβ production are incompletely understood. Fassbender et al. [14] have shown that simvastatin and lovastatin reduce intracellular and extracellular levels of Aβ 42 and Aβ 40 in primary cultures of hippocampal and mixed cortical neurons. In addition, guinea pigs treated with high-dose simvastatin showed a reduction in cerebral Aβ, levels including the Aβ 42 isoform [22].

Kojro et al. [32] have shown that inhibition of cholesterol production increases α-secretase, favoring trafficking of APP through a nonamyloidogenic pathway. These investigators have determined that the cholesterol content of the cell membrane directs the fate of APP either toward α-secretase (low membrane cholesterol) or β/γ-secretase (high membrane cholesterol), the latter being a predominantly amyloidogenic pathway. Therefore, depletion of membrane cholesterol may represent a mechanism by which cholesterol-lowering drugs reduce Aβ production [12, 14, 32].

Follow-up studies in humans have recently suggested that patients receiving statin therapy have a reduced incidence of dementia. Cholesterol lowering with statins may have potential therapeutic benefit in AD. Simvastatin has been shown to reduce plasma levels of apoE in patients with senile AD, though cerebrospinal fluid levels of apoE were not significantly changed [43, 56]. As a causative relationship between apoE levels and AD has not been shown, the clinical significance of this finding is unclear. Moreover, lovastatin has been shown *in vitro* to reduce production of components of the senile plaques in AD. Senile plaques consist primarily of aggregated forms of Aβ, which are released by proteolytic processing of APP, a transmembrane precursor protein. Addition of lovastatin to APP-transfected human embryonic kidney cell lines lowered intracellular cholesterol, which caused a reduction in β-secretase processing of APP to β-amyloidogenic fragments [15]. Lovastatin reduced cellular formation of Aβ in living hippocampal neurons by 70%, and this effect was reversed by the re-addition of cholesterol to previously depleted cells [59]. Finally, two recent follow-up studies provide preliminary epidemiological evidence to support a potential therapeutic role for statins in AD. The first demonstrated a 60–73% lower prevalence of probable AD in patients taking lovastatin or pravastatin, but not simvastatin, compared to patients taking other cardiovascular medications [28, 43–45, 73]. The second study showed that patients taking statins had a 71% relative reduction in the likelihood of being clinically diagnosed with dementia or AD compared to controls, including those taking other lipid lowering agents [28].

One concern regarding the use of statins in patients with AD is the suggestion of possible deleterious cognitive effects from cholesterol-lowering. A recent small, randomized trial comparing lovastatin to placebo in healthy adults with elevated LDL cholesterol levels demonstrated a small but significant negative effect on measures of psychomotor speed and attention in the patients receiving lovastatin [44]. Another small trial involving cholesterol lowering through dietary modification showed a negative effect on a single measure of cognitive function, in patients with the greatest decline in cholesterol levels showing the most impairment [70]. The much larger, ongoing PROSPER trial mentioned below, will provide more data on potential cognitive effects of statin treatment.

Epidemiological studies of different designs and patient populations have shown a 40–70% reduction in the risk of AD associated with statin intake [28, 53, 73]. Despite the inherent limitations of these data to ascribe a causative link between statin use and a decreased risk of AD, the magnitude and consistency of the observed effect are remarkable. Two recent randomized placebo-controlled trials have failed to show a benefit of statin therapy on age-associated cognitive decline [21, 58]. The short follow-up of 3 years and 5 years in both trials [21, 58], and the elderly population studied in the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) trial [58] might have undermined the ability of these studies to show an effect. Also, age-associated cognitive decline is at best a poor surrogate for AD [4].

Antiinflammatory effects of statins have been recently confirmed by an analysis of the CARE study

showing a reduction of cerebral and coronary ischemic events, during pravastatin treatment, related to reduced CRP levels [8]. In the same article [8], Di Napoli et al. have indicated that one important action of statins is their ability to scavenge oxygen-derived free radicals in a concentration-dependent manner. A variety of statins, including simvastatin, fluvastatin, atorvastatin, pravastatin and cerivastatin, share this property (Tab. 1). These antioxidant effects have been considered as a possible explanation for the reported reduction of chronic cerebral degenerative diseases, such as AD, by statins [8].

Statins and multiple sclerosis

Evidence has emerged that statins, which are inhibitors of HMG-CoA, have immunomodulatory effects. Recent reports have shown that statins prevent and reverse chronic and relapsing experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis (SM). Furthermore, *in vitro* experiments with human immune cells have documented an immunomodulatory profile of statins comparable to that of interferon β. An open label clinical trial of simvastatin and atorvastatin for SM revealed a significant decrease in the number and volume of new MRI lesions and a favorable safety profile [46]. A shift from proinflammatory to anti-inflammatory conditions was consistently observed [1, 46, 75]. Furthermore, *in vitro* studies with human peripheral blood lymphocytes revealed that the anti-inflammatory effects of simvastatin, lovastatin, and mevastatin are strong and comparable with those of interferon β-1b. Some proinflammatory effects caused by heightened secretion of interferon γ and interleukin 12 were also observed. The combination of statins and interferon β-1b had additive anti-inflammatory effects [1, 75].

In addition, statins curtail T-cell proliferation, lower expression of activation surface markers and induce production of the cytokine IL-4. Currently, simvastatin is being tested in a phase II clinical trial in SM [45].

Statin neuroprotection in animal models

Pharmacological and genetic approaches in animal models of cerebral ischemia have clearly demonstrated that eNOS and vascular NO play a prominent role in maintaining cerebral blood flow and preventing neuronal injury [7, 13, 24, 25, 55].

Evidence from animal models of cerebral ischemia suggests that statins improve stroke outcome through eNOS-dependent mechanisms, which might explain the 'statin-withdrawal syndrome' [17, 35, 67]. Chronic pretreatment with statins decreased expression of markers of platelet activation and increased cerebral blood flow in both ischemic and non-ischemic murine brain tissue. Additional intravenous infusion of L-arginine further augmented blood flow in statin-treated animals. Both, functional impairment and tissue injury were significantly reduced in wild-type animals chronically treated with statins following occlusion of the middle cerebral artery and reperfusion. These protective effects were completely eliminated in eNOS knockout mice, indicating that eNOS up-regulation might be the main mechanism of protection [12]. This effect was independent of changes in cholesterol level and was reversible by co-treatment with mevalonate or geranylgeranylpyrophosphate, highlighting the biological relevance of intermediates in the cholesterol biosynthetic pathway. In addition to this prophylactic effect, the therapeutic benefit of statins extends to a 3-h post-stroke treatment paradigm [60] and can also be observed in stroke-prone spontaneously hypertensive animals [30].

In a similar study, atorvastatin has been shown to reduce stroke size in normocholesterolemic mice through cholesterol-independent mechanisms [38]. Interestingly, lovastatin inhibits cytokine-mediated up-regulation of inducible NO synthase and production of NO in rat astrocytes and macrophages [50]. Thus, statin therapy may provide an important approach to suppression of cytokine responses during ischemia and reperfusion. Statins may directly reduce the *in vivo* induction of inflammatory mediators, such as inducible NO synthase, interleukin-1β, and tumor necrosis factor-α, in astrocytes and macrophages. Summarizing, these observations imply dually protective effects of statins through simultaneous up-regulation of eNOS and inhibition of inducible NO synthase in the vasculature of the ischemic brain [66]. It demonstrates that statins may be anti-inflammatory by decreasing isoprenylation of proteins involved in inflammatory cascades.

The statins' effects modifying endothelial control of vasomotor function in both the coronary and peripheral circulation [2, 18, 48, 57, 63, 69] were com-

pletely absent in eNOS-deficient mice and were reversed by co-treatment with cholesterol precursors (mevalonate or geranylgeranylpyrophosphate), suggesting that statins act by selective up-regulation of eNOS and that intermediates in cholesterol biosynthesis may modulate eNOS. Cucchiara et al. [6] have proved that statins appear to have a potential dual synergistic neuroprotective role in cerebral ischemia by simultaneously inhibiting iNOS and up-regulating eNOS.

Combination of simvastatin with cytotoxic chemotherapy resulted in synergistic anti-tumor activity in animal models of neuroblastoma and glioma [61]. Medulloblastoma and neuroblastoma cells appear to be highly dependent on the HMG-CoA reductase pathway, and lovastatin has been shown to inhibit growth and promote apoptosis of these cells *in vitro* [9, 42]. The specific intermediate of the HMG-CoA reductase pathway responsible for these effects on cellular proliferation is unknown [42]. Other effects of statins, including their role in NO production and their antioxidant properties as described above, may also have an impact on tumors. The data suggest that treatment of some CNS malignancies with statins holds promise, and further research in this area appears warranted.

Neuroprotective effects of statins are summarized in Table 1.

Tab 1. Neuroprotective properties of statins in different CNS diseases

Statins	Alzheimer's disease	Multiple sclerosis	Stroke	References
Atorvastatin	+	+	+	[8, 37, 45, 46]
Cerivastatin*	+	ND	ND	[8]
Fluwastatin	+	ND	+	[8, 57]
Mevastatin	ND	+	+	[1, 37, 46, 75]
Lovastatin	+	+	+	[1, 8, 14, 15, 18, 28, 32, 37, 43–46, 50, 65, 69, 73, 75]
Pravastatin	+	ND	+	[2, 4, 8, 21, 28, 52, 58, 65, 73]
Rosuvastatin	ND	ND	+	[37]
Simvastatin	+	+	+	[1, 8, 14, 22, 28, 37, 43, 45, 46, 48, 56, 64, 73, 75]

* Food and Drug Administration withdrew cerivastatin from United States market because of reports of sometimes fatal rhabdomyolysis, a severe muscle adverse reaction from this cholesterol-lowering product. + – action present, ND – not determined

Conclusions

This review has discussed recent experimental evidence suggesting that some mechanisms of action of statins can protect from ischemic stroke. Statins and other modalities that selectively augment endothelial NO production, like physical activity, steroid hormones and nutrients, reduce injury in animal models of cerebral ischemia.

Although several important pros and cons, such as eNOS uncoupling and the dual role of NO in brain ischemia, have to be considered, eNOS targeting is an attractive approach to preventing and treating stroke in humans [20, 29]. NO-mediated effects can explain some of the cholesterol-independent protective effects of statins. In humans, improvement of endothelium function is one of the earliest clinical effects after initiation of statin treatment and clearly proceeds lowering of cholesterol levels. Indeed, the protective effects of statins are apparent within days and weeks [20, 29].

Further investigation using neuroimaging studies and serial cognitive evaluation studies are warranted to explore preliminary observations suggesting the neuroprotective properties of statins. If potential cholesterol-independent effects of statins are proved to be clinically important in humans, then this class of drugs will find wide-ranging application in the management of a variety of cerebrovascular disease events in patients with and without hypercholesterolemia.

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