Statins and stroke: evidence for cholesterol-independent effects

P. Di Napoli1, A. A. Taccardi1, M. Oliver2 and R. De Caterina1

1University Cardiology Division and Chair of Cardiology, ‘G. d’Annunzio’ University, Chieti, Italy; 2London, UK

Introduction

Many drugs, even when they modulate one single metabolic step, have multiple effects, due to the different functions that the affected pathway plays in different organs or tissues. Therefore pleiotropy (from the Greek πλείων for ‘multiple’ and τρόπος for ‘direction or turn’) is common in pharmacology. Far from being ideal Paul Ehrlich’s ‘magic bullets’, drugs usually exert multiple effects on the organism. These may be related or unrelated to the primary mode of action of the drug. Pleiotropic effects may emerge during pre-clinical and clinical studies in drug development but, more often, are discovered a posteriori, long after the therapeutic agent is marketed. They may be undesirable and recognized as adverse side effects, they may be neutral, or they may be beneficial, enhancing the desirable effect of the drug.

In recent years, major clinical trials and ancillary mechanistic studies have provided growing evidence that some of the impressive effects of statins, the most widely prescribed lipid-lowering agents, on the occurrence or recurrence of vascular events could be ascribed to their pleiotropism. First introduced into clinical practice in the late 1980s, several years after the discovery of their lipid-lowering effects[1], the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) reduce cholesterol synthesis through competitive inhibition of HMG-CoA reductase, an enzyme catalyzing the rate-limiting conversion step of HMG-CoA to mevalonate[2]. This leads to increased expression of low-density lipoprotein (LDL) receptors[3], ultimately leading to reduction of LDL plasma concentration.

Ample epidemiological data have suggested that hypercholesterolaemia is a powerful risk factor for coronary heart disease (CHD)[4,5]. In addition, different cholesterol-lowering drugs or non-pharmacological treatments can significantly reduce morbidity from CHD[6–14], thus proving a causal role for cholesterol in coronary events. Cholesterol seems, however, to play little or no role in the pathogenesis of cerebrovascular accidents, including stroke[15,16], currently one of the commonest causes of death and long-term severe disability. Yet recent clinical trials and surrogate end-point data strongly suggest that statins protect against stroke[9,14,17–21]. We will here analysise this apparent paradox and review the evidence for pleiotropic effects of statins playing a substantial role in preventing cerebrovascular accidents. As for many drugs, it is unlikely that pleiotropic effects always go in the ‘right’ direction, favourable for the patient. This is well illustrated by the recent discovery that some statins are potentially lethal due to rhabdomyolysis and renal failure[22]. We will show, however, that statins’ effects independent of cholesterol reduction are likely to play a beneficial role in cerebrovascular disease.

Statins exert most of their effects on CHD by reducing cholesterol

The Framingham Heart Study[4], the Multiple Risk Factor Intervention Trial (MRFIT)[23] and the Prospective Cardiovascular Münster (PROCAM) study[24] all established a positive relationship between elevated blood cholesterol concentrations and CHD. Data from the MRFIT and Pooling Project[25] have clearly established that there is an exponential increase in the risk of CHD with the increase of plasma cholesterol. Subsequently, large-scale lipid-lowering intervention trials, predominantly in patients with established CHD, demonstrated that lowering plasma cholesterol and, specifically, low-density lipoprotein (LDL) cholesterol, can halt progression and even induce some regression of pre-existing coronary atherosclerosis[26,27] and significantly decrease the risk of clinical coronary events[28–35]. The effectiveness of lipid-lowering therapy

Key Words: HMG-CoA reductase inhibition, stroke, cholesterol, neuroprotection, nitric oxide.
in reducing CHD morbidity was first established in a series of intervention trials using either dietary interventions or now less used first-generation hypolipidemic drugs, such as fibrates, niacin or colestipol[6,28,36–38]. The strongest and most compelling evidence of the impact of lipid-lowering therapy on progression (and even regression) of pre-existing coronary atherosclerosis and decreased risk of clinical coronary events has come, however, from intervention trials with statins[9–12,14,26,27,39]. Here for the first time intervention trials with a lipid-lowering treatment have shown a significant reduction in coronary and all-cause mortality. This important conclusion is most likely due to the large size of recent statin trials and the magnitude of cholesterol reduction achieved. A recent comparative meta-analysis of six statin and nine non-statin trials, in both primary and secondary prevention, has shown that the degree of cholesterol lowering is an excellent predictor of cardiovascular event reduction, independent of the intervention (statin or non-statin)[40]. This conclusion is unchanged with the inclusion of the more recent Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS)[30] and the MIRACL trial[21].

A subanalysis of AFCAPS/TexCAPS based on the levels of C-reactive protein (CRP) at entry has recently shown efficacy of lovastatin in patients with below-median LDL cholesterol at entry but above-median CRP levels[41]. Although it may be that inflammation, mirrored by high levels of CRP, may mark an increased cardiovascular risk, which can be reduced by lovastatin through cholesterol-independent mechanisms, such conclusions still would pertain to patients with low HDL/LDL ratio, which may mark a statin-sensitive subset of patients even in the presence of ‘normal’ LDL levels[42].

In Fig. 1 we show that the beneficial effect of statins (% reduction of CHD events as a function of % reduction of cholesterol) is on a similar regression line as that of other, non-statin, cholesterol-lowering interventions, and therefore fully explained by cholesterol lowering[43,44]. Data obtained from recent meta-analyses[45,46] suggested that the relationship between total cholesterol
concentration and coronary events is curvilinear or log-linear, with no further decrease in events expected below 150 mg. dl$^{-1}$ (corresponding to an LDL concentration of 110 mg. dl$^{-1}$), a result that is very similar to that found within the CARE trial. When LDL cholesterol levels are in this range, further lowering with statin therapy elicits diminishing returns in terms of coronary event rates.

**The uncertain relationship of cholesterol and stroke**

In contrast to its established role in the pathogenesis of CHD, raised plasma cholesterol and LDL cholesterol are less well established as important risk factors for cerebrovascular disease[16,47,48]. The reasons are likely to be many. Nearly all CHD events are linked to coronary atheroma, but less than half of strokes are due to large vessel atheroma. Non-atheromatous causes of strokes, such as cardiac arrhythmias leading to cerebral emboli, cardiomyopathies, or intracranial vessel diseases are responsible for most of the rest. Nevertheless, the majority of ischaemic strokes is caused by thromboemboli arising from atheromatous disease (outside the brain, either from the carotid arteries or the aortic arch), where hypercholesterolaemia is commonly present. The contention that cholesterol lowering is effective in reducing carotid atheroma is supported by ultrasound measurements of carotid artery intima-media thickness, independently shown to be a predictor of the occurrence of stroke[49]. Such measurements indeed show that reductions of plasma LDL cholesterol by 25% or more prevented any detectable progress in carotid intima-media thickness and reduced the development of new lesions both in asymptomatic people and in patients <75 years old[50,51] — as for coronary atheroma. Thus, the reduction of CHD with cholesterol-lowering therapy would be still expected to be accompanied by some reduction in the incidence of stroke.

Yet, there are several studies which suggest that raised total cholesterol levels do not predict stroke-related death. In some studies cholesterol levels are actually inversely related to stroke death[52]. In the Framingham Heart Study, cholesterol was related positively to stroke mortality only in women <55 years old[46]. In the same study, there was actually an inverse relationship between cholesterol and short-term mortality from stroke for women >70 years old. In a study from Israel, patients with stroke had lower cholesterol levels[53].

This apparent contradiction may be due to the composite aetiology of stroke and to the fact that, in earlier studies, thrombotic and haemorrhagic strokes were not differentiated. Indeed, epidemiological data from the MRFIT[23] and the Helsinki Heart Study[54] suggest that hypercholesterolaemia is a risk factor for non-haemorrhagic stroke. Observational data from the Honolulu Heart Program[55] and the Multiple Risk Factor Intervention Trial[56] suggested that there is a J-shaped relation between serum cholesterol and total stroke, with increased risk only at very low and very high cholesterol. One explanation for this relation may be that high cholesterol levels predispose an individual to atherothrombotic cerebral infarct, whereas low cholesterol levels may independently increase the risk for the less common variety of haemorrhagic stroke. However, the relationship between low cholesterol levels and haemorrhagic stroke needs further support[51], and is also related to the presence of elevated blood pressure. Whenever stroke aetiologies are not classified and simply pooled, there is clearly no relationship between cholesterol levels and stroke. A recent analysis of 45 prospective observational cohorts, reporting 13 397 strokes (independent of aetiology) in 450 000 people, showed no independent association of baseline total cholesterol level and the cumulative risk of stroke[57]. In aggregate, even the relationship between raised total cholesterol and isolated thrombotic stroke is far less strong compared with CHD[16].

**Cholesterol lowering and strokes — the statin paradox**

Two meta-analyses have reviewed the results of trials reported before 1995[58,59], suggesting that lowering serum cholesterol through dietary modification or non-statin drugs does not reduce stroke mortality or morbidity. Clofibrate, which has a more potent triglyceride-lowering and HDL-elevating effect rather than those of other cholesterol-lowering drugs, actually appeared to increase the risk of fatal stroke (odds ratio: 2.64, 95% CI: 1.42-4.92, $P=0.002$) in spite of an 8% reduction of total serum cholesterol[29]. In the Program on the Surgical Control of the Hypercholesterolemia (POSCH) Study, an intervention with partial ileal bypass surgery in patients with hypercholesterolaemia and a previous myocardial infarction significantly reduced total and LDL-cholesterol levels (by 23.3 and 37.7%, respectively) and the combined double endpoint (death due to CHD and confirmed non-fatal myocardial infarction) by 35% ($P<0.001$), but failed to show any trend to reduced cerebrovascular accidents ($P=0.69$)[31].

In contrast, 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. Ischaemic, non-embolic strokes and transient ischaemic attacks were reduced by 51% and 35%, respectively[59]. In the Cholesterol and Recurrent Events (CARE) Trial, the pravastatin group had a 31% lower incidence of all strokes ($P=0.03$), although again the incidence of fatal strokes was about the same. Of note, there was no increase in the rate of haemorrhagic stroke[19]. In the
Table 1  Statin therapy and stroke: results from main meta-analyses

<table>
<thead>
<tr>
<th>References</th>
<th>Sample size</th>
<th>Strokes (fatal and non-fatal)</th>
<th>Relative reduction in rates, %</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Statin</td>
<td>Placebo</td>
<td>Statin</td>
<td>Placebo</td>
</tr>
<tr>
<td>Crouse et al. [13]</td>
<td>PTT</td>
<td>3908</td>
<td>48</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>SPT</td>
<td>5862</td>
<td>134</td>
<td>192</td>
</tr>
<tr>
<td></td>
<td>PSPT</td>
<td>9770</td>
<td>182</td>
<td>248</td>
</tr>
<tr>
<td>Hebert et al. [14]</td>
<td></td>
<td>16 826</td>
<td>193</td>
<td>261</td>
</tr>
<tr>
<td>Ross et al. [15]</td>
<td></td>
<td>10 387</td>
<td>74</td>
<td>97</td>
</tr>
<tr>
<td>Blow et al. [16]</td>
<td></td>
<td>10 314</td>
<td>106</td>
<td>159</td>
</tr>
</tbody>
</table>

PPT=Primary prevention trials; SPT=Secondary prevention trials; PSPT=Primary and secondary prevention trials.

recent Long-term Intervention with Pravastatin in Ischemic Disease Study (LIPID), a secondary prevention trial, pravastatin significantly reduced the incidence of stroke (19%, P=0.022) [14,20]. Only in the West of Scotland Coronary Prevention Study (WOSCOPS), a primary prevention trial, was the number of strokes similar in the pravastatin and in the placebo groups (46 vs 51; P=0.67) [11]. The reduction of strokes by statins was also confirmed in some short-term clinical studies [17,18]. Levels of total cholesterol <160 mg . dl⁻¹ (4.16 mmol . l⁻¹) that in MRFIT were associated with higher risk of haemorrhagic stroke did not lead to any increase in haemorrhagic stroke in the statin trials.

Four meta-analyses [60–63] have accumulated the number of strokes in all trials using statins to prevent CHD (Table 1). In aggregate, statin treatment appears to reduce the risk of stroke significantly. Secondary prevention trials demonstrated a significant reduction in cerebrovascular events, whereas primary prevention trials only showed trends towards reductions in the stroke rate, probably due to the lesser risk of stroke in this setting [60]. In the most recent meta-analysis, in 21 303 patients, statin treatment significantly reduced all-cause death (−24%, P<0.0001) and non-fatal strokes (−32%), while the number of fatal stokes was similar to placebo [63]. These results were confirmed in one of the most recently reported clinical trials, the MIRACL study, that has shown that atorvastatin, initiated 24–96 h after an acute coronary syndrome, reduces recurrent ischaemic events in the first 16 weeks. In these patients there were no significant differences in the risk of death, non-fatal myocardial infarction, and cardiac arrest between the atorvastatin group and the placebo group, although the atorvastatin group had a lower risk of symptomatic ischaemia requiring emergency rehospitalization (6.2% vs 8.4%; RR 0.74; 95% CI, 0.57–0.95, P=0.02). More interesting, there were significantly 50% fewer strokes in the atorvastatin than in the placebo group (12 vs 24, respectively; P=0.045) [21]. Preliminary data from the Heart Protection Study (HPS) also confirm the reduction of stroke by statins and also suggest the absence of a relationship between cholesterol levels at entry and the reduction of stroke. In 20 556 patients at high risk of vascular events, simvastatin treatment significantly reduced the risk of vascular events (relative risk reduction: −24%, P<0.0001) and all-cause stroke (relative risk reduction −27%; P<0.0001). This also occurred in patients with relatively low levels of LDL and total cholesterol (LDL cholesterol <116 mg . dl⁻¹ (3.0 mmol . l⁻¹) and total cholesterol <193 mg . dl⁻¹ (5.0 mmol . l⁻¹) (data available from www.hpsinfo.org).

These benefits of statin therapy appear unrelated to cholesterol reduction [64], as shown (for both statin and non-statin trials) in Fig. 2. From a comparison with Fig. 1, one can appreciate the difference between the slopes of regression lines in CHD and stroke. These argue against cholesterol reduction as an explanation for any of the effects of interventions in stroke. It might be wise, therefore, to consider other ways in which statins might protect against strokes.

Cholesterol-independent neuroprotective properties of statins

Statins competitively inhibit HMG-CoA reductase, an early step in cholesterol biosynthesis, thus decreasing the production of mevalonate and other subsequent intermediates in the mevalonate pathway (Fig. 3). These include isopentenyl pyrophosphate, involved in transfer RNA synthesis, dolichol, which is involved in plasma membrane fluidity, ubiquinone, involved in mitochondrial respiration, and geranyl- and farnesylpyrophosphate, which are involved in the post-translational modification of a number of intracellular regulatory proteins [3,65]. Therefore the inhibition of HMG-CoA reductase will probably have multiple effects (Fig. 3). Some of these are likely candidates to explain statin effects in stroke prevention and in reduction of brain damage (Table 2) [66,67].

Statin effects maintaining protection from stroke are those causing stabilization/regression of atherosclerosis in both the coronary and carotid arterial system (‘cholesterol-dependent’ effects), and effects on endothelial function and the inflammatory response elicited by ischaemic and peroxidative damage (‘cholesterol-independent’ effects) [66–68] (Fig. 4).
These can be grouped into (1) effects explained by the influence of statins on nitric oxide bioavailability; (2) antiinflammatory effects; (3) antithrombotic effects; and (4) antioxidant effects.

**Nitric oxide (NO)-dependent neuroprotective effects of statins**

Among various cholesterol-independent statin effects, those on nitric oxide are likely to be highly clinically relevant. In the cerebral endothelium and parenchyma, statins may regulate all the different isoforms of nitric oxide synthase (NOS)[66,69]. Nitric oxide (NO) produced by the constitutive endothelial NOS (eNOS, NOS III) has a protective role in ischaemic conditions, regulating leukocyte and platelet adhesion and activation[70], inducing vasodilatation and reducing post-ischaemic hyperpermeability[67], and maintaining the antithrombotic properties of the vessel wall[71–76]. The importance of eNOS activity in the cerebral circulation is shown by the fact that eNOS knockout mice, thereby lacking the gene for this enzyme, have larger cerebral infarcts after middle cerebral artery occlusion and the administration of nitro-l-arginine induces a reduction of infarct size[77]. In spontaneously hypertensive rats, enhanced NO availability, either by the administration of NO donor (3-morpholinosydnonimine)[78] or the eNOS substrate l-arginine[79], also confers protection from stroke. The inducible form of NOS (iNOS, NOS II), produced by astrocytes, neutrophils and the microglia after stimulation with a series of proinflammatory mediators, also contributes to the inflammatory response in conjunction with cytokines such as tumour necrosis factor-a (TNF-a), interleukin (IL)-1β and IL-6[66,72,76]. Iadecola et al. recently reported that mice lacking the iNOS gene have a significant reduction in the volume of cerebral infarcts[80]. An excessive NO production following inflammatory stimulation leads to an enhanced reaction of NO with superoxide anion[67,81], with increased production of peroxynitrite, and may in turn be responsible for increased peroxidative damage in the brain parenchyma and the vascular wall. This occurs through enhanced neuronal and endothelial cell apoptosis and the promotion of oxidative damage of cellular DNA/RNA, proteins and membrane lipids[66,67,82]. The neuronal NOS (nNOS, NOS I) is also actively involved in ischaemia–reperfusion injury of brain parenchyma, being able to promote oxidative damage and glutamate-mediated cytotoxicity[83]. Huang et al. have reported that nNOS contributes to the development of ischaemic brain necrosis[84]. Statin therapy favourably modifies the balance of NO production. Statins augment cerebral blood flow, reducing cerebral infarct size and improving neurological outcome in normocholesterolaemic mice; this is
concomitant with increased eNOS activity, without any changes in nNOS expression \[^80\]. The blood flow and neuroprotective effects of statins, evident also in stroke-prone strains in normocholesterolaemic rodents \[^85\], are completely absent in eNOS-deficient mice \[^77\], indicating that enhanced eNOS activity by HMG-CoA reductase inhibitors is the predominant mechanism by which these agents protect against cerebral injury.

Recent observations also suggest that statin therapy reduces the cytokine-mediated upregulation of iNOS \[^86\]. This inhibition may suppress the inflammatory response accompanying acute ischaemia, which contributes to the ischaemic damage of brain parenchyma. These observations are consistent with those in several experimental models of ischaemia or hypoxia. In human endothelial cells exposed to hypoxia, statins increase NO production by modulating eNOS mRNA stability \[^87,88\]. In normocholesterolaemic rat hearts, statins decrease polymorphonucleate adhesion after ischaemia–reperfusion \[^89\]. Statins also reduce myocardial damage in isolated-perfused working hearts from normocholesterolamaic rats by increasing eNOS and reducing iNOS expression \[^90,91\]. These effects on NOS and cerebral microvasculature may also contribute to explaining the recent observed reduction of the risk of dementia in patients treated with statins \[^92\].

These effects of statins on NOS are likely to be due to a modulation of isoprenoids synthesis. Isoprenylation is important for the vesicular targeting of membrane proteins, including eNOS \[^93\]. In particular, farnesylation is necessary for the anchoring of G-proteins such as p21 \[^94\] to cell membrane. This modulates receptor-mediated eNOS activity through effects on membrane fluidity and cell growth \[^94\]. Farnesylation of the \(\beta\)-subunit of certain G-proteins may also be involved in the modulation of Ca\(^{2+}\) entry, an important step in reperfusion injury \[^95,96\]. 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 \[^96,97\]. Rho proteins, in particular, have a role in accelerating eNOS mRNA degradation \[^98\]. An inhibition of rho occurs by reduced prenylation, as a consequence of HMG-CoA reductase inhibition by statins, and therefore leads to...
reduced eNOS mRNA degradation and higher levels of eNOS protein and activity\(^99,100\). The eNOS mRNA stabilization by statins may also be due to L-mevalonate stimulation of proteins that bind to a sequence motif (AUUUA) in the 3'-untranslated region of eNOS mRNA, which is known to mediate mRNA destabilization via protein–mRNA interactions\(^101\). In addition, since eNOS mRNA stability is cell-cycle dependent, statins may stabilize eNOS mRNA indirectly by regulating the cell cycle of vascular endothelial cells\(^87\).

**Antinflammatory and antiatherothrombotic effects of statins**

The relationship between inflammation and atherosclerosis and its complications has received increasing attention. Inflammatory cell infiltration is commonly observed in atherosclerotic plaques\(^102\). A variety of circulating markers of inflammation, including C-reactive protein (CRP), serum amyloid A protein (SAA), heat shock protein 65, IL-6, and a number of leukocyte adhesion molecules\(^103-106\), have been shown to predict either the extent of atherosclerosis or the risk of vascular events. Atherosclerosis is prevented when genes of some mediators of the inflammatory response (such as monocyte chemoattractant protein-1 (MCP-1)\(^107\), interleukin-8 or macrophage-colony stimulating factor (M-CSF)) are eliminated by gene knockout in atherosclerotic-prone dyslipidemic mice\(^108,109\).

A pivotal role in the origin, progression and destabilization of atheroma is played by several protein families involved in the adhesion and activation of inflammatory cells into vessel wall, such as selectins, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)\(^109\). Inflammatory cells play a key role in promoting plaque destabilization: they stimulate matrix degradation and inhibit smooth muscle cell (SMC) function or survival, and may lead to thrombosis by increasing tissue factor expression\(^105\). Inflammatory cells can erode the fibrous cap of atheroma by releasing various matrix metalloproteinases and inhibiting matrix synthesis by SMCs. In particular, it has been shown that interferon-γ, an inflammatory cytokine produced by activated T cells, can down-regulate collagen synthesis by human SMCs in vitro\(^110\). Inflammatory cells are also cytotoxic to SMCs and can induce apoptotic cell death\(^109\). There are also suggestions for a role of viral\(^111\) or bacterial (i.e. *Helicobacter pylori*\(^112\) or *Chlamydia pneumoniae*\(^113\)) infections in promoting inflammation and plaque destabilization, but these need confirmation\(^114,115\).

HMG-CoA-reductase inhibitors have been shown to inhibit several pathways in inflammatory processes occurring in plaque destabilization, cerebral hypoxia, ischaemia and reperfusion (Table 2). Statins reduce leukocyte–endothelium interactions occurring either in hypercholesterolaemic\(^116\) or in normocholesterolaemic conditions\(^68,89\), and inhibit monocyte adhesion to the endothelium by reducing the endothelial expression of P-selectin, ICAM-1 and VCAM-1, as well as the number of monocytes expressing Mac-1, one of the ICAM-1
In hypercholesterolaemic humans, simvastatin and lovastatin reduce monocyte expression of CD11b/CD18, another ICAM-1 ligand[103,117]. These effects, mediated through reduced isoprenylation of leukocyte G-proteins or reduced isoprenoid-dependent anchoring or dimerization of adhesion molecules such as CD11b/CD18 on monocytes, are involved in reducing ischaemic cell damage after cerebral artery occlusion. These anti-adhesion molecule effects appear to be dependent upon enhanced NO production, since statins failed to ameliorate the high leukocyte-rolling and adherence in eNOS-deficient mice[118]. Antiinflammatory effects of statins have been recently confirmed by an analysis of the CARE study showing a reduction of cerebral and coronary ischaemic events, during pravastatin treatment, related to reduced CRP levels[119]. Although not always clearly demonstrated in normocholesterolaemic conditions, these effects are likely to occur, at least in part, independent of cholesterol lowering[41].

Statins also modulate central nervous system production of cytokines such as TNF-α, IL-1β and IL-6. The demonstration that these effects are reversible with the co-administration of mevalonate or farnesyl pyrophosphate also suggests the involvement of decreased isoprenylation by statins of proteins implicated in intracellular signaling and inflammation[65,85]. Recently, antiinflammatory effect of statins have also been shown in human immune cells exposed to Chlamydia pneumoniae[120], an agent linked to increased cardiovascular risk.

Antithrombotic effects of statins

Statins also exert beneficial antithrombotic effects. Acute ischaemic stroke has been shown to be associated with elevation of prothrombotic markers such as fibrinogen, plasminogen activator inhibitor-1 (PAI-1) and thrombin-antithrombin III complexes[67,121]. Statins reduce enhanced platelet reactivity accompanying hypercholesterolaemia, although it is not clear if such effect occurs independent of cholesterol reduction[122], and the thrombogenic potential of atheroma[123], also independent of cholesterol levels[124]. Statins also directly influence the local fibrinolytic balance (inhibiting PAI-1 expression and increasing tissue-type plasminogen activator, tPA, within the vessel wall), promoting fibrinolysis and thus reducing the thrombotic risk after plaque rupture[125,126]. By reducing the local expression of PAI-1 by SMCs within vascular lesions while increasing tPA expression by luminal endothelial cells, statins may tip the local fibrinolytic balance toward...
Figure 5  Schematic representation of the main mechanisms for the eNOS mRNA stabilization during statin therapy. Three main mechanisms contribute to eNOS mRNA instabilization through the mevalonate pathway: (1) L-mevalonate stimulates proteins that bind to a sequence motif (AUUUA) in the 3'-untranslated region of eNOS mRNA; (2) geranyl-geranylation is necessary for the proper function of Rho GTPases, favoring eNOS mRNA degradation; (3) isoprenoids contribute to cell proliferation, which favors eNOS mRNA degradation (eNOS mRNA stability is cell-cycle dependent). By inhibiting these mechanisms, statins stabilize eNOS mRNA leaving eNOS mRNA synthesis unaltered. The net result is an increase in eNOS mRNA steady-state concentration and protein.
increased fibrinolysis, which would limit the extent of thrombus formation that follows plaque rupture. On the other hand, increased local fibrinolysis may also promote extracellular matrix degradation (activation of matrix metalloproteinases) that, in turn, may destabilize advanced atherosclerotic plaques[126]. However most experimental results show that statins actually inhibit matrix metalloproteinase activity, thus probably leading to a reduced risk of plaque rupture[127,128].

Antioxidant effects of statins

Statins may also exert neuroprotection through antioxidant effects. These may contribute to reduced early atherogenesis, where low-density lipoprotein (LDL) oxidation plays a key role contributing to cholesterol accumulation in macrophages, foam cell formation, cytotoxicity, thrombosis and inflammation[129–131]. Moreover, in the presence of oxidized LDL, the G protein-dependent stimulation of NO release is interrupted, and the physiological action of NO is directly blocked by lipid peroxidation products[130]. Oxidative injury is also directly involved in the development and progression of neuronal cell death in acute and chronic diseases of the central nervous system. Oxidative stress to the central nervous system predominantly causes enhanced lipid peroxidation because of the high content of lipids, and, particularly, of polyunsaturated fatty acids, which are highly susceptible to oxidation. The central nervous system is particularly rich in the highly unsaturated docosahexaenoic acid, with six double bonds, therefore very liable to peroxidation[132].

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[66]. Fluvastatin has been claimed to scavenge hydroxyl radicals[131]. Some of the superoxide scavenging actions of statins may be due to their reduction in the biosynthesis of isoprenoids. Some superoxide-generating systems (e.g. the NAD(P)H oxidase system) are indeed isoprenylated proteins, and the inhibition of isoprenylation by statins would reduce the catalytic efficiency of these free radical generators[66]. Through still unclear mechanisms, statins have also been shown to reduce leukocyte-induced LDL oxidation[133], increase the a-tocopherol/total cholesterol ratio[134], and preserve the HDL-paraoxonase enzymatic system, which is consumed during HDL oxidation[134]. Metabolites of atorvastatin have been shown to protect HDL against oxidation and have a paraoxonase-sparing effect, protecting HDL-associated paraoxonase activity against oxidative degradation[135]. Finally, statins may exert broader antioxidant effects through preservation of superoxide dismutase activity[136] and the inhibition of cytokine-mediated upregulation of iNOS[86], which would otherwise promote the inflammatory response and oxidative damage through the increased production of peroxynitrite accompanying acute ischaemia. These antioxidant effects have been considered as a possible explanation for the reported reduction of chronic cerebral degenerative diseases, such as Alzheimer's disease, by statins[92,137].

Conclusions

There is persuasive evidence that statins protect from ischaemic stroke. This effect is probably multifactorial and due not only to atheroma stabilization in extracranial arteries and a reduction of CHD (reducing sources for thromboembolism), but also to direct neuroprotection. The latter appears to be largely independent of LDL cholesterol reduction and probably linked to reduction of isoprenoid intermediates in the mevalonate pathway.

Clinical and experimental data confirm the existence of endothelial protective, antiinflammatory, antithrombotic and antioxidant properties of statins that could extend the usefulness of this class of drugs in the management of cerebrovascular disease in patients with or without hypercholesterolaemia.

The effects of statins on stroke are presently the best available evidence for the existence of clinically relevant cholesterol-independent 'pleiotropic' effects of these drugs.

References

[40] Colquhoum D, Hicks B, Glassziou P. Comparative meta-analysis of 6 statins and 9 non-statinis coronary regression trials. Evidence that cholesterol lowering is the key to outcomes. Circulation 1996; 94: 579.


[105] Lim L, Manser E, Leung T, Hall C. Regulation of phosphor-


[108] Rajavashisth T, Qiao JH, Tripathi S et al. The role of oxidized lipoprotein, lipid mediators and statins on reducing susceptibility to atherosclerosis in mice that over-


with hypocholesterolemic effect of the drug and its binding to LDL. Atherosclerosis 1997; 128: 11–18.

