Statins and their role in vascular protection

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Abstract

The statins reduce cholesterol synthesis through inhibition of HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase and are widely prescribed for hyperlipidaemia to reduce the risk of atherosclerotic complications. The beneficial effect of lipid lowering by statins in the treatment of coronary heart disease has been demonstrated in large clinical trials. However, statins appear to have additional benefits on vascular function above and beyond their lipid lowering effects. Through inhibition of farnesyl pyrophosphate and geranylgeranyl pyrophosphate. Isoprenylation is important in the post-translational modification of a variety of proteins, including the small GTPases Rho, Rac and Ras, and hence plays an integral role in cellular signalling. Moreover, interference with isoprenylation underlies many of the beneficial actions of the statins on vascular endothelium, which include increased endothelial nitric oxide synthase expression, pro-angiogenic effects, increased fibrinolytic activity, immunomodulatory and anti-inflammatory actions, including increased resistance to complement. This has led to interest in the use of this class of drugs outside the realm of cardiovascular disease.

Introduction

Inhibitors of HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase, the statins, significantly reduce cholesterol synthesis. Their efficacy in reducing cardiovascular morbidity and mortality has been demonstrated in large intervention trials [1]. However, debate continues as to whether the beneficial effects of statins can be ascribed purely to their ability to reduce cholesterol or whether additional actions, independent of cholesterol lowering, play a significant role [2–5]. Clinical trial evidence demonstrates that lipid-lowering therapy reduces the progression of atherosclerosis and may lead to plaque regression and reduction in stenosis [6]. The potent cholesterol-lowering effect of the statins and the known close association of cholesterol levels with coronary atherosclerosis [7] suggests that the beneficial effects on cardiovascular mortality can be completely explained by lipid reduction. However, a recently reported study of pravastatin [8] suggests that, although the benefits of the drug in CHD (coronary heart disease) can be largely explained by lipid-lowering,
liver-independent actions may also have a significant role. Furthermore, detailed analysis of a number of large clinical trials has shown that the benefits of statins occur early, extend to patients within the normal LDL (low-density lipoprotein)-cholesterol range for Western populations [9] and exceed those of other lipid-lowering drugs, despite comparable falls in total cholesterol [1,10,11]. In addition, data from angiographic studies [6,10] suggest that the clinical benefit observed with statins is disproportionate to the rather modest improvement seen in luminal diameter. Although some of this apparent discrepancy may relate to the effects of lipid reduction on plaque stability, the demonstration of clinical benefits within 4 months of acute coronary events would appear to be too early to reflect significant changes in plaque anatomy due to reduced cholesterol [11,12]. Moreover, despite the fact that large trials have failed to demonstrate correlation between serum cholesterol levels and ischaemic CVA (cerebrovascular accident) [13,14], statins have been reported to reduce the incidence of ischaemic stroke [15], suggesting the presence of an additional protective mechanism.

These observations have led to the hypothesis that statins exert additional beneficial actions, independent of lipid lowering, now widely described as their ‘pleiotropic’ effects [2–5,16]. A wide variety of predominantly in vitro studies have been performed in order to identify these phenomena [17]. These have supported the hypothesis that statins exert significant vascular modulatory and anti-inflammatory actions, independent of their ability to lower cholesterol [2,4,5,18]. Although the pleiotropic effects of the statins are the subject of intense scientific interest, the debate between the clinical significance of the lipid-lowering and lipid-independent effects of the statins is far from resolved and remains an area of intense interest [3,16,19]. A number of concerns underlie the scepticism that has been expressed in some quarters.

First, statin therapy will reduce lipid levels in the vast majority of patients and it is difficult in clinical trials to separate cholesterol-lowering from non-cholesterol-lowering effects. Indeed, reduction in lipid levels might contribute significantly to statin effects such as improvements in endothelial function, immunomodulation and decreased thrombogenicity [3]. Furthermore, there is now considerable evidence to suggest that LDL-cholesterol is not the most appropriate index of the future risk of cardiovascular disease, nor indeed the best measure of response to statin therapy [20]. Results from the AFCAPS/TexCAPS (Air Force/Texas Coronary Atherosclerosis Prevention Study) and LIPID (Long-term Intervention with Pravastatin in Ischemic Disease) trials suggest that apoB (apolipoprotein B) levels and the apoB/apoA-1 (apolipoproteinA-1) ratio are better predictors of vascular disease in patients on statins than concentrations of LDL-cholesterol or the LDL/HDL (high-density lipoprotein) ratio [8,20,21]. Hence some of the benefits of statins in patients with ‘normal’ levels of LDL-cholesterol may reflect changes in these indices. Indeed, in the LIPID trial changes in apoB explained 87% of the proportion of treatment effect for pravastatin [8]. However, it should also be noted that the effects of statins can often be detected in rodent models in which there is no change in lipid levels.

Secondly, many of the in vitro studies of the effects of statins have used concentrations that exceed those achieved in the plasma in pharmacokinetic studies [22,23]. However, this may reflect reduced responsiveness of cultured cells to statins, similar to that seen with some cytokines, and it has yet to be determined how the concentrations of statins used in vitro relate to plasma and tissue levels achieved in vivo following oral dosage. Thirdly, it appears that hydrophilic statins, such as pravastatin and rosuvastatin, exert similar effects on the vasculature as do lipophilic statins, including atorvastatin and cerivastatin, which would be expected to have greater cell penetration [24]. A possible explanation for this is that inhibition of hepatic HMG-CoA reductase, leading to reduced circulating isoprenoids, may mediate cholesterol-independent effects and thus not require direct entry to vascular cells [23]. Finally, the relative lack of in vivo studies confirming in vitro observations has been a cause for justified concern [3,16,19]. This is, in part, related to the difficulties in adequately controlling for changes in plasma lipids in clinical trials. Although this may be achieved by the use of experimental animal models, these may not always accurately represent human pathophysiology [17]. Notwithstanding this, potentially important biological effects of the statins, independent of lipid lowering, have been demonstrated in well designed in vitro and in vivo studies and these will be discussed below.

This review will focus on the evidence for pleiotropic effects of the statins with particular focus on vascular inflammation and will relate, where possible, in vitro findings to supportive in vivo evidence.

**STATIN PHARMACOLOGY**

HMG-CoA reductase is the rate-limiting enzyme of the mevalonate pathway, through which cells synthesize cholesterol from acetate moieties [25]. Inhibition of HMG-CoA reductase, and the subsequent reduction in cholesterol synthesis, induces hepatocytes to increase their surface expression of LDL-receptors, so increasing uptake of LDL and reducing plasma LDL and cholesterol. In addition, the rate at which apoB particles are secreted by the liver is reduced. The statins exert their action through their ability to bind more potently to HMG-CoA reductase than HMG-CoA [26]. However, differences exist between statins with respect to their ability to bind HMG-CoA, their potency and
Pleiotropic effects of statins

Figure 1 The mevalonate pathway

Inhibition of HMG-CoA reductase by statins has multiple downstream effects, including inhibition of cholesterol biosynthesis and of isoprenoid intermediates such as FPP and GGPP. Geranylgeranyl and farnesyl are lipid attachments important in the post-translational modification of signalling proteins, including the small GTPases Rho, Rac and Ras and G-proteins. PP, pyrophosphate.

geranylgeranylation has been implicated in Rho translocation. Evidence to date suggests that many of the cholesterol-independent effects of statins are mediated via inhibition of Rho isoprenylation [32–34].

ENDOTHelial FUNCTION

Chronic abnormalities in vascular endothelial function, induced by factors such as smoking, hyperlipidaemia, systemic hypertension, diabetes or hyperhomocysteinaemia, play an important role in the pathogenesis of atherosclerosis [35,36]. Endothelial dysfunction, as measured by impaired flow-mediated vasodilatation, is an early manifestation of atherogenesis and is also present in inflammatory diseases such as SLE (systemic lupus erythematous), RA (rheumatoid arthritis) and Behcêt syndrome [37–40].

Raised levels of LDL have been associated with impaired endothelial function [41] and reduction of LDL by statins or following plasma apheresis reverses this [35,42,43]. However, beneficial effects on vascular endothelium following treatment with pravastatin and simvastatin have been reported within 1 month of starting treatment and in the absence of lipid lowering [44–47]. These effects include preservation of myocardial perfusion, coronary micropermeability and the structure of coronary adventitial vasa vasorum [48,49]. Thus it seems likely that improved vascular endothelial function following treatment with statins reflects not only their ability to lower cholesterol, but also lipid-independent effects.

Clinical trials

This hypothesis is supported by two small clinical trials. In the first, the short-term actions of cerivastatin on the endothelial function of elderly diabetic patients was investigated [50]. Twenty-seven patients with or without mild hypercholesterolaemia were treated with cerivastatin for 3 days. Endothelium-dependent flow-mediated dilatation was significantly improved by cerivastatin in the absence of any change in the lipid profiles. The beneficial effect was related, at least in part, to an increase in NO (nitric oxide) bioavailability. The second study was performed on eight normocholesterolaemic men who were treated with 80 mg of atorvastatin daily [51]. An increase in endothelium-dependent forearm blood flow was seen within 24 h and before any significant decrease in serum cholesterol or CRP (C-reactive protein). Moreover, withdrawal of atorvastatin after 30 days treatment led to a rapid return to the pretreatment level of forearm blood flow.

Role of NO

NO plays a central role in the maintenance of normal endothelial function and is generated in response to
lamellar shear stress. Endothelial NO is a vasodilator, inhibits smooth muscle proliferation, platelet aggregation, endothelial adhesion molecule expression and leucocyte–EC interactions [52]. The demonstration that statins are able to enhance local NO generation in ECs, by increasing the half-life of eNOS (endothelial NO synthase) mRNA, was fundamental to the acceptance of the emerging evidence for lipid-independent effects [53]. Statins retain their ability to increase eNOS in the presence of oxidized LDL and under hypoxic conditions [32,53]. In addition, statins exert further beneficial effects on the endothelium through their inhibition of the expression of the potent vasoconstrictor endothelin-1 [54]. These actions have now been demonstrated for a number of different statins, including simvastatin, lovastatin, atorvastatin, pravastatin and fluvastatin in in vivo and in vitro studies [18].

Detailed analyses of the means by which statins influence eNOS and endothelin-1 expression have revealed that addition of GGPP, but not FPP, squalene or cholesterol, reverses the effects of the statin [54,55]. Inhibition of Rho geranylgeranylation results in enhanced eNOS mRNA stability, but has no effect on eNOS gene transcription [55]. In vivo studies in animal models have suggested that statin-induced eNOS up-regulation may be of clinical significance, resulting in reduced cerebral ischaemia and neuroprotection [31,56] and limiting cardiac contractile dysfunction in myocardial ischaemia/reperfusion [57]. Confirmation that statins increase NO biosynthesis subsequent to their effect on eNOS has also been obtained in vivo [57,58]. Moreover, the importance of NO is demonstrated by the failure of statins to protect against ischaemic CVA or to inhibit leucocyte–EC interactions in eNOS-deficient mice [56,59].

THROMBOSIS AND FIBRINOLYSIS

HMG-CoA reductase inhibitors have various effects on haemostasis and thrombosis, favouring fibrinolytic over prothrombotic mechanisms, a function that may be especially important at sites of atherosclerotic plaque rupture [16,60]. Statins exert an antithrombotic action by preventing TF (tissue factor) expression and activity. This effect was first observed in monocytes and macrophages in vitro [61,62] and subsequently in vivo [63]. Similar results have been reported in human aortic ECs and VSMCs (vascular smooth muscle cells) in which simvastatin prevents TF induction by thrombin, at least in part, through inhibition of Rho-kinase-dependent Akt dephosphorylation [64]. The inhibition of TF by statins has also been reported in vivo [65], with fluvastatin reducing TF expression in carotid lesions in cholesterol-fed rabbits. Clinical studies have also supported an antithrombotic action of the statins independent of their cholesterol-lowering effects [66].

An important additional antithrombotic action is inhibition of platelet activation and function, reflecting both the lipid-dependent and -independent influence of statins. Potential mechanisms include increased eNOS and local NO, reduced thromboxane A2 synthesis and changes in the cholesterol content of platelet membranes [2,67]. In addition, a recent study [34] has reported that lovastatin increases Ecto-5′-Nu (ecto-5′-nucleotidase) activity and membrane expression in ECs, thus enhancing the inhibition of platelet aggregation at the EC surface through the action of the Ecto-5′-Nu product adenosine. This action appears to be independent of changes in membrane cholesterol. The inhibition of platelet function has been demonstrated in vivo in both animal [68,69] and human studies [70,71]. Furthermore, atorvastatin and rosuvastatin, through their actions on eNOS and platelet activation, protect against cerebral ischaemia and CVA in normocholesterolaemic mice [69,72].

In addition to their effects on haemostasis and thrombosis, the statins encourage fibrinolysis. Lovastatin, fluvastatin, atorvastatin and simvastatin have been shown [73–75] to increase the profibrinolytic factor tPA (tissue plasminogen activator), while inhibiting synthesis of the antifibrinolytic plasminogen activator inhibitor-1 by ECs, macrophages and VSMCs. These actions are mimicked by the RhoA inhibitor C3 exoenzyme [73] and prevented by GGPP, but not FPP, suggesting that the statin effect is mediated by inhibition of geranylgeranylation [30,73].

While it is tempting to assume that all statins act similarly, it is important to note that, although most studies support a predominantly antithrombotic action of the statins, some opposite or neutral effects have been reported [60]. Furthermore, not all statins affect fibrinolysis to the same degree and comparative studies have demonstrated some differences [60,76]. Thus, although results to date suggest that the statins may induce lysis of thrombus at sites of plaque rupture, further clinical trials are required to confirm this.

VASCULAR INFLAMMATION AND LEUCOCYTE TRAFFICKING

The concept of atherosclerosis as an inflammatory disease [77] and the evidence for cholesterol-independent effects of the statins raises the question of whether inhibition of HMG-CoA reductase has anti-inflammatory sequelae. This is of relevance not only to the treatment of atherosclerosis, but to vascular inflammation in general and to a broad range of diseases, including inflammatory arthritis, SLE, the vasculitides, glomerulonephritis, diabetes mellitus and multiple sclerosis. There has been intense recent interest in this area, with significant in vitro findings and some emerging in vivo supportive evidence [2,4,36].
Treatment of cultured vascular ECs with pravastatin, simvastatin, fluvastatin and cerivastatin reduces the synthesis of the proinflammatory cytokines IL-1β, TNFα (tumour necrosis factor α) and IL-6 and also the inducible form of cyclo-oxygenase (COX-2) [78]. Similar results have been reported in VSMCs following treatment with atorvastatin [79] and in leucocytes, with reports of reduced secretion of TNFα, IL-1β, IL-6, IL-8, MCP-1 (monocyte chemotactic protein-1) and MMPs (matrix metalloproteases) [63,80–84]. This, in turn, may result in changes in adhesion molecule expression, including inhibition of endothelial P-selectin, ICAM-1 (intercellular cell-adhesion molecule 1) and VCAM-1 (vascular cell-adhesion molecule) up-regulation [85,86] and reduced neutrophil and monocyte β2 integrin expression and activation [57,87,88]. A recent study [89] has demonstrated that simvastatin, administered 18 h before endotoxin, results in a 50% increase in eNOS, significant reduction in endothelial P-selectin expression and attenuation of leucocyte rolling and transmigration in the rat mesentery. Furthermore, microarray analysis is beginning to reveal the range of genes that may be regulated by statin therapy. A study [90] of men with cholesterol levels >5 mmol/l and/or triacylglycerols (triglycerides) >2 mmol/l and treated with atorvastatin has demonstrated that more than 200 genes are regulated by the statin. Among these are genes associated with inflammation, including those involved in lipid metabolism, leucocyte recruitment, thrombosis, apoptosis and atherosclerosis.

Treatment with simvastatin or fluvastatin has been shown to reduce significantly leucocyte adhesion to the endothelium of mesenteric post-capillary venules in both normocholesterolaemic and hypercholesterolaemic rats [89,91,92]. In studies of leucocyte–endothelial interactions under physiological flow, pretreatment of U937 monocytc cells with either cerivastatin or atorvastatin reduced firm adhesion [93,94]. This was the result of down-regulation of leucocyte integrins, inhibition of RhoA translocation and a reduction in F-actin content. Paradoxically, simvastatin has also been reported to potentiate the induction of E-selectin, ICAM-1 and VCAM-1 on TNFα- or IL-1β-treated ECs in vitro [95].

Mechanisms of action

The mechanisms responsible for the effect of statins on cytokine-induced cellular-adhesion molecule expression and leucocyte adhesion remain to be fully determined and appear to be multifactorial. The inhibition by statins of NF-κB (nuclear factor κB) is likely to be important [96–98], as is up-regulation of the nuclear receptors PPARα and PPARγ (peroxisome proliferator-activated receptors α and β) [78,99]. Moreover, the up-regulation of eNOS and NO production by statins reduces leucocyte adhesion to endothelium [89,100], and this may, in part, reflect NO-mediated inhibition of adhesion molecule expression/function [57,85,101]. An additional mechanism was proposed in a recent study [86] in which atorvastatin, cerivastatin and pravastatin all inhibited TNFα/IFNγ (interferon γ)-stimulated CD40 expression by ECs, resulting in a decrease in CD40-ligand induced IL-12 expression. Transcription factor analysis suggested that this was the consequence of inhibition of NF-κB and STAT (signal transducer of transcription)-1-dependent synthesis of interferon regulatory factor-1 [86]. Finally, of particular interest is the observation that lovastatin is an allosteric inhibitor of the function of the β2 integrin LFA-1 (leucocyte function antigen-1; CD11a/CD18). Lovastatin, simvastatin and mevastatin bind to a site in the I domain of LFA-1 locking the receptor in an inactive conformation, thereby preventing binding to its counter-receptor ICAM-1, an adhesion mechanism important for leucocyte adhesion to endothelium and T-cell costimulation [102].

In vivo studies

In a rabbit model of atherosclerosis, atorvastatin reduced neointimal inflammation with inhibition of NF-κB activation, MCP-1 synthesis and macrophage infiltration into the vascular wall [96]. Recent studies [103,104], using simvastatin and pravastatin, have confirmed that these effects are independent of cholesterol lowering. The atherosclerotic lesions in statin-treated animals had less IL-1β. VCAM-1 and TNF and reduced macrophage infiltration [104]. The role of NO in mediating the anti-inflammatory effects of statins has been demonstrated in vivo in studies using rosuvastatin [59], cerivastatin and pravastatin [105].

Evidence is also emerging to suggest that statins have important anti-inflammatory effects in other settings, including carrageenan-induced inflammation [103,106], inflammatory arthritis [107] and renal and myocardial ischaemia/reperfusion injury [108–110]. Likewise, in central nervous system inflammation, lovastatin and inhibitors of protein prenylation both ameliorated experimental encephalitis [111,112]. The potential of the statins as a therapy for inflammatory diseases has been demonstrated further in a recent study [113] of atorvastatin in experimental autoimmune encephalomyelitis, a Th1-mediated model of multiple sclerosis. Oral atorvastatin, at doses relevant to human therapy, was able to prevent or reverse chronic and relapsing paralysis. Atorvastatin promoted a Th2 bias, inducing STAT-6 phosphorylation and secretion of Th2 cytokines, while inhibiting STAT-4 and synthesis of Th1 cytokines [113]. Statin treatment also promoted differentiation of Th0 cells to Th2 and transfer of these cells conferred disease resistance.

Taken together, results from animal models suggest that statins possess potent effects against acute and chronic inflammation, which are cholesterol-independent and interfere with the leucocyte adhesion cascade [114]. It is now important to determine whether statins have
anti-inflammatory effects in human studies. The rapid beneficial effect of statins in some clinical trials suggests that this may be the case [11], as have recent studies of CRP [118–117], levels of which are reduced by statins independently of their effects on cholesterol.

**IMMUNOMODULATION AND TRANSPLANTATION**

Hypercholesterolaemia is common in transplant patients and the immunosuppressive drugs frequently used, including both cyclosporin and corticosteroids, also have a tendency to increase lipid levels [118]. Therefore the combination of potent lipid-lowering, anti-inflammatory and immunosuppressive actions suggests that the statins might be particularly useful in organ transplant recipients, and recent work in the transplantation field has reinforced this view [19]. However, not all of the studies of the use of statins in transplantation have controlled cholesterol levels, making it difficult to establish whether lipid-independent actions are important.

**Mechanism of action**
The ability of statins to inhibit monocyte/macrophage function [80,84], B lymphocyte activation and proliferation [119] and T lymphocyte and natural killer cell cytotoxicity [120,121] is relevant to the prevention of graft rejection. Furthermore, pravastatin, lovastatin and atorvastatin have been shown [122] to inhibit IFN-γ-induced expression of MHC class II in macrophages, endothelial and VSMCs, but not B cells or dendritic cells. The mechanism for this action is through inhibition of promoter IV of CIITA (MHC class II transactivator), which regulates MHC class II gene transcription [122]. Although this response required high doses of statins and remains to be confirmed in vivo, it may represent an important action of these drugs [123]. The reduced MHC class II expression on arterial ECs, SMCs (smooth muscle cells) and infiltrating macrophages is likely to reduce T-cell proliferation and differentiation. Given the emerging importance of the immune system in the pathogenesis of atherosclerosis [124], this is likely to have an important influence on disease progression. Moreover, the benefits may extend to other inflammatory diseases such as RA, multiple sclerosis and chronic graft rejection.

**Animal models**
Simvastatin and pravastatin have been shown [125–127] to reduce chronic rejection and the accelerated atherosclerosis of graft vessels in rat cardiac and hepatic transplant models. These drugs also attenuated the deleterious effects of ischaemia/reperfusion in the myocardium of normocholesterolaemic rats and in a renal model, in which elevated eNOS was also found [57,128]. In addition to the immunomodulatory and anti-inflammatory effects described above, the ability of statins to inhibit VSMC proliferation [33,129] may play an important role in attenuating transplant-associated arteriosclerosis [130]. In vitro studies suggest that statins arrest the cell cycle at the G1 to S phase transition in PDGF (platelet-derived growth factor)-treated VSMCs [33]. This effect on VSMC proliferation was reversed by GGPP, but not FPP or cholesterol [33,129]. Furthermore, inhibition of Rho by C3 exoenzyme reproduced the effect of statins. These studies suggest the presence of a cholesterol-independent mechanism contributing to the protective effects of statins post-transplantation, but this remains to be confirmed in vivo.

**Clinical trials**
A number of clinical trials in heart transplant patients have reported improved outcomes in terms of survival and graft arteriosclerosis. Comparison of pravastatin-treated patients with controls receiving conventional immunosuppression alone demonstrated increased 1-year survival and a reduction in acute rejection [131]. Likewise, simvastatin has been reported to improve 4-year survival, reducing local release of pro-inflammatory cytokines, including TNFα, reducing vasculopathy [132] and improving endothelial function [133]. Preliminary results [134] also suggest that statin treatment improves patient survival by 24% following kidney transplantation.

**ANTIOXIDANT EFFECTS OF STATINS**
It is well established that hypercholesterolaemia is associated with increased LDL oxidation and oxygen radical formation [135,136]. The endothelium seems to be the source of the increased superoxide anion, which inactivates NO, resulting in impaired endothelial function [135]. Reduction of plasma lipids by drugs or dietary manipulation can reverse this by reducing oxidative stress and changing LDL composition, so rendering it less susceptible to oxidation [135,137,138]. Lipid-independent actions of statins may also play an important role in this regard by increasing endothelial NO biosynthesis and reducing generation of reactive oxygen species through inhibition of NAD(P)H oxidase activity. This results in reduced LDL oxidation and intracellular oxidative stress [5,18]. A potential additional mechanism is statin-enhanced Ecto-5′-Nu activity, which is increased further by hypoxia [34]. This has also been demonstrated in vivo [139], where pravastatin, at a dose which did not normalize serum cholesterol, increased Ecto-5′-Nu activity in the ischaemic rabbit myocardium and reduced infarct size. Extracellular adenosine production by Ecto-5′-Nu has been shown [140] to be important in the protection of the vasculature against hypoxic stress.
The ability to scavenge oxygen-derived free radicals is shared by many statins, including simvastatin [141], fluvastatin[142], atorvastatin, pravastatin and cerivastatin [143], and has been demonstrated in a variety of cell types, including macrophages, neutrophils, VSMCs and ECs [141,144]. The functional effects of reduced oxidative stress are varied. Simvastatin, fluvastatin and pravastatin inhibit lysosphatidylcholine-induced oxidative stress in cultured VSMCs and this was mirrored by the ability of these drugs to inhibit VSMC migration in vitro [145]. Atorvastatin prevented hyperglycaemia-enhanced superoxide formation in coronary artery segments [146]. In vivo, simvastatin may attenuate hypoxia and oxidative stress in the coronary artery wall, preserving endothelial function in coronary epicardial vessels and arterioles independently of cholesterol lowering [47,49]. Cytoprotection has also been demonstrated with rosuvastatin, which increases vascular eNOS production and attenuates myocardial necrosis following ischaemia/reperfusion in wild-type, but not eNOS-deficient, mice [109]. Furthermore, withdrawal of statin therapy induces oxidative stress and endothelial dysfunction in mice. The mechanism underlying this involves activation of gp91phox-containing NAD(P)H oxidase by Rac-1 resulting in generation of superoxide anions, which scavenge eNOS [147].

The Rho family member Rac1 is a regulatory component of NAD(P)H oxidase and inhibition of Rac1 isoprenylation by statins inhibits release of reactive oxygen species in ECs [143]. Statins exert similar effects on VSMCs and cardiac myocytes, inhibiting Ang II (angiotensin II)-induced reactive oxygen species through inhibition of Rac1 geranylgeranylation [148,149]. These actions may have important consequences, as demonstrated by the ability of simvastatin to inhibit cardiac hypertrophy in rats treated with Ang II or subjected to transaortic constriction [148].

The action of statins on Ang II-mediated responses may be due to both lipid-lowering and lipid-independent actions of the statins. LDL may induce AT1 (Ang II type 1 receptor) up-regulation in VSMCs in vitro [150], and both hypercholesterolaemic rabbits [151] and humans have increased expression of AT1 [152]. Moreover, in the latter study the reduction of lipids by statins down-regulated AT1 expression. Statins have also been shown to reduce AT1 expression and so interfere with signalling mechanisms downstream of the receptor independently of cholesterol lowering [148,149,153].

**VASCULAR CYTOPROTECTION AGAINST COMPLEMENT-MEDIATED INJURY**

The complement cascade plays a central role in defence against infection and in the modulation of inflammatory responses [154,155]. However, complement has also been implicated in the pathogenesis of many inflammatory diseases, including glomerulonephritis, RA, SLE and atherosclerosis. In order to prevent bystander injury to host tissues following complement activation, a variety of soluble and membrane-bound complement regulatory proteins have evolved. These include the cell-surface proteins DAF (decay-accelerating factor; CD55), CD46 (membrane cofactor protein), CD59 (protectin) and CD35 (complement receptor 1) [156]. DAF is inducible on the EC surface and this results in enhanced cytoprotection against complement-mediated injury during inflammation, thrombosis and angiogenesis [157–159]. Furthermore, treatment of human umbilical vein, aortic and dermal microvascular ECs with atorvastatin or simvastatin leads to a significant increase in DAF expression and inhibits complement-mediated cell lysis [160] (Figure 2).

**Mechanism of action**

The pleiotropic effect of statins on vascular endothelium have been associated predominantly with increased eNOS-derived NO and/or activation of PI3K (phosphoinositide 3-kinase)/Akt [5]. In contrast, the up-regulation of DAF by statins was not linked to these pathways. DAF up-regulation required gene transcription, increased steady-state mRNA and de novo protein synthesis, but was not attenuated following inhibition of NOS or PI3K/Akt. Up-regulation was independent of the inhibitory effects on cholesterol synthesis and was reproduced by C3 exoenzyme and a geranylgeranyl transferase inhibitor (GGTI 286) and was reversed by GGPP, but not FPP [160]. Although statin-induced up-regulation of DAF remains to be confirmed in vivo, these findings may represent a means by which vascular endothelium can be therapeutically conditioned for the treatment and prevention of atherosclerosis and other vascular inflammatory diseases involving complement activation.

**Role of complement in vascular disease**

The role of complement in the pathogenesis of atherosclerosis, myocardial infarction and the accelerated atherosclerosis following transplantation is well described, and a number of mechanisms by which complement may be activated in the arterial wall have been identified [161–163]. These include activation by CRP, which colocalizes with the C5b–9 MAC (C5b–9 membrane attack complex) in the atherosclerotic vessel wall [164,165] and has been shown [166] to activate complement both in vitro and in vivo. Thus CRP may represent one means by which chronic inflammation can be sustained in the arterial wall. Levels of CRP in the upper quintile of the normal range are associated with a significant increased risk of MI (myocardial infarction) and CVA amongst healthy individuals [167]. Moreover, a variety of statins have now been shown...
Figure 2 Statin treatment protects ECs against complement-mediated injury

Human umbilical vein ECs (HUVECs) were treated with atorvastatin (1 µmol/l) for 24 h and, following harvesting, were incubated with the anti-endoglin monoclonal antibody RMAC8 or medium alone for 30 min at 4 °C. HUVECs were then washed in Hanks balanced salt solution/1 % (w/v) BSA prior to addition of 20 % (v/v) normal human serum for 3 h at 37 °C. Binding of C3 was detected by flow cytometry using FITC-conjugated rabbit anti-(human C3) antibody. (A) Percentage of C3 binding to unstimulated and atorvastatin-treated HUVECs, with binding to unstimulated HUVECs shown as 100 %. Values are means ± S.D. (n = 3). The negative control (Neg) represents C3 binding in the presence of heat-inactivated human serum. (B) C3 binding to unstimulated (NHS) and atorvastatin (Atorva)-treated HUVECs in the presence of the DAF-inhibitory monoclonal 1H4. Results are expressed as relative fluorescence intensity and are means ± S.D. (n = 3). (C) Unstimulated (hatched bar) and atorvastatin-treated (black bar) HUVECs were loaded with calcein acetoxymethyl ester and opsonized with RMAC-8 prior to exposure to rabbit serum for 45 min at various concentrations. Calcein release was measured and the percentage of HUVEC lysis was calculated (values are means ± S.D.). *P < 0.05 and **P < 0.01. This Figure is reproduced from Mason, J. C., Ahmed, Z., Mankoff, R., Lidington, E. A., Ahmad, S., Bhatia, V., Kinderlerer, A., Randi, A. M. and Haskard, D. O. Statin-induced expression of decay-accelerating factor protects vascular endothelium against complement activation, Circulation Research 91(8), 696–703 with permission. © (2002) Lippincott, Williams & Wilkins.
mid-range therapeutic doses of these drugs in humans are likely to be angiogenic and high doses angiostatic. In vitro studies demonstrated that the low-dose range enhanced EC proliferation and migration, via release of VEGF (vascular endothelial growth factor), whereas higher doses reduced VEGF and resulted in apoptosis. The low-dose effects were mediated by activation of PI3K/Akt and the high-dose effects by inhibition of geranylgeranylation [179–181]. Moreover, the stabilization of eNOS mRNA was only achieved by the higher dose range [180]. These dose-dependent actions held true in murine models of angiogenesis in which cerivastatin decreased tumour growth and vascularity in a Lewis lung carcinoma model [179]. However, a recent study [182] suggests that this paradigm may not hold true in all situations. The authors report that low concentrations of cerivastatin (0.005 M) inhibit advanced glycation end-product-induced angiogenesis in vitro through a mechanism dependent upon inhibition of FPP and not GGPP.

**Therapeutic potential**

The ability of statins to modulate angiogenesis has led to speculation about their therapeutic potential in cardiovascular disease, cancer, inflammatory arthritis and diabetic retinopathy [171–173,178,179]. Although these proposals are exciting, they must be explored further in carefully controlled clinical trials, not least because it remains unclear whether current lipid-lowering doses of statins in humans are predominantly pro- or anti-angiogenic. Furthermore, in atherosclerotic disease there are potential benefits of angiostatic effects, leading to impaired development of intraplaque microvessels, but also of pro-angiogenic actions, encouraging growth of collateral vessels [183,184]. Moreover, absence of results suggesting increased malignancy in patients treated with current recommended doses of statins might be interpreted as demonstrating that any pro-angiogenic effects in vivo are weak [185]. However, it remains imperative to carefully define the effects on angiogenesis of currently available statins, so as to fully establish the beneficial actions and potential risks.

**ATHEROSCLEROTIC PLAQUE STABILIZATION**

Angiographically analysed trials of lipid-lowering therapy in CHD have demonstrated that the reduction in future cardiac events greatly exceeds that expected from the minimal increase in coronary artery luminal diameter [1,186]. This led to the concept of plaque stabilization, which describes changes in the biology of the plaque, including a thicker collagenous fibrous cap, a smaller lipid pool, increased SMCs and fewer inflammatory cells [16,77]. As a consequence of these changes, the risk of plaque rupture and subsequent thrombosis leading to acute coronary syndromes is reduced.

There is substantial evidence that reduction of lipid levels by measures, including dietary manipulation, statin or non-statin therapy, encourages accumulation of mature VSMCs, reduces local vascular inflammation, cholesterol accumulation in macrophages and biosynthesis of MMPs by macrophages and VSMCs, all features that may result in plaque stabilization [187–191]. The ability of statins to facilitate plaque stabilization is well established in animal models. Therapy results in reduced macrophage accumulation and activation, with an associated reduction in the expression of TF, synthesis of pro-inflammatory mediators and MMPs in cholesterol-fed rabbits [63,96,192,193]. There is also some evidence for similar beneficial effects in patients treated with pravastatin and simvastatin. Analysis of carotid plaques demonstrated increased collagen deposition and reduction in lipid content, leucocyte infiltration and MMPs [194].

Although the benefits of lipid-lowering therapies are without dispute, the rapidity of their actions suggests lipid-independent effects are important in plaque stabilization [2,16]. These may explain the ability of the statins to protect against ischaemic CVA, despite levels of LDL–cholesterol being a poor predictor of such events [195]. Pravastatin has been reported to improve acetylcholine-induced vasodilatation of the coronary artery in non-human primates and to alter plaque histology to favour stability, independently of changes in plasma lipids [46]. A recent study [104] has extended these findings, demonstrating that pravastatin and simvastatin reduce inflammation and enhance features associated with plaque stability, including increased collagen and SMC content and reduced MMP synthesis. Similar findings have been reported in apolipoprotein E-deficient mice in which simvastatin promoted plaque stability independent of cholesterol lowering [196].

The doubts about the clinical significance of the lipid-independent effects of statins on plaque stability remain [3], not least because of the difficulty in separating these effects from those of reduced cholesterol in clinical trials [17]. However, carefully performed studies in experimental models under controlled conditions, such as those above, are beginning to suggest that both cholesterol lowering and pleiotropic effects of the statins contribute to plaque stability and the reduction in clinical events seen in patients treated with these drugs.

**CONCLUSION**

The explosion of studies describing pleiotropic effects of the statins (Figure 3) are the subject of intense and justified interest. They highlight the fact that the potential use of these drugs is diverse, both within the spectrum of cardiovascular diseases, such as CHD, ischaemic CVA,
A wide range of effects of statins on the vasculature have been reported, including anti-inflammatory, immunomodulatory, pro-angiogenic and antithrombotic actions. Many of these appear to be dependent, at least in part, on cholesterol-independent actions of the statins.

ischaemia/reperfusion and cardiac transplantation, and outside, where evidence is emerging to suggest that statins may have direct therapeutic benefits in the treatment of Alzheimer’s disease, osteoporosis and multiple sclerosis [113,197,198].

The role of the statins in the management of inflammatory disease is also the focus of considerable interest. It is now clear that systemic inflammatory diseases such as RA and SLE, in addition to their primary features, have secondary effects, including premature atherosclerosis and increased cardiovascular mortality [169,199]. This observation raises the question of whether statins should be used prophylactically in such patients, regardless of their lipid profile, in order to reduce the risk of cardiovascular mortality. This is supported by the reports of cholesterol-independent anti-inflammatory and vasculoprotective actions. Moreover, the use in the same patients of bisphosphonates, for the prophylaxis of steroid-induced osteoporosis, could be considered an analogous scenario. However, the jury is still out and too many uncertainties remain to recommend the widespread use of such an approach in normocholesterolaemic patients. Notwithstanding this, when considering the use of a statin in patients with chronic inflammatory disease, the assessment should take into account the effect of the underlying disease as a significant CHD risk factor, much in the way that diabetes mellitus is currently assessed.

It is also important to accept that the diverse actions of the statins, although exciting, are also potentially harmful and may even exacerbate disease [24]. Further work is required to define the relevant doses of statins for each indication, as these may differ from those resulting in optimal lipid lowering. Furthermore, although these drugs are relatively safe, the recent withdrawal of cerivastatin is a reminder of the continuing need for vigilance. Hence on-going research aimed at the design of specific new drugs based on the statins and with defined targets is particularly important [114]. Moreover, the results of further randomized controlled clinical trials, required to differentiate the cholesterol-dependent and -independent effects of currently available statins and to establish their potential use in a variety of different disease states, are keenly awaited.

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