Review

Do the pleiotropic effects of statins in the vasculature predict a role in inflammatory diseases?

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Abstract

Pleiotropic effects are now described for the 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (or statins) that might have utility in the context of chronic inflammatory autoimmune disease. Here we discuss the pharmacology and established uses of statins and in this context describe potential anti-inflammatory and immune-modulatory effects. An extensive *in vitro* data set defines roles for statins in modifying endothelial function, particularly with respect to adhesion molecule expression and apoptosis. Broader effects on leukocyte function have now emerged including altered adhesion molecule expression, cytokine and chemokine release and modulation of development of adaptive immune responses via altered MHC class II upregulation. *In vivo* data in several inflammatory models, including collagen-induced inflammatory arthritis and experimental autoimmune encephalomyelitis, suggest that such effects might have immune-modulatory potential. Finally, a recent clinical trial has demonstrated immunomodulatory effects for statins in patients with rheumatoid arthritis. Together with their known vasculoprotective effects, this growing body of evidence provides compelling support for longer-term trials of statin therapy in human disease such as rheumatoid arthritis.

Introduction

Statins were developed and tested clinically on the basis of their capacity to suppress cholesterol biosynthesis and thereby modify an important vascular risk factor. Numerous clinical studies have demonstrated efficacy in this respect, both in secondary and primary prevention strategies. A significant recent advance in understanding vascular risk has identified the utility of C-reactive protein (CRP) and, by implication, inflammation as an important pathogenetic factor in atherogenic pathogenesis. In parallel, there has been increasing recognition that the vasculoprotective effects of statins might reside not only in lipid modification but also in direct effects on inflammation manifested presumably through direct effects on the vascular lesion, or via secondary modification of the hepatic acute-phase response and constituent moieties, particularly CRP. CRP measured in this context is typically of low concentration measured via high-sensitivity assays. A logical question arising from such studies concerns the capacity of statins, or statin-sensitive pathways, to operate in the context of 'high-grade' inflammation such as that characteristically seen in autoimmune diseases such as rheumatoid arthritis (RA).
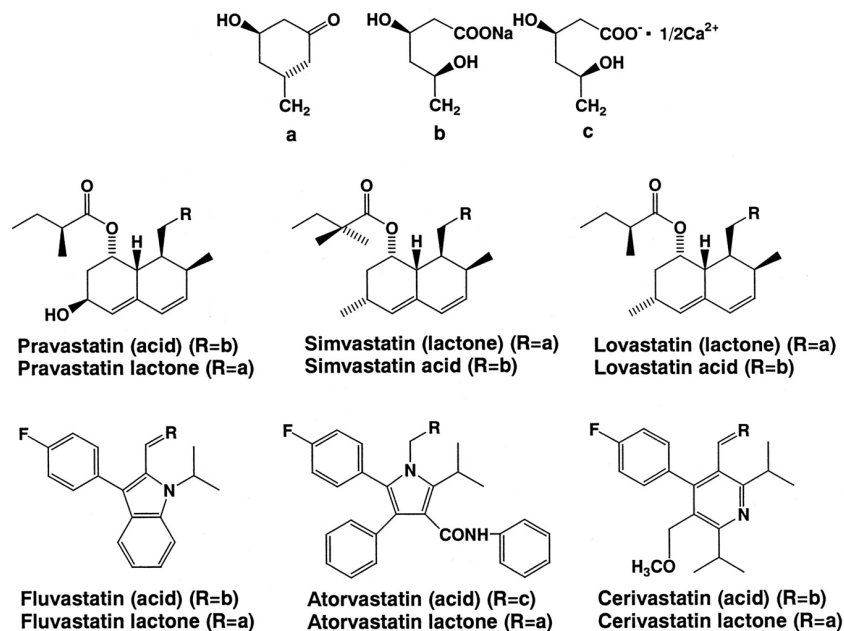
Pharmacology of the HMG-CoA reductase inhibitors

The enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase catalyses the conversion of HMG-CoA to mevalonic acid and is a rate-limiting step in the cholesterol biosynthetic pathway. Statins are selective, competitive inhibitors of this enzyme and are effective lipid-lowering drugs in humans. They decrease hepatic cholesterol synthesis, promoting the upregulation of low-density lipoprotein (LDL)-cholesterol receptors and increasing the removal of LDL-cholesterol from the plasma [1]. Numerous derivatives generated in this pathway, including squalene-derived moieties, farnesyl pyrophosphate and geranyl-geranyl pyrophosphate (GGP), in turn might interact with additional cell signalling pathways, some of which might have immune-modulatory potential. Five statins are currently available within the UK: pravastatin, simvastatin, fluvastatin, atorvastatin and rosuvastatin; in addition, lovastatin is available in other countries. Cerivastatin has been withdrawn from sale because of concerns over adverse events [2] (Fig. 1).

Lovastatin is a fungal metabolite, of which pravastatin and simvastatin are semi-synthetic derivatives, whereas fluvastatin, atorvastatin and rosuvastatin are entirely synthetic [1]. Lovastatin and simvastatin are of the lactone

Figure 1

Molecular structures of some of the HMG-CoA reductase inhibitors. (From [3]; reproduced by permission of The American Society for Pharmacology and Experimental Therapeutics.)

pro-drug form, whereas atorvastatin, fluvastatin and pravastatin are presented in the active (acid) form [3]. Rosuvastatin belongs to a novel group of methane-sulphonamide pyrimidine- and *N*-methanesulphonyl pyrrole-substituted 3,5-dihydroxy-6-heptenoates [4]. All of the drugs have high oral bioavailability, are subject to significant first-pass metabolism and have active metabolites. All of the statins except for pravastatin and rosuvastatin are relatively lipophilic [3].

Efficacy of the HMG-CoA reductase inhibitors in vascular disease

Statins are now established in the first-line treatment of hyperlipidaemia refractory to dietary intervention [5]. Their primary effect is to decrease LDL-cholesterol and total cholesterol; however, they have also been shown to effect benefit by decreasing apolipoproteins B, C-II, C-III and E, and by modestly increasing high-density lipoprotein-cholesterol [5], an effect that might be linked to their ability to activate peroxisome-proliferator-activated receptor (PPAR)-α. Decreases in triglycerides are particularly striking with atorvastatin, and this effect is thought to be attributable to increased binding and clearance of very-low-density lipoprotein particles in which most of the triglycerides are transported [6]. The decrease in LDL-cholesterol is dose-dependent and is typically in the range 20–45%, although larger decreases can be achieved with higher doses [7].

Although the statins were developed as lipid-lowering drugs they are now used mainly in the primary and

secondary prevention of vascular events. The 4S trial [8] showed for the first time the benefits of statins in secondary prevention of coronary events in patients with elevated cholesterol levels. In this study, 4,444 patients with angina pectoris or previous myocardial infarction, and moderately elevated cholesterol levels (5.5–8.0 mM), received either simvastatin or placebo and were followed up for a mean of 5.4 years. The simvastatin-treated group were significantly less likely to die (all causes and cardiac mortality) and underwent significantly fewer major coronary events. A role for statins in the primary prevention of cardiovascular events was observed in the WOSCOPS trial [9]. Pravastatin was shown to decrease cardiovascular events and mortality by about 30% in middle-aged male patients with a moderate degree of hyperlipidaemia but no prior personal history of cardiovascular disease. The value of statin therapy in patients with known coronary artery disease and normal lipid profiles is perhaps best defined by virtue of the Heart Protection Study [10], among others. This study clearly demonstrated that the relative risk reduction for vascular events was the same irrespective of baseline cholesterol level, and that even patients with cholesterol levels less than 5 mM received significant protection from simvastatin. Furthermore, it is clear that statin therapy has even short-term benefits when administered in the setting of the acute coronary syndrome in patients with normal cholesterol levels. The MIRACL study demonstrated that atorvastatin in this context reduced the risk of recurrent events in the first 16 weeks after an index event [11].

Effects of statins beyond lipid lowering in cardiovascular disease?

The increasing body of evidence for the efficacy of statins in protecting against vascular events irrespective of cholesterol status has prompted investigation of the pleiotropic effects of these drugs. Many data now suggest a key role for inflammation in the pathogenesis of atherosclerosis [12]. This provides a context in which various anti-inflammatory and immune-modulatory effects of statins have been explored. *In vivo* in clinical trials it has been shown that markers of inflammation in serum, in particular CRP, are decreased by statin therapy [13]. It has been suggested that inflammatory markers could be used as indicators of cardiovascular risk, although the relative level of prediction given by CRP remains in debate [14]. Statin-induced reductions in CRP have been shown both in patients with and without established cardio-vascular disease [15]. Interestingly, these anti-inflam-matory effects are not correlated with the lipid-lowering effects of statins, suggesting novel mechanisms of action [12]. Commensurate with this, broad effects have now been demonstrated across distinct components of the host immune and inflammatory response.

Statins modify endothelial dysfunction – effects on inflammatory initiation and perpetuation via endothelial cells

Effects of statins on endothelial cells have been studied primarily in the context of the endothelial dysfunction that typically predates atherosclerosis. High-resolution vascular ultrasound studies demonstrate that atorvastatin reduces endothelial dysfunction in patients with type 2 diabetes and, critically, that this improvement is correlated with a decrease in CRP measured by high-sensitivity assay but not with changes in blood lipid profiles [16]. Plasma intercellular cell-adhesion molecule (ICAM)-1, vascular cell-adhesion molecule (VCAM)-1, E-selectin and P-selectin function as surrogate markers for endothelial dysfunction. Both simvastatin and atorvastatin decrease circulating soluble ICAM-1, E-selectin and P-selectin significantly in patients with established coronary artery disease [17]. This study showed no consistent effect on levels of VCAM-1, although other studies have shown similar decreases in this molecule with statin treatment [18]. It is proposed that statins decrease the expression of LOX-1, a receptor for oxidised LDL-cholesterol, and hence decrease adhesion molecule expression. Oxidised LDL-cholesterol treatment of human coronary artery endothelial cells upregulates the expression of VCAM-1, ICAM-1, E-selectin and P-selectin through a LOX-1-dependent pathway, and statins block this effect [18]. Statins also modify inflammatory gene expression locally in the vascular endothelium. In human umbilical-vein endothelial cells (HUVEC) atorvastatin decreases levels of mRNAs for interleukin (IL)-8 and monocyte chemotactic protein (MCP)-1 while promoting the expression of endothelial

nitric oxide synthase [19]. Statins also decrease cytokine-stimulated CD40 expression, in both human cultured endothelial cells and monocytes, thus potentially attenuating CD40 ligand-induced proinflammatory responses in atherosclerosis [20].

Effects of statins on various haemostatic parameters provide further evidence for beneficial effects on endothelial function. Both simvastatin and atorvastatin have been shown to promote a pro-fibrinolytic state with increases in serum D-dimer levels and tPA activity and a concomitant decrease in tPA antigen [21]. Both fluvastatin and atorvastatin decrease the expression of tumour necrosis factor (TNF)-α-induced plasminogen activator inhibitor-1 (PAI-1) in cultured human endothelial cells [22]. Simvastatin also reduces the expression of PAI-1 by cultured smooth muscle cells and endothelial cells [23]. This study confirmed that simvastatin promoted a twofold increase in tPA release from endothelial cells. These effects have been replicated in various studies and are apparently reversed by mevalonic acid and, hence, are dependent on HMG-CoA reductase inhibition [24]. Statins have also been shown to downregulate the expression of tissue factor, a potent pro-thrombotic agent [25].

Complement-mediated vascular damage is central to the initiation and perpetuation of inflammation, and this might also be ameliorated by statin therapy. Treatment of HUVEC with either atorvastatin or simvastatin promoted an up to fourfold increase in expression of decay-accelerating factor (DAF), thereby resulting in a significant decrease in C3 deposition and complement-mediated lysis of antibody-coated endothelial cells [26]. This effect was reversible by co-administration of GGP, a metabolite downstream from HMG-CoA reductase that is critical in the activation of RhoA signalling. DAF expression (or lack thereof) has recently been proposed as a critical tissue localising factor in immune-complex-mediated arthritis in the KBxN arthritis model [27], and complement-mediated promotion of synovial inflammation is well recognised in RA.

Effects on monocytes

The atherogenic plaque is reminiscent of chronic inflammatory lesions more akin to RA and Crohn's disease [28]. In particular there is widespread monocyte recruitment and macrophage activation manifest in cytokine expression. Several studies have therefore addressed the statin-mediated modulation of monocyte function. Atorvastatin activates nuclear receptor PPAR-γ in primary human monocytes in culture, in turn decreasing TNF-α production [29]. Pravastatin has also been shown to increase PPAR-γ expression and to suppress the translocation of nuclear factor κB (NFκB) in monocytes, thereby inhibiting the uptake of oxidised LDL. One comparative *in vitro* study demonstrated that all of the currently available statins inhibited lipopolysaccharide-

induced NFκB activation and that the effect was most profound with atorvastatin and simvastatin [30].

Statins might also mediate anti-inflammatory effects in part through their actions on cyclooxygenase-2 (COX-2). In a rabbit model of atherosclerosis [31], atorvastatin downregulated COX-2 expression, both *in vivo* and *in vitro*, that correlated with reduction in neointimal size, macrophage infiltration to the atherosclerotic plaque and decreases in expression of other inflammatory mediators such as IL-8 and matrix metalloproteinase-3. Effects on chemokine-mediated monocyte recruitment have also been suggested. Administration of atorvastatin at a modest dose (10 mg daily) to patients presenting with acute coronary syndromes significantly decreased circulating MCP-1 levels [32]. These findings were paralleled by similar findings *in vitro*. Statins also upregulate the expression of the scavenger receptor CD36 on monocytes [33]. Intriguingly, this effect was augmented by the co-administration of PPAR-γ ligands. Again, this statin effect was reversed by GGP, suggesting the critical importance of the inactivation of Rho GTPases. Together these studies suggest direct effects of statins on monocyte/macrophage function that can impinge on chemokine and cytokine release, on prostaglandin expression and on effector phagocytic function.

Effects on polymorphonuclear cell lineages

Relatively little is known about the effects of statins on neutrophils. Cerivastatin and simvastatin were shown, by using neutrophils from healthy volunteers, to reduce antineutrophil cytoplasmic antibody-induced respiratory burst activity *in vitro*, possibly by inhibition of ERK activation [34]. Statins also seem to decrease the expression of endothelial nitric oxide synthase in neutrophils [35]. Little work has been done to characterise the effects of statins on eosinophils; however, we recently observed a decrease in eosinophilia in bronchoalveolar lavage fluid in a murine model of allergic asthma [36]. However, direct effects on eosinophil function remain to be definitively demonstrated.

Statins and the adaptive immune response

Beyond these various effects on the innate immune response, several data now suggest effects for statins in acquired immune responses. Kwak and colleagues first showed that statins inhibit interferon-γ (IFN-γ)-inducible MHC-II expression in various cell types including endothelial cells and macrophages, thereby inhibiting MHC-II-mediated T cell activation [37]. This was mediated through the inhibition of the inducible promoter IV of the class II transactivator (CIITA). This effect was reversed in the presence of mevalonic acid, and no effect was observed in cells constitutively expressing MHC-II. Some statins also seem to have allosteric properties that allow them to block cell–cell interactions directly. Lovastatin and simvastatin bind to the L-site on the β2 integrin leukocyte function antigen-1 (LFA-1) [38] and selectively block the LFA-1-mediated adhesion and co-stimulation of lymphocytes. It is proposed that statins block direct cell contact mediated by LFA-1 on the T cell and ICAM-1 on the endothelial cell, thus inhibiting T cell adhesion, activation and recruitment to the atherosclerotic plaque. Because LFA-1 is also implicated in cytokine-activated T cell-mediated bystander amplification of inflammation in many tissue lesions including RA, this provides a central pathway whereby statins could critically modulate T cell activation and subsequent downstream effector function [39].

Moreover, direct effects on human dendritic cells are also reported. Human monocyte-derived dendritic cells incubated with simvastatin or atorvastatin and subsequently stimulated with a cytokine cocktail (TNF-α, IL-1β, prostaglandin E$_2$) exhibited an immature phenotype and a significantly lower expression of CD83, CD40, CD86, HLA-DR and CCR7 than controls. This effect was reversed by mevalonate or GGP. This was accompanied by a decreased ability to induce T cell proliferation, suggesting relevance of function [28]. Atorvastatin administration *in vitro* also inhibited IFN-γ-inducible transcription at multiple MHC CIITA promoters and suppressed class II upregulation in microglial cells [40]. IFN-γ-inducible expression of CD40, CD80 and CD86 co-stimulatory molecules was also suppressed. Statins might also effect changes in T cell polarisation, presumably in part via the above-mediated pathways. Studies both *in vitro* and *in vivo* suggest that statins tend to promote a T helper (Th) type 2 response and to suppress Th1 cytokine production [41]. *Ex vivo* and *in vitro* data from studies discussed below lend weight to this assertion [40,42]. That said, some investigators have shown enhanced IFN-γ release in human peripheral blood cultures exposed to statins *in vitro*, and the context of statin treatment might therefore be of some importance.

In vivo effects of statins in models of inflammation

Several groups have now investigated the enticing possibility that these various anti-inflammatory and immune-modulatory effects might have utility in disease states beyond atherogenesis. Sparrow and colleagues demonstrated that simvastatin had a comparable anti-inflammatory effect to that of indomethacin in the carrageenan-induced foot pad oedema inflammatory model [43]. A large dose of simvastatin (100 mg/kg) was required to achieve this effect, despite which there was no significant change in plasma cholesterol in the treated animals. This is in part explained by the upregulation of hepatic expression of HMG-CoA reductase in rodents when challenged with statins. We reported recently that simvastatin was effective both in preventing murine collagen-induced arthritis when given prophylactically and

in ameliorating established disease [42]. However, high doses (40 mg/kg) of parenterally administered simvastatin were required to obtain this effect. Greater than 50% inhibition of disease acquisition was achieved in the prophylactically treated animals. *Ex vivo* re-challenge of draining lymph node cells from treated animals with type II collagen showed that simvastatin had mediated an antigen-specific Th1-inhibitory effect with no evidence of a compensatory Th2 response. Interestingly, CIITA mRNA levels in lymph nodes were unaltered, suggesting that a general suppression of inducible class II MHC expression was unlikely to explain the effects observed. However, this effect, although confirmed for simvastatin, has not been observed with atorvastatin or rosuvastatin given orally in the collagen-induced arthritis model [44], and the extent to which these data are instructive in translating to human disease remains unclear.

However, results from models of other diseases are encouraging. Statins have been shown to inhibit the production of TNF-α and inducible nitric oxide synthase by microglia and astrocytes [45], generating interest in the possibility that they might be beneficial in diseases such as multiple sclerosis. Youssef and colleagues used the experimental autoimmune encephalomyelitis model that oral atorvastatin therapy prevented or reversed chronic and relapsing paralysis [40]. Commensurate with findings of Kwak and colleagues [37] they also showed that atorvastatin inhibited IFN-γ-inducible MHC-II expression in microglial cells and provided evidence that atorvastatin promoted Th2 differentiation in Th0 cells. Furthermore it was elegantly demonstrated by means of an adoptive transfer model that these Th2-differentiated cells protected recipient mice from developing the disease. These data provide further clear evidence that statins mediate antigen-specific, protective, immune-modulatory effects.

Other *in vivo* model studies are emerging. In a murine model of allergic asthma, we recently showed potential benefits of statin therapy on inflammatory airway disease. After priming and intra-nasal ovalbumin challenge, reductions in inflammatory cell infiltrate and eosinophilia in broncho-alveolar lavage fluid were observed with both oral and intraperitoneal administration of simvastatin [36]. Continuing studies are addressing the local cell-specific pathways subserving these *in vivo* observations. Finally, statins have recently been shown to prevent atrial fibrillation in a canine model of sterile pericarditis [46] and to be protective in a model of renal ischaemia–reperfusion injury [47].

Statins as immune-modulatory agents in human disease
Until recently, the only clinical evidence of beneficial immune-modulatory effects of statin therapy had come from the field of solid organ transplantation, and these sparse data were contradictory. A pilot study in kidney transplant recipients showed a significant reduction in the rejection rate in patients treated with pravastatin [48]. More recently, an international, multicentre, randomised, placebo-controlled trial with fluvastatin failed to replicate these findings [49]. These conflicting results might be explained by a lack of class effect in the immune-modulatory properties processed by statins or by other factors such as trial design. Two studies in heart transplant recipients have also reported conflicting results. Wenke and colleagues found increased long-term survival and lower rates of graft vessel disease but could not show any significant effect on rejection rates in a 4-year follow-up study with simvastatin [50]. In contrast, Kobashigawa and colleagues showed an improvement in rates of severe rejection with haemodynamic compromise with pravastatin, but no effect on mild or moderate rejection episodes [51].

Statins in RA?
Striking parallels may be drawn between the atherosclerotic plaque and synovitis in RA at the tissue level [52]. Similar populations of proinflammatory cells, notably activated macrophages and T cells, drive a primarily Th1-mediated response in both disease processes. It is also increasingly clear that uncontrolled inflammation in the context of various rheumatological disorders predisposes to atherogenesis, contributing to an increased burden of cardiovascular co-morbidity and premature mortality [53]. It is therefore of critical importance to develop increasingly effective anti-inflammatory therapeutic agents and to devise strategies for reducing parallel vascular risk in RA.

Statins may offer dual beneficial effects in modifying rheumatoid disease activity itself and are likely to be beneficial in the long-term management of patients at higher risk of cardiovascular disease. Our group recently reported the findings of a double-blind, randomised, placebo-controlled trial of atorvastatin in RA with predefined primary outcome measures in RA disease activity and secondary outcomes including surrogate markers of vascular risk [54]. We noted that atorvastatin significantly decreased lipids and several other risk factors predictive of coronary heart disease (fibrinogen and plasma viscosity). More importantly, at 6 months, the disease activity score using 28 joints (DAS28) improved significantly, albeit modestly, on atorvastatin compared with placebo (difference between groups −0.52, 95% confidence interval (CI) −0.87 to −0.17, $P = 0.004$). The DAS28 European League Against Rheumatism response was also more likely to be achieved in the atorvastatin group (odds ratio 3.9, 95% CI 1.42–10.72, $P = 0.006$). In line with the above data, C-reactive protein and erythrocyte sedimentation rate declined by 50% and 28%, respectively, relative to placebo ($P < 0.0001$, $P = 0.005$, respectively). Finally, swollen joint count also decreased (−2.69 versus −0.53; mean difference −2.16, 95% CI

−3.67 to −0.64, $P = 0.0058$). These data showed for the first time that statins can mediate modest but clinically apparent anti-inflammatory effects with modification of vascular risk factors in the context of high-grade autoimmune inflammation.

Although the results of our trial concurred with our hypothesis, we recognised many limitations in our study, including its modest size and duration. As a result, further studies of statin in RA are required in order to establish long-term benefits, in particular with respect to protection against cardiovascular events. Such studies are being planned. Moreover, whereas adverse events occurred with similar frequency in patients allocated atorvastatin and placebo in our trial [54], further larger studies are required to confirm definitively the safety of statins in patients with RA, many of whom are on multiple drugs with their own liver toxicity risk. Importantly, statins should not be recommended for use on the basis of this study in RA for disease-modifying purposes. Finally, long-term studies should also address which patients with RA would benefit most from statin use and also the issue of cost, because statins are not inexpensive and their widespread use in patients without RA is already consuming large portions of health budgets.

Conclusions

There are increasingly compelling data showing that statins possess significant anti-inflammatory and immune-modulatory properties that might be of importance to their efficacy in the prevention and treatment of cardiovascular disease. With the recognition of the critical role of vascular risk in the increased mortality associated with a variety of chronic inflammatory diseases, such properties might render statins an attractive adjunct to therapy. Various laboratory studies and one recent clinical trial now support the notion that these pleiotropic effects might have utility as direct immune modulators in other chronic, inflammatory, autoimmune conditions. Statins are widely used in practice and possess a favourable toxicity profile, suggesting that even modest efficacy might provide a beneficial therapeutic ratio. Further longer-term clinical studies are required to confirm our recent observations and to assess fully the extent to which this class of drugs might be of benefit to patients in these two crucial respects.

Competing interests

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