Pleiotropic vasoprotective effects of statins: The chicken or the egg?

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Abstract: Statins (3-hydroxy-3-methyl glutaryl coenzyme A [HMG-CoA] reductase inhibitors) are the most commonly used lipid-lowering drugs. Their main lipid-lowering effect is achieved by an increase in the expression of low-density lipoprotein cholesterol receptors associated with inhibition of cholesterol synthesis through inhibition of HMG-CoA reductase – the first and rate-limiting step in cholesterol synthesis. However, beyond cholesterol synthesis inhibition, inhibition of the HMG-CoA reductase affects as well the synthesis of other molecules with significant roles in different, yet often intercalating, metabolic pathways. On this basis, and supported by an increasing series of advocating epidemiological and experimental data, an extended dialogue has been established over the last few years regarding the nonlipid or “pleiotropic” actions of statins.

Keywords: statins, immunomodulatory, pleiotropic effects

Introduction

Statins are the most widely prescribed lipid-lowering drugs worldwide. They have been persistently shown to decrease serum low-density lipoprotein (LDL) cholesterol (by as much as 70%), total cholesterol and serum triglycerides and increase serum high-density lipoprotein (HDL) cholesterol. Statins target mainly hepatocytes and inhibit 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase, the enzyme that converts HMG-CoA into mevalonic acid, a cholesterol precursor. The mevalonate pathway then branches out before the synthesis of squalene and cholesterol (Figure 1). Other biologically important products are dolichols, which are involved in lipoprotein synthesis, ubiquinone, and the isoprenoids, farnesyl-pyrophosphate (PP) and geranylgeranyl-PP. Isoprenoids can be covalently bound to proteins, a process termed prenylation, and play an important role in the post-translational modification of regulatory proteins, such as G proteins, Ras, Rho, and Rab, that influence the polymerization, the membrane anchoring and intracellular trafficking and thus the biologic activity of these proteins.

Large-scale epidemiologic studies, such as 4S, WOSCOPS, CARE, LIPID, AFCAPS/TexCAPS, REVERSAL, AVERT, PROVE IT-TIMI 22, HPS, and ASCOT-LLA have provided solid data showing statins’ preventive effects on the progression of atherosclerosis and its clinical sequelae. The beneficial effects of statins on vascular clinical events had been attributed solely to their lipid-lowering action until recently. However, the results from these and several other recent clinical studies...
have been surprising and intriguing. In particular, statins’ efficacy even in patients with normal cholesterol levels,\textsuperscript{11,13} as well as the rapidity and magnitude of their action have suggested that other factors are at play. Careful comparison of the cardiovascular event rates in patients on statins with the rates expected for those with similar lipid levels from epidemiological data has shown that statin treatment decreases the rate below that expected.\textsuperscript{4,14} Furthermore, although some regression in lesion size has been achieved with statins,\textsuperscript{15} it is not of such a magnitude that could explain their clinical efficacy. The increasing perception of the inflammatory pathology of atherosclerosis has led to further research for the multipotentiality of these drugs. The term “pleiotropic effects” signifies effects of statins other than cholesterol-lowering, though for many of these effects it has not been proved that they are really independent of their hypcholesterolemic action.

**Mechanisms of action**

**Direct lipid actions**

**Inhibition of HMG-CoA reductase**

Statins target hepatocytes and inhibit HMG-CoA reductase, the enzyme that converts HMG-CoA into mevalonic acid, a cholesterol precursor (Figure 1). Through the inhibition of this rate-limiting step in cholesterol synthesis statins achieve an increase in the expression of LDL cholesterol receptors, which results in increased take up by hepatocytes of LDL cholesterol from the circulation and a decrease in

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**Figure 1** The mevalonate pathway.

*Abbreviations:* Acetyl-CoA, acetyl-coenzyme A; HMG-CoA, 3-hydroxy-3-methyl glutaryl coenzyme A; PP, pyrophosphate.
plasma LDL cholesterol level. Statins do not simply exert a competitive action against the normal substrate in the enzyme active site; instead, they bind reversibly HMG-CoA reductase and result in a conformational change in the enzyme active site, preventing HMG-CoA reductase from attaining a functional structure. The resultant increase in hepatic cell LDL receptors determines the reduction of circulating LDL and of its precursors (intermediate-density [IDL] and very low-density [VLDL] lipoproteins). Statins’ efficacy in the reduction of triglyceride concentration parallels LDL cholesterol reduction. HMG-CoA reductase inhibition by statins results also in the inhibition of hepatic synthesis of apolipoprotein B-100 and, thus, in the reduction of the synthesis and secretion of triglyceride rich lipoproteins, as well as in an increase in the production of receptors for apolipoprotein B. Statins have only modest effect on HDL increase and no influence on the concentration of lipoproteins.

Reduction of LDL susceptibility towards oxidation
At least five mechanisms have been proposed to explain statins’ antioxidant properties: (a) the reduced lipoprotein cholesterol and reduced level of oxidation substrate, as a result of their hypocholesterolemic effect; (b) the inhibition of the generation of superoxide by macrophages, which results in the decrease of cell oxygen production. The attenuation of the formation of superoxide anion in endothelial cells is also achieved by statins by preventing the prenylation of p21 Rac protein; (c) the preservation of the activity of the endogenous antioxidant system, like superoxide dismutase; (d) the binding of statins to phospholipids on the surface of lipoproteins (fluvastatin and lovastatin bind to LDL phospholipids) preventing the diffusion towards the lipoprotein core of free radicals generated during oxidative stress; and (e) the potent antioxidative potential of the metabolites (ie, atorvastatin and fluvastatin metabolites).

Inhibition of the expression of type A scavenger receptor
The inhibition of the expression of type A scavenger receptor in THP-1 cells and in human monocytes decrease the receptor-mediated degradation of oxidized LDL (oxLDL). Statins also reduce mRNA level and CD36 expression on the cell surface, as well as LDL binding to human U937 monocytes.

Intracellular signaling pathways
Several of the pleiotropic effects of statins, which affect endothelial function and redox equilibrium as well, have been attributed to the prenylation of regulatory proteins, such as the G proteins, and the enzymes involved in this prenylation have been proposed as potential targets for therapeutic intervention. Prenylated proteins may interact with specific membrane-bound receptor proteins and hence prenylation mediates protein–protein interactions. The complex process of cell signaling is very important for intercellular communication. Extracellular signaling molecules, which are water-soluble and have high molecular weight, need to bind to specific receptors on the cell surface, which transduce the extracellular signals into the cell by intracellular signaling pathways. Many intracellular signaling molecules are prenylated proteins. The specific receptors on the cell surface are associated with trimeric G protein, or have Ser/Thr/Tyr kinases activities. The trimeric G protein has a geranylgeranylated subunit (gamma), allowing this signaling protein to be inserted in the cell membrane near specific membrane receptors and to receive extracellular signals, which are then transferred to the secondary signaling molecules in the cell. Another important class of prenylated signaling molecules are the components of Ras family, which are farnesylated and intermediate Ser/Thr/Tyr kinases activities of membrane receptors from the cell surface. The small monomeric G proteins, such as Rho, are also regulated by prenylation. It has been known for some time that Rho is a regulator of actin-containing stress fibers of the cytoskeleton and more recently of focal adhesion sites, a cell membrane component connected to stress fibers. These sites are foci where integrins congregate and through which a cell makes adhesive contacts with extracellular components, either other cells or extracellular matrix proteins. They also contain the proteins focal adhesion kinase (pp125 FAK), p130, and paxillin. Recent evidence confirmed the essential role of Rho in the assembly of the focal adhesion sites. Other G protein pathways may also be involved in focal adhesion formation, as the lipoxigenase-derived arachidonic acid metabolite 5-hydroxyeicosatetraenoic acid can activate neutrophil self-adhesion via the G protein pathway Raf-1/Mek/Erk. In the case of the β1 integrins, which are involved in leukocyte adhesion to extracellular matrix, there is direct evidence that adhesion of U937 cells involves geranylgeranylated signaling proteins.

Despite the fact that several effects of statins were shown to be independent of cholesterol lowering in vitro, extensive evidence links hypercholesterolemia in vivo with increased lipid peroxidation and increased oxidative stress, and increased oxygen radical formation accompanying hypercholesterolemia influences many of the same factors that are modulated by statins via inhibition of prenylation.
It is well established that multiple signaling pathways regulating the expression of atherogenic genes are oxidation-sensitive, either because oxLDL activates them by binding to cell surface receptors or because increased extracellular lipid oxidation causes a shift in the intracellular redox balance. Among the many oxidation-sensitive pathways that affect cell growth, secretory activity, and death, three are particularly relevant in inflammation and atherogenesis. The first of these is nuclear factor-κB (NFκB), which regulates adhesion molecules and growth factors, including vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and monocyte chemoattractant protein-1 (MCP-1), important contributors to monocyte and T cell recruitment into the arterial intima. The second is the apoptotic signaling pathway that is activated through Fas/FasL and tumor necrosis factor (TNF) receptors and regulates the expression of caspases and other effectors of apoptosis. The third oxidation-sensitive pathway is the peroxisome proliferators-activated receptor γ (PPARγ) pathway. PPARγ is a nuclear receptor that regulates fat cell development and glucose. It is also highly expressed in macrophage/foam cells of atherosclerotic lesions. Activation of PPARγ by oxLDL or synthetic ligands upregulates the expression of the ABC-A1 transporter involved in reverse cholesterol transport from peripheral cells, but it also downregulates a number of pro-inflammatory factors, including TNFα, interleukin-1α (IL-1α), IL-6, the inducible nitric oxide synthase (iNOS), and gelatinase B, one of the metalloproteinases thought to promote plaque rupture. Therefore, many of the factors which hypercholesterolemia and its associated oxygen radical formation affect are modulated by statins via inhibition of prenylation. For example, the conversion of NO into the less active peroxynitrate is the mechanism through which hypercholesterolemia induced LDL oxidation interferes with NO-mediated vasodilatation. Statins act on this site by decreasing the activity of NAD(P)H oxidase and therefore by increasing endothelial NO production and decreasing the production of reactive oxygen species (ROS), reducing thus both LDL oxidation and intracellular oxidative stress.

**Pleiotropic effects**

Recent evidence revealed a multitude of actions of statins, other than lipid-lowering, on different types of cells, which have been addressed with the term “pleiotropic” and are predominantly vasoprotective. These include inhibition of smooth muscle cell growth, inhibition of neointima formation, induction of apoptosis in smooth muscle cells, reduction of leukocyte adhesion to and transmigration through endothelial cells, induction of endothelial nitric oxide synthase, inhibition of endothelin and MCP-1 expression in endothelial cells, inhibition of MCP-1, tissue factor and matrix metalloprotease-9 expression in macrophages. Figure 2 summarizes the mechanisms through which statins exert their vasoprotective effects.

**Effects on endothelial dysfunction and inflammation**

Atherosclerosis is a complex inflammatory process characterized by the presence of monocytes, macrophages, and T lymphocytes in the atheroma. Endothelial dysfunction is one of the earliest manifestations of atherosclerosis, occurring well before the presence of any angiographic evidence of disease. Studies in animals and humans have shown that the combination of hemodynamic strain and the accumulation of lipids may initiate an inflammatory process in the artery. Activated endothelial cells express several types of leukocyte adhesion molecules, which cause blood cells rolling along the vascular surface to adhere at the site of activation. An important characteristic of endothelial dysfunction is the paradoxical vasoconstriction caused by acetylcholine because of the impaired synthesis, release, and activity of endothelium-derived nitric oxide (NO). Statins reverse endothelial dysfunction through the reduction of both extracellular LDL oxidation (by reducing substrate availability) and intracellular oxidative stress (by cholesterol-independent effects on NO and, indirectly, by reducing oxLDL). Geranyl-geranylation of the GTP-binding protein Rho decreases endothelial cell nitric oxide synthase (eNOS) expression and inhibits nitric oxide-induced vascular relaxation. By blocking synthesis of geranyl-geranyl-PP, statins decrease geranylation of Rho and upregulate eNOS. Nitric oxide generation in endothelial cells is also promoted by another mechanism. Statins activate the protein kinase Akt/PKB, which results in enhanced phosphorylation (activation) of its natural substrate, eNOS. Regarding the effects of statins on Akt activation and nitric oxide, Laufs and colleagues showed that statins prevent the hypoxia-induced downregulation of NOS 3 in human endothelial cells via inhibition of mevalonate synthesis. Furthermore, in human, increased circulating NO was found in response to fluvastatin treatment, which correlated with decreased circulating soluble P-selectin and ICAM-1 levels. Kureishi and colleagues found that simvastatin activates the signaling molecule Akt in human umbilical vein endothelial cells (HUVEC), an effect requiring phosphatidylinositol 3-kinase (PI3-kinase), which indeed is normally the upstream activator of Akt, and being inhibited by the specific PI3-kinase inhibitor wortmannin.
Normally, PI3-kinase activity is suppressed by mevalonate, so that the decrease in mevalonate concentration caused by statin action would be expected to increase PI3-kinase activity. Akt itself undergoes phosphorylation by PI3-kinase, thereby phosphorylating NOS 3 (one of several substrates for Akt) and thus increasing NO production. Enhanced NOS 3 activity, and hence NO production, will give rise not only to vasodilatation but also to other potentially beneficial and vasoprotective effects: inhibition of atherogenesis, inhibition of platelet activation and aggregation, attenuation of endothelial cell apoptosis, and promotion of angiogenesis. Increased formation of NO promotes arterial vasodilatation and inhibits atherogenesis. NO is therefore emerging as a prime target for pharmacologic intervention. Resistance to the inhibitory effect of oxLDL on NOS 3 activity has also been elicited by statin treatment in vitro. By contrast with the induction of NOS 3 by statins, which has a generally beneficial action, lovastatin inhibited NOS 2 (the inducible isoform of NOS) expression in rat astrocytes, microglia, and macrophages. This may be advantageous, as the large quantities of NO generated by NOS 2 may not be as benign as the smaller amounts generated through NOS 3. In addition to preventing the Rho-mediated downregulation of eNOS, other effects of statins have been linked to geranyl-geranylation, such as inhibition of proliferation and induction of apoptosis in SMCs, inhibition of integrin-dependent leukocyte adhesion and increased fibrinolytic activity. The observation of some of these effects, eg, improved vascular function, in the absence of hypercholesterolemia supports the notion that they are cholesterol-independent. Unequivocal evidence that many of the above effects are due to protein prenylation has been provided by the fact that they are reversible by addition of geranylgeranyl-PP (or farnesyl-PP), which does not restore cholesterol synthesis, but not by addition of squalene or cholesterol.

Recent evidence suggests that statins possess anti-inflammatory properties because of their ability to reduce the number of inflammatory cells in atherosclerotic plaques. The mechanisms have yet to be fully elucidated but seem to involve inhibition of adhesion molecules expression, reduction of leukocyte adhesion, as well as inhibition of inflammatory and chemotactic cytokine production. Lovastatin and simvastatin have been found to reduce the production of MCP-1 in human peripheral blood mononuclear cells or endothelial cells following exposure to lipopolysaccharide (endotoxin), other bacterial products, or the inflammatory cytokine IL-1.

Figure 2. Pathways of the effects exerted by statins.
Abbreviations: LFA-1, leukocyte function antigen-1; MHL-II, major histocompatibility antigen-II; NO, nitric oxide; oxLDL, oxidized low-density lipoprotein; ROS, reactive oxygen species; SMC, smooth muscle cells.
Likewise, they reduced the exudates content of MCP-1 and the degree of leukocyte accumulation in a mouse air-pouch inflammation model.\textsuperscript{49} The expression of MCP-1 in both endothelial cells and monocytederived macrophages of atherosclerotic plaques is believed to be important in mediating monocyte chemotaxis and hence in stimulating atherogenesis. Indeed, it has been found that statins reduce the expression of adhesion molecules on leukocytes,\textsuperscript{50} inhibit leukocyte-endothelium interactions,\textsuperscript{51} and reduce inflammatory cell number within atherosclerotic plaques.\textsuperscript{52} Xenios and colleagues\textsuperscript{53} have shown in human iliac artery endothelial cell cultures that fluvasatin caused a substantial reduction in ICAM-1 expression, through the upregulation of eNOS and AMP kinase. Cicha and colleagues\textsuperscript{54} in their study on HUVEC cultures found that statins reversed the shear stress-induced expression of adhesion molecules on endothelial cells. Moreover, Lin and colleagues\textsuperscript{55} found in human aortic endothelial cell cultures that statins caused a similar reversion of homocystein-induced VCAM-1 expression upregulation. Interestingly, Liang and colleagues\textsuperscript{56} found in HUVEC cultures that simvastatin reduced VCAM-1 expression on endothelial cells as well as the adhesiveness of monocytes, through inhibition of NF-κB activation by C-reactive protein (CRP).

Integrins are activated through a conformational change of their molecule and it has been suggested recently that the principal regulation of integrins in vivo is by receptor clustering into adhesion complexes.\textsuperscript{57} Rho geranylgeranylation by HMG-CoA reductase products is likely to play a crucial role in this procedure.\textsuperscript{58} Cerivastatin was shown by Yoshida and colleagues\textsuperscript{59} to reduce human monocyte cell line adhesion to endothelial cells under physiological flow conditions via RhoA-dependent mechanisms. Similarly, atorvastatin was found to reduce the adhesion of U937 cells to HUVEC and to decrease RhoA and FAK activation also in those cells.\textsuperscript{60} It is likely that HMG-CoA reductase inhibitors can inhibit focal adhesion complex formation and thereby inhibit leukocyte adhesion. In the absence of an activating signal, the β2 integrin leukocyte function antigen-1 (LFA-1) does not associate with lipid rafts. After its activation in T-lymphocytes, LFA-1 is mobilized to the lipid raft domains. The association between LFA-1 and lipid rafts is required for LFA-1-dependent adhesion to occur, and similar results were obtained with α4β1 integrin.\textsuperscript{61} The recent work of Lum and colleagues\textsuperscript{62} provided direct evidence of the involvement of these events in leukocyte-endothelial adhesion. RhoA molecule, beyond its involvement in these events of integrin activation that take place in leukocytes, with particular relevance to atherosclerosis, it is also involved in endothelial cells in creating receptor clusters, which allow adhesion to monocytes. Association with the actin cytoskeleton is required, but the formation of stress fibers is not.\textsuperscript{53} Monocyte adhesion and spreading on human endothelial cells is dependent on Rho regulated receptor coupling in the latter. Therefore, both types of cell involved in the monocyte-endothelial interaction could possibly be affected by statin-mediated modification of Rho signaling, though evidence from cellular adhesion studies suggests that it is mainly the monocyte that is affected in vivo. Surprisingly, lovastatin, simvastatin, and other statins were found capable of binding to a novel site on the I-domain of LFA-1.\textsuperscript{64} This domain is probably involved in activation changes that allow binding to ICAM-1. Interestingly, even the lactone forms of the statins were capable of binding despite having no activity against HMG-CoA reductase. This binding inhibits the adhesive activity of LFA-1 while, a novel high affinity statin- related compound, LFA703, was found to have powerful anti-inflammatory activity. However, this field remains controversial, as long as statin-LFA-1 interaction was not confirmed by others.

Further parallel evidence was obtained from the suppression of IL-8 and MCP-1 production by cerivastatin in macrophages.\textsuperscript{65,66} Similarly, in endothelial cells, the more lipophilic statins upregulate PPARγ, resulting in decreased expression of IL-1, IL-6, and cyclooxygenase (COX)-2.\textsuperscript{67} The decrease in IL-6 production could provide an explanation for the decreased production of CRP observed in vivo in patients on statin therapy, as IL-6 is the principal inducer of its synthesis in the liver.\textsuperscript{68} More recently, it was shown in an in vitro system that human adipocytes can release CRP under inflammatory conditions and that this phenomenon may be modulated by statin treatment,\textsuperscript{69} a finding which might explain in part the beneficial cardiovascular effects of these drugs.\textsuperscript{70} Furthermore, recently it was found that statins reduce IL-6 induced CRP directly in hepatocytes via inhibition of protein geranylgeranylation and of the phosphorylation of the transcription factor STAT3.\textsuperscript{71,72} Other probable beneficial effects of statins include an increase in the number and mobilization of circulating endothelial progenitor cells (EPCs), which are cell with reparative action on sites of ischemic injury, the inhibition of the migration and proliferation and the induction of apoptosis of vascular smooth muscle cells (SMCs)\textsuperscript{73} and the inhibition of leukocyte–endothelial cell interactions.\textsuperscript{74} Statin treatment has also been found to reduce effectively experimental atherosclerosis as well.\textsuperscript{75–77}
Effects on immune responses

Although strictly speaking not yet proven, there is substantial evidence that statins may modulate immune responses. These include effects on the intimal recruitment, differentiation, proliferation, and secretory activity of a number of immune cells, mainly monocyte/macrophages and T cells. Recently, statins were found to inhibit the expression of class II major histocompatibility antigens (MHC-II) on human macrophages, endothelial cells and SMCs stimulated by interferon γ (IFN-γ). Precise regulation of MHC class II gene expression plays a pivotal role in the control of the immune response especially after transplantation. The expression of MHC-II on the surface of antigen-presenting cells together with processed antigens and cellular cofactors results in activation of the T cell receptor. Statins’ effect on the expression of MHC-II is exerted by both lipophilic and hydrophilic statins at nanomolar to micromolar concentrations, but it is limited to antigen-presenting cells requiring co-stimulation by IFN-γ, an effect which is dose-dependent for both MHC class II protein as well as mRNA. Whereas a limited number of specialized antigen-presenting cells express MHC class II constitutively, numerous other cells become MHC class II-positive upon induction with IFN-γ. This means that professional antigen-presenting cells constitutively expressing MHC-II, eg, B cells and dendritic cells are not affected. This complex regulation is under the control of the class II transactivator CIITA. Statins were found to inhibit promoter IV of the MHC-II transactivator CIITA, which regulates transcription of MHC-II and thus synthesis of the MHC-II protein. In contrast, statins did not affect the expression of MHC class I, pointing to specific actions in the MHC class II signaling cascade. This discovery has been proposed to provide a firm scientific rationale to recommend the use of this drug as an immunosuppressor. The reduction of MHC-II on the surface of antigen-presenting cells together with the consequent acute clinical events. Plaques with a high degree of smooth muscle cell proliferation, giving rise to well-developed tough fibrous caps on the luminal side of the lesions, rarely are complicated by thrombosis and are termed stable plaques. On the other hand, lesions rich in inflammatory macrophages and lipid deposits are mechanically weaker, as metalloproteinase enzymes from macrophages digest the extracellular matrix and weaken the wall.

Antioxidant effects

Another class of actions for statins and a potential mechanism by which statins may improve endothelial function is through their antioxidant effects. In this regard, a recent study showed that pravastatin therapy caused an early significant decrease in serum malondialdehyde concentration, an index of lipid peroxidation and plaque instability, and increase in flow-mediated dilation of the brachial artery, before any substantial reduction in blood lipid levels, in 37 patients with unstable angina. These findings suggest that pravastatin exerts pleiotropic effects on endothelial dysfunction during the early phase of the acute coronary syndrome, that are independent of the degree of plasma cholesterol lowering; however, it has never been unequivocally demonstrated in humans that prolonged statin treatment exerts effects independent of LDH cholesterol lowering because the use of statins always resulted in reduced LDH cholesterol levels. In a recent study, Pretnar-Oblak and colleagues found a significant improvement of the cerebrovascular reactivity to intravenous application of L-arginine, a perceived index of cerebral endothelial function, as well as flow-mediated dilatation of the brachial artery, after a three-month treatment with atorvastatin in patients with lacunar cerebral infarctions, a state of endothelial impairment. In their elegant study, Landmesser and colleagues showed that despite the similar reduction in LDH cholesterol levels caused by simvastatin and ezetimibe, a novel cholesterol absorption inhibitor, only simvastatin resulted in beneficial effects on endothelial function.

Effects on plaque stability

It is now established that atherosclerotic plaques are heterogeneous and vary in their tendency to undergo thrombosis and the consequent acute clinical events. Plaques with a high degree of smooth muscle cell proliferation, giving rise to well-developed tough fibrous caps on the luminal side of the lesions, rarely are complicated by thrombosis and are termed stable plaques. On the other hand, lesions rich in inflammatory macrophages and lipid deposits are mechanically weaker, as metalloproteinase enzymes from macrophages digest the extracellular matrix and weaken the wall.
They frequently crack, so exposing circulating platelets to an abnormal vascular wall that rapidly induces activation and a thrombotic mass.94 These are unstable plaques, the inflammatory nature of which is central to their clinically hazardous behavior. Coronary events are the result of unstable atherosclerotic lesion rupture and thrombus formation.95 High circulating cholesterol, apart from promoting atherogenesis, may also give rise to an increase in thrombogenesis through an increase in tissue factor expression.96 Fluvastatin has been shown to decrease this prothrombotic tendency with an associated reduction in tissue factor, and this may be partly through cholesterol lowering but also through non-cholesterol-mediated actions, such as reduced prenylation of the Rho protein Cdc42. Statins influence plaque stability by preventing macrophage activation, reducing the uptake and endogenous synthesis of cholesterol and the production of metalloproteinases by macrophages.97 Metalloproteinases are the enzymes responsible for weakening the plaque’s fibrous cap, thereby increasing the risk of rupture. In addition, lipid lowering by statins contributes to stability by reducing plaque size, by modifying the physicochemical properties of the lipid core,98 by combined reduction in lipids, lipid oxidation, matrix-metalloproteinase-2, inflammation, and cell death, or by increases in tissue inhibitor of metalloproteinase-1 and collagen content.99

**Effects on thrombogenesis and thrombolysis**

It is now established that atherosclerotic plaques are heterogeneous and vary in their tendency to undergo thrombosis and the consequent acute clinical events. Plaques rich in inflammatory macrophages and lipid deposits are mechanically weaker (“unstable plaques”), as metalloproteinase enzymes from macrophages digest the extracellular matrix and weaken the wall, they frequently crack, so exposing circulating platelets to an abnormal vascular wall that rapidly induces activation and a thrombotic mass. High circulating cholesterol, beyond promoting atherogenesis, may also give rise to an increase in thrombogenesis through an increase in tissue factor expression, in platelet reactivity, in thromboxane A₂ (TXA₂) synthesis, in platelet α, adrenergic receptor density, in platelet cytosolic calcium, and changes in platelet membrane phospholipids and cholesterol. Platelets play a critical role in the development of acute coronary syndromes.102 Although the precise mechanisms involved are not fully understood, statins have been shown to influence platelet function,101 probably through reduction in the production of TXA₂, increased synthesis of prostacyclin,102 and modifications in the cholesterol content of platelet membranes. Notably, one of the well-characterized effects of endothelial NO is its inhibition of platelet aggregation. Thrombosis on mildly damaged swine arteries at high shear rate was reduced by half with atorvastatin.101 Likewise, blood from patients on statins showed significant reduction in platelet thrombus formation,104 and platelet-derived thrombin generation was decreased.108 Both lipid-lowering and nonlipid-related effects are likely to contribute, as patients with hypercholesterolemia have hyperreactive platelets, which would be normalized with lipid lowering.106 Furthermore, multiple other routes of action have been detailed.107 Hypercholesterolaemic patients also have increased circulating coagulation factors and increased soluble CD41 ligand, a cell-activating factor derived from activated platelets; these components are reduced by pravastatin or cerivastatin treatment.108 Mechanisms involved in the antithrombotic action of statins may include the augmented production of NO from endothelial cells, as described above. In addition, atorvastatin administered to mice was found to enhance platelet production of NO, and this was accompanied by a decrease in circulating markers of platelet activation,109 which is probably related to the reduction of geranylation of Rap1b, a protein involved in platelet aggregation.110,111 The effect of statins on other cells involved in thrombosis also appears to play a part. Human aortic smooth muscle cells in vitro were found to increase their expression of COX-2 and production of prostacyclin, a platelet inhibitory agent, under the influence of mevastatin or lovastatin.102 In addition, statins have been shown to mitigate platelet stimulation in a time- and dose-dependent manner, to decrease the prothrombin fragments F1+2 in plasma from patients with type 2 diabetes, independent of cholesterol levels, and to act as inhibitors of tissue factor-dependent thrombin generation.112 The authors of a recent study hypothesized that statins decrease the exocytosis of Weibel–Palade bodies, which are endothelial cell granules whose contents promote thrombosis and vascular inflammation.113 Simvastatin decreased thrombin-stimulated Weibel–Palade body exocytosis by 89%, in part by increasing the synthesis of NO, which induced S-nitrosylation of the N-ethylmaleimidesensitive factor, a critical regulator of exocytosis. Simvastatin treatment also decreased the myocardial infarct size by 58% in wild-type but not eNOS knockout mice. Furthermore, simvastatin decreased endothelial exocytosis and neutrophil infiltration into the ischemic reperfused myocardium, which was mediated in part by the P-selectin contained in the Weibel–Palade bodies. However, simvastatin did not affect the exocytosis and inflammation in the myocardial infarcts of eNOS knockout mice. Inhibition of endothelial exocytosis is
a novel mechanism by which statin inhibitors reduce vascular inflammation, inhibit thrombosis, and protect the ischemic myocardium. Recently, an in vivo study investigated the effects of rosvastatin on vascular remodeling and thrombosis after arterial injury in apoE−/− mice. In the rosvastatin treated mice, the size of the neointimal area and the severity of the luminal stenosis were significantly reduced, and these effects were independent of systemic lipid lowering.114

Furthermore, statins exert antithrombotic effects on monocytes through reduction of the synthesis of plasminogen activator inhibitor (PAI)-1, a change likely to result in enhanced fibrinolysis and thrombus dissolution.155,116 The same result may be achieved in another route in endothelial cells, as statins cause an increase in expression of tissue plasminogen activator (t-PA). Activation of the coagulation pathway may also be impeded, as tissue factor expression has been found to be prevented by statins in human endothelial cells.117 There is increasing evidence from in vitro studies that statins positively affect the fibrinolytic system of cultured smooth muscle cells as well as endothelial cells. In these studies a decrease in PAI-1 and an increase in t-PA were observed after co-treatment with statins in endothelial cells.118 It seems likely that further pathways remain to be discovered. Remarkably, statins may have a direct influence on the coagulation pathway itself, as patients treated with simvastatin were seen to have decreased rates of stimulated activation of fibrinogen, prothrombin, factor V, and factor XIII.119 These changes did not relate to cholesterol lowering. Finally, initiation of thrombosis may be substantially inhibited by the stabilization of plaques by statin therapy. Furthermore, fluvastatin has been shown to decrease prothrombotic tendency with an associated reduction in tissue factor,120 and this may be partly through cholesterol lowering but also through noncholesterol-mediated actions, such as reduced prenylation of the Rho protein Cdc42.

Clinical studies
Statin therapy has been shown to reduce cardiovascular risk even in patients without vascular disease.121 Large epidemiologic studies have shown that CRP is a predictor of cardiovascular disease in the general population122–125 as well as special patient populations, such as patients with diabetes mellitus and patients with end-stage renal disease.126–129 Statin therapy lowers high sensitivity-CRP (hs-CRP) levels in patients with hypercholesterolemia.130 Long-term therapy with pravastatin in the Cholesterol and Recurrent Events (CARE) trial also reduced the levels of CRP,131 a change which was not found to correlate with the reduction in LDL cholesterol levels. The latter finding seems to be confirmed by recent trials, such as the PRINCE study.132 In the more recent JUPITER study,133 a four-year treatment with rosvastatin resulted in considerable decrease in hs-CRP levels and cardiovascular morbidity in healthy adults without hyperlipidemia. However, any potential clinical benefits conferred by the lowering of hs-CRP levels are difficult to separate from those of the lipid-lowering effects of statins without further clinical studies and, even in the JUPITER study in patients with no hyperlipidemia, hs-CRP lowering was escorted by a significant reduction in LDL cholesterol levels below normal levels during the first year of the study. Lately, the independent effects of statins on CRP are debated.134 In their extensive meta-analysis, Genser and colleagues135 found a close correlation between statin-induced reductions in LDL cholesterol and CRP which has not been evident from individual studies. This discrepancy could be attributed to the great variability and the non-normal distribution of the values of CRP. As long as serum CRP originates predominately from the liver and secondly form the sites of inflammation, different degrees of inflammation could probably stimulate different sites of CRP production. If this hypothesis is true, CRP measured in low degrees of inflammation, such as in the general population, could originate from local production in atherosclerotic plaques, while CRP measured in higher degrees of inflammation, such as in end-stage renal disease patients, could originate predominantly from liver production. Statins effects might differ in each situation, and their effects on the CRP produced by the liver might be linked somehow with the inhibition of HMG-CoA reductase, while peripherally produced CRP might be genuinely independent. Unfortunately, in CARE study there has not been a separate analysis of the correlation between CRP and lipid decrease in those patients with the highest levels of CRP, it is however characteristic that these patients enjoyed the maximal cardiovascular benefits. However, in PRINCE study such a correlation was indeed found in the patients with hsCRP values within the highest quartile. Other studies revealed several other beneficial effects of statins, most notably their immunosuppressant effects in heart transplant recipients. These studies have suggested a better transplant outcome in patients taking statin therapy136,137 as well as improved endothelial function and reduced inflammatory cytokine release.138 In renal transplant recipients, some evidence for similar beneficial effects of statins on endothelial functions has been noted139,140 and, probably, on the rate of acute rejection reactions,141 although the later is still debatable.142–144 The effect of serum lipids on monocyte adhesion molecules has not been investigated until recently.
The monocyte adhesion molecules CD11b and CD14 have been found to be elevated and L-selectin to be decreased (usually, this occurs as a consequence of cell activation) in patients with hypercholesterolemia compared with control subjects; furthermore, the levels of each of these adhesion molecules were found to correlate with those of LDL. Simvastatin was found to correct the levels of each of these towards normal. The exposure of normal leukocytes to LDL induced changes similar to those in the hypercholesterolemic patients. It is important to note that changes in monocyte adhesion molecule expression in the patients can be a direct consequence of their exposure to increased LDL levels. Reversal with statins may therefore be attributable both to their hypercholesterolemic effect and to another direct effect on the cells unrelated to lipid lowering. Finally, several in vivo studies have also investigated the effect of statin therapy on the regulation of the fibrinolytic system. Although the results are inconsistent, in some studies statins decrease PAI-1 plasma levels.

Conclusions

In recent years several in vitro and in vivo studies have provided solid evidence suggesting that statins exert multiple vasoprotective effects. Some of them are likely to be independent of the lipid-lowering effects of these drugs. However, caution is needed when an effect is to be disconnected from others, as long as it is now clear that there are multiple interconnections between lipid- and nonlipid-lowering pathways and the endothelium is the center where many different physiologic intercalating pathways converge. Furthermore, hypercholesterolemia itself has an inflammatory effect on the vascular endothelium and liver. To this extent, the correlation between different effects of statins appears to be like the causality dilemma, “which came first, the chicken or the egg?” and it wouldn’t be surprising if future research revealed a correlation of effects which nowadays seem independent. Given all the evidence that research has come up to, it is now tempting to question the multiple-dispersed “pleiotropic” effects of statins in favor of a unifying theory that would bind together the pieces of the puzzle.

Disclosure

The authors report no conflicts of interest in this work.

References


