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The lipid and non-lipid effects of statins

Anthony S. Wierzbicki[a], Robin Poston[b], Albert Ferro[b,*]

[a]Department of Chemical Pathology, GKT School of Medicine, King's College London, London, UK
[b]Centre for Cardiovascular Biology and Medicine, GKT School of Medicine, Room 2.36B New Hunts House, King's College London, Guy's Hospital Campus, London Bridge, London SE1 1UL, UK

Abstract

The 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitors, more commonly known as statins, are a class of drug widely used for the treatment of hypercholesterolaemia in patients with established cardiovascular disease as well as those at high risk of developing atherosclerosis. Their predominant action is to reduce circulating levels of low-density lipoprotein (LDL) cholesterol; to a smaller degree, they also increase high-density lipoprotein (HDL) cholesterol and reduce triglyceride concentrations. In recent years, however, there has been an increasing body of evidence that their effects on lipid profile cannot fully account for their cardiovascular protective actions: their beneficial effects are too rapid to be easily explained by their relatively slow effects on atherogenesis and too large to be accounted for by their relatively small effects on plaque regression. Experimental models have revealed that statins exert a variety of other cardiovascular effects, which would be predicted to be of clinical benefit: they possess anti-inflammatory properties, as evidenced by their ability to reduce the accumulation of inflammatory cells in atherosclerotic plaques; they inhibit vascular smooth muscle cell proliferation, a key event in atherogenesis; they inhibit platelet function, thereby limiting both atherosclerosis and superadded thrombosis; and they improve vascular endothelial function, largely through augmentation of nitric oxide (NO) generation. The relative importance of the lipid- and non-lipid-related effects of the statins in the clinical situation remains the subject of much continuing research.

Keywords: Statins; 3-hydroxy-3-methyl glutaryl coenzyme A reductase; Lipids; Inflammation; Cardiovascular system

Abbreviations: CRP, C-reactive protein; EAE, experimental allergic encephalomyelitis; ER, endoplasmic reticulum; FXR, farnesoid-X receptor; HDL, high-density lipoprotein; HMG-CoA, 3-hydroxy-3-methyl glutaryl coenzyme A; HUVEC, human umbilical vein endothelial cells; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; LDL, low-density lipoprotein; LFA-1, leucocyte function antigen-1; LXR, lectin-like oxidized LDL receptor; MCP-1, monocyte chemoattractant protein-1; MTP, microsomal transfer protein; NOS 3, nitric oxide synthase type 3; PI3-kinase, phosphatidylinositol 3-kinase.

Contents

* Corresponding author. Tel.: +44-20-7848-6233; fax: +44-20-7848-6220.
E-mail address: albert.ferro@kcl.ac.uk (A. Ferro).

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1. Introduction

Statins (3-hydroxy-3-methyl glutaryl coenzyme A [HMG-CoA] reductase inhibitors) are the most commonly used lipid-lowering drugs. It is commonly supposed that statins act by blocking cholesterol synthesis through inhibition of HMG-CoA reductase—the first and rate-limiting step in cholesterol synthesis (Istvan et al., 2000; Istvan & Deisenhofer, 2001). Their actual mechanism of action is to increase the expression of low-density lipoprotein (LDL) cholesterol receptors through inhibition of cholesterol synthesis. However, this is an oversimplification as inhibition of the first (rate-limiting) step of cholesterol synthesis also affects the synthesis of other compounds with a variety of metabolic roles. Hence, controversy exists as to whether statins have other actions, which do not relate to lipid lowering (Farmer, 2000; Comparato et al., 2001).

1.1. Biochemistry of the cholesterol synthesis pathway

Cholesterol synthesis occurs in both peroxisomes and endoplasmic reticulum (ER) (Fig. 1) and shows a diurnal rhythm peaking at 0300–0500 (Rusnak & Krisans, 1987; Krisans, 1996; Wanders & Tager, 1998; Aboushadi et al., 1999; Hogenboom et al., 2002; Kovacs et al., 2002). While the steps around squalene synthase are well localised in the ER, the HMG-CoA reductase enzyme is found both in ER and in peroxisomes (Hogenboom et al., 2002). In some bacteria, these are separate enzyme isoforms with little homology, but whether this is so in man is unknown (Breitling & Krisans, 2002). It is often assumed that both forms of HMG-CoA reductase are equally inhibited by statins; although given a peroxisomal localisation for one subtype, this implies that these drugs are transported into that organelle (Breitling & Krisans, 2002). If true, then, since many statins possess 3-methyl side chains, one would expect these drugs to be β-oxidised by the peroxisome as well as being metabolised by the cytochrome *P*450/glucuronidation pathways (Tokui et al., 1999).

It is interesting to note that in peroxisomal biogenesis disorders, where peroxisomes are absent or present as "nonfunctional ghosts," cholesterol levels are only mildly reduced despite the fact that many enzymes for cholesterol synthesis, from HMG-CoA lyase to geranyl pyrophosphate synthase, are located in that organelle (Hogenboom et al., 2002). This implies that either this pathway exists in "ghost" peroxisomes or it is active when dispersed in the cytosol. Similarly, controversy exists about the higher enzymes in the pathway after lathosterol, as these may be located either in the peroxisome or on the ER (Bae et al., 1999). Certainly, additional control points regulated by cholesterol or its precursors exist in the cholesterol synthetic pathway, as the treatment of Smith-Lemli-Opitz syndrome with statins results in a large paradoxical increase in plasma cholesterol as well as a significant decrease in the toxic 7-dehydrocholesterol (Jira et al., 2000).

1.2. Other effects of inhibiting the cholesterol synthetic pathway

1.2.1. Isoprenoid prenyl intermediates

The complexity of the pathway is such that a reduction in cholesterol synthesis by statins, and hence in levels of the intermediate geranylgeranyl pyrophosphate and its free acid geranylgeranoic acid, is likely to result in alterations in other biochemical intermediates (Kovacs et al., 2002).

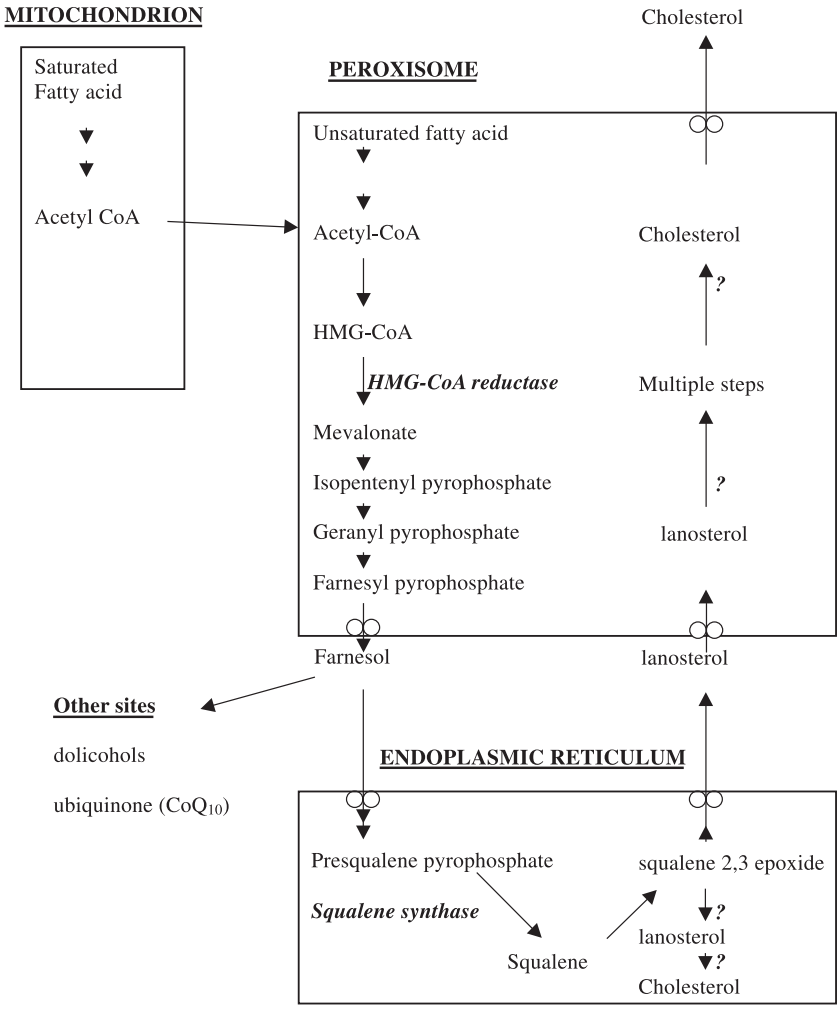

Fig. 1. Compartmentation and simplified biochemistry of cholesterol synthesis. Drug targets are shown in bold italics.

Protein prenylation by farnesylation or geranylation is a major regulator of biochemical processes, implying that statins may have profound actions on cellular regulatory pathways, including apoptosis via cyclin-G and Rho kinase amongst many others (Zhang & Casey, 1996; Kovacs et al., 2002). This will be discussed in more detail in Sections 4–6.

1.2.2. Dolichols and ubiquinone

Other compounds derived from isoprenoid intermediates of cholesterol synthesis include dolichols (involved in neuronal membrane function), ubiquinone (coenzyme Q_{10}, which is an electron transport shuttle vector in the mitochondrion), and the synthesis of glycosphingolipids. Statins have variable effects on plasma coenzyme Q_{10} levels, but few data exist on intracellular levels of coenzyme Q_{10} or on effects on mitochondrial function as assessed by nuclear magnetic resonance spectroscopy studies (Wierzbicki, 2002). Many clinicians use coenzyme Q_{10} supplementation therapeutically to combat the myalgia caused by statins, and there are anecdotal reports of success. However, the effect has not been proven in placebo-controlled clinical trials.

1.3. Mechanisms of statin-induced toxicity

The principal side effects of statins are gastrointestinal disturbance, abnormal liver transaminases, and myalgia-myositis and in different clinical studies occur in 0.1–5% of patients. Statins have to enter cells to exert their actions on cholesterol synthesis and may need to enter subcompartments to be fully active. They are transported into cells by the organic anion transporter (OAT), and some may be excreted by the action of the multiple drug resistance protein MDR-2 whose action they may also inhibit (Wang et al., 2001). Many need to be activated to acids from prodrug lactone compounds after uptake (Prueksaritanont et al., 2002a). Therefore, differences in uptake and activation may largely explain the variability in tissue-specific side effects between drugs and between individuals.

1.3.1. Liver side effects of statins

Many patients complain of gastrointestinal disturbance (nausea, bloating, diarrhoea, and constipation), and some have to discontinue statin therapy due to liver transaminase levels exceeding 3 times the upper reference limit ($\sim$150 IU/L). The mechanisms behind these side effects are unclear, but a few possibilities have been suggested. It has been noted that statins can induce a transient acute phase response on initiation, especially at high doses (Wierzbicki et al., 1998, 2001), and this may represent a transient chemical hepatitis due to disturbance of the cholesterol-bile acid pathways. Similarly, disturbances in bile acid metabolism could be responsible for the gastrointestinal side effects of statins, as in many ways these side effects resemble those commonly seen with the bile acid sequestrants, which also lower plasma cholesterol levels. The mechanism of these side effects is likely to involve derivatives of cholesterol. Oxidised cholesterol is the prime ligand for the novel lectin-like oxidised LDL receptor (LXR) (Mehta & Li, 2002; Rosenson & Brown, 2002), while 7-α-cholesterol hydroxylase (CYP7A1) is the rate-limiting step for the control of bile acid synthesis through the farnesoid-X receptor (FXR), which may be regulated by the cholesterol intermediate lanosterol as well as bile acids (Tu et al., 2000; Ekins et al., 2002). Statins reduce levels of oxidised cholesterol and cholesterol substrate for the hydroxylase and thus are likely to affect these other metabolic pathways, with both positive outcomes and possible negative ones in terms of side effects. The complexity of interactions within the LXR and FXR pathways could give rise to the variable nature of statin side effects both within and between individuals.

1.3.2. Muscle side effects of statins

The mechanism of rhabdomyolysis and myositis (painful myalgia with a significant creatine kinase release [>2000 IU/L]) induced by statins remains obscure, though contributors include low muscle mass, age (different muscle subfibre type), hypothyroidism, and concomitant medications (Wierzbicki, 2002).

1.3.3. Neuropathies induced by statins

Case reports of demyelinating neuropathies caused by statins suggest that, in some individuals, the actions of statins in reducing dolichols or glycosphingolipid synthesis may be significant (Jeppesen et al., 1999). This also remains to be proven.

2. Clinical pharmacology and metabolism

The early "natural" statins—lovastatin, pravastatin, and simvastatin—are modified fungal extracts (Dujovne & Moriarty, 1996). The newer statins—fluvastatin, cerivastatin, atorvastatin, (p)itavastatin (NK-104), and rosuvastatin—are synthetic compounds (Table 1). The most lipophilic compounds are simvastatin and lovastatin, while pravastatin and rosuvastatin are the most hydrophilic. Most are highly protein bound in plasma. Food has no effect on bioavailability of the agents, except for lovastatin where it is increased. All except atorvastatin (14 hr) and rosuvastatin (22 hr) have a short plasma half-life, though for the statins this is a somewhat misleading concept. The active compounds are the acids derived from hydrolysis of the precursor drugs. The plasma half-life of these drugs indicates their first-pass metabolism; in fact, most of the biologically active product recirculates through the enterohepatic circulation in company with cholesterol (Desager & Horsmans, 1996; Igel et al., 2001).

2.1. Metabolism of statins

The statins have a variety of excretion pathways. Pravastatin shows significant renal excretion, while the rest are metabolised by the liver to active hydroxy acids and excreted in the bile. Rosuvastatin is glucuronidated for excretion, while simvastatin, lovastatin, and atorvastatin are metabolised by cytochrome *P*450 3A4. Cerivastatin is metabolised by *P*450 3A4 and 2C8 and fluvastatin is metabolised by 2C9. These interactions were thought to

Table 1
Pharmacokinetics of statins

Drug	Rat HMG-CoA			BA (%)	Protein binding (%)	Half-life (hr)	Excretion	Metabolism	Dose	ΔLDL (%)
	IC_{50} (nM)	ED_{50} (mg/kg)	F/H (log)							
Atorvastatin	15.2	1.3	2.2	14	98	14 (20–30)[1]	90% liver, 2% renal	CYP 3A4	10–80 mg	44–59
Cerivastatin[2]	13.1	0.01	−0.14	60	99.1–99.5	2–3	60% liver, 30% renal	CYP 2C8, CYP 3A4	100–400 μg	20–38
Fluvastatin	17.9	0.25	−0.04	19–29	98	0.5–0.7	90% liver, 5% renal	CYP 2C9	20–80 mg	22–38
Lovastatin	150	0.3	1.3	30	>95	3	83% liver, 10% renal	CYP 3A4	20–80 mg	22–42
(P)itavastatin[3]	6	0.13	3.2	80	90	11	90% liver, 5% urine	nil/glucuronide	1–4 mg	33–47
Pravastatin	55.1	22.3	3.4	18–34	50	1.3–2.7	70% liver, 20% renal	nil	10–40 mg	19–34
Rosuvastatin	11.8	0.8	0.16	?	95	18–20	90% liver, 10% renal	nil/glucuronide	10–40 (80) mg	51–65
Simvastatin	18.1	1.2	0.41	5	95	1.9	60% liver, 13% renal	CYP 3A4	10–80 mg	38–47

Abbreviations: BA=bioavailability; F/H=rat fibroblast/hepatocyte IC_{50} ratio.

[1] Atorvastatin has active metabolites.

[2] Cerivastatin was voluntarily withdrawn in 2001 after reports of rhabdomyolysis.

[3] This compound is in phases 2 and 3 development.

be of theoretical importance only, except in patients on cyclosporine or HIV protease inhibitors, until the clinically important interaction between gemfibrozil and cerivastatin caused the voluntary withdrawal of the latter (Prueksaritanont et al., 2002a, 2002b). Following on from this episode, the use of statin-fibrate combination therapy by clinicians has lessened measurably, and concerns have recently been raised with early reports of rhabdomyolysis in patients treated with the combination of gemfibrozil and lovastatin (Wierzbicki et al., 2003a). The interaction between statins and gemfibrozil turns out to be complicated. Gemfibrozil displaces lovastatin, simvastatin, and particularly cerivastatin from protein binding sites. In addition, it inhibits the OAT-2 for cellular excretion of statins, inhibits statin glucuronidation (UDPG-1), and interacts via cytochrome *P*450 2C8 to increase statin concentrations (Prueksaritanont et al., 2002b). These actions appear to be unique to gemfibrozil and do not seem to occur with other fibrates.

3. Lipid-lowering actions

3.1. Statins and low-density lipoprotein cholesterol

Statins were initially trialled at doses of 1 mg daily and shown to reduce LDL cholesterol by around 10%. As confidence has grown with these agents, the dose ranges have been extended for most drugs in the class. The current statins fall into two dose-efficacy groups. Pravastatin and fluvastatin produce reductions of up to 30% in LDL at top dose (40 and 80 mg, respectively). Lovastatin, simvastatin, atorvastatin, and rosuvastatin reduce LDL by 30–70% depending on dose (10–80 mg). All statins follow the "5–7 rule," whereby a doubling of dose results in a further 5–7% increment in LDL reduction (Stein et al., 1998; Wierzbicki et al., 2000). All are limited above licensed top doses by an increasing incidence of chemical hepatitis (alanine transaminase or aspartate transaminase elevations >150 IU/L), myalgia, or myositis.

3.2. Statins and triglycerides

All statins reduce triglycerides in proportion to their effect on apolipoprotein B or LDL reduction and to baseline triglyceride level (Stein et al., 1998; Wierzbicki et al., 2000). Statins work by increasing hepatocyte and other cell surface LXR expression in response to a fall in intracellular cholesterol levels. Thus, they are of limited efficacy in patients homozygous for null alleles (truncation/major deletion) of the LXR (homozygous familial hypercholesterolaemia). Their intracellular action seems to involve reduction in the size of the cholesterol pool that is incorporated into hydrophobic pockets of the apolipoprotein B gene. Apolipoprotein B is made in excess, and any that is not stabilised by the addition of cholesterol is ubiquitinated and routed for destruction in the proteasome. The intracellular lipid pool also seems to contain triglycerides, given its dependence on microsomal transfer protein (MTP). Pharmacological inhibition of MTP results in a profound fall in both LDL (80%) and triglycerides (65%), though none of the MTP inhibitors have been clinically licensed due to secondary hepatic steatosis (Wetterau et al., 1998). Part of the effect of niacin also appears to be mediated through effects on the triglyceride portion of the intracellular cholesterol pool (Jin et al., 1999). Thus, reduction in the size of the intrahepatocyte lipid pool leads to fewer and smaller very-low-density lipoprotein (VLDL) particles containing less triglyceride and cholesterol (Davis, 1999; Chan et al., 2000; Cardozo et al., 2002).

3.3. Statins and high-density lipoprotein cholesterol

Statins also increase high-density lipoprotein (HDL) cholesterol to a small degree, and patients with lower HDL levels exhibit a greater increase in HDL with statin therapy. The determinants of this response are drug specific and differ from those for triglycerides (Wierzbicki et al., 2000). Indeed, 80 mg of atorvastatin daily has been shown to decrease HDL and apolipoprotein A-1 levels in some studies, as compared with a similar dose of simvastatin, which has the reverse effect (Wierzbicki et al., 1999; Illingworth et al., 2001; Wierzbicki & Mikhailidis, 2002). The mechanism behind the HDL-raising effects of statins has not been fully clarified, but it is likely that it involves gene transcription and phosphorylation of peroxisomal proliferating activator receptor-α (PPAR-α) by isoprenoid intermediates of cholesterol synthesis acting through Rho kinase. This action shows a different dose dependence to the LDL-lowering effect of statins (Martin et al., 2001).

Statins show a marked variation in clinical efficacy in individuals, and the pharmacogenetics of the response profile has been clarified to some extent. The efficacy of statins seems to depend on their actions on genetic variants in cholesterol ester transfer protein (Kuivenhoven et al., 1998) and hepatic lipase (Zambon et al., 2001).

4. Effects apparently unrelated to lipid lowering

The clinical success of statins has brought to light the intriguing realisation that their action is not through lipid lowering alone. This is because their beneficial effects appear to be too rapid to be explained by their relatively long-term effects on atherogenesis and too large to be explained by their relatively small effects on plaque regression. The increasing understanding of atherosclerosis as an inflammatory disease has provided the background in which the non-lipid-lowering actions of statins have been understood as being principally anti-inflammatory effects. This topic has received extensive attention recently and has been the subject of several recent reviews (Palinski, 2001; Takemoto & Liao, 2001; Lefer, 2002; Ridker, 2002).

4.1. Evidence for the independent role of inflammation in the pathogenesis of atherosclerosis

4.1.1. Inflammatory nature of the lesions

It is now well recognised that there is an important inflammatory aspect to atherosclerosis. The migration of monocytes from the blood into the arterial intima occurs through binding to specific adhesion molecules and the action of chemoattractants. The accumulation of lipid-laden macrophages within the lesions then follows. These events are a modified form of chronic inflammation. Macrophages can oxidise LDL in addition to producing inflammatory cytokines, and both these classes of factors may activate local arterial endothelial cells to express adhesion molecules and chemokines, which is likely to allow further macrophage recruitment and self-perpetuation of the lesion.

Multiple adhesion molecules are involved in the interaction of blood monocytes with the arterial wall to initiate the lesions. Initial rolling adhesion is likely to be mediated by P-selectin, which is well expressed on the endothelial cells of atherosclerotic plaques that binds to specific oligosaccharides of glycoproteins on the monocytes. The β_2 integrins are important monocyte adhesion molecules that mediate the subsequent tight binding of monocytes to endothelial cells by interacting principally with intercellular adhesion molecule-1 (ICAM-1). This family of heterodimeric molecules is characterised by having the common β_2 chain, otherwise defined as CD18. Two members of the family are thought to be important in the atherosclerotic process: the leucocyte function antigen-1 (LFA-1) with the α chain CD11a and the MAC-1 with the α chain CD11b. Another monocyte adhesion molecule is CD14, which may have a major role in atherosclerosis (Poston & Johnson-Tidey, 1996). Its endothelial ligand has not been completely defined but is probably heat shock protein 60, a stress-induced molecule. This monocyte-endothelial cell adhesion is part of the emigration process by which these cells leave the circulation in inflammation. The same mechanisms apply to emigration of monocytes into the intima of both fatty streaks and atherosclerotic plaques. The β_2 integrins are molecules that are normally inactive; on the other hand, with cellular activation, for instance, by chemokines, they undergo a molecular change to allow active binding. However, it is only recently that the regulation of this process has been understood, and it is given detailed consideration below, as its modification by statins may be a major mechanism for the anti-inflammatory activity of these drugs.

As follows from the integrin mechanisms just described, cytokines, chemokines (chemotactic cytokines), and other inflammatory mediators have an important role in facilitating the adhesion process, either by inducing integrin activation and hence tight adhesion or by setting up a gradient directing the cellular emigration. The chemotactic cytokine, the monocyte chemoattractant protein (MCP-1), is a principal monocyte-selective chemotactic agent that has been shown to have an important role in atherosclerosis.

4.1.2. Stable and unstable plaques

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 lumenal 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 (Davies et al., 1993). These are unstable plaques; the inflammatory nature of which is central to their clinically hazardous behaviour.

High circulating cholesterol, as well as promoting atherogenesis, may also give rise to an increase in thrombogenesis through an increase in tissue factor expression (Camera et al., 2002); this may provide an additional mechanism whereby thrombosis occurs on an underlying atheromatous plaque. 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 (Camera et al., 2002).

4.1.3. Circulating acute phase proteins

The blood concentrations of C-reactive protein (CRP), fibrinogen, and several other circulating acute phase proteins have been found to be predictive of future vascular events (Ridker, 2001; Ridker et al., 2001a; Engström et al., 2002). These are proteins that are synthesised in the liver in response to cytokine signals such as interleukin (IL)-6, which can originate from foci of inflammation. Indeed, circulating levels of the macrophage-derived cytokines IL-6 (Ridker et al., 2000a), tumour necrosis factor α (Ridker et al., 2000b), and IL-18 (Blankenberg et al., 2002) are all risk factors predicting vascular events. These proteins may be of significance in two possible ways. On the one hand, they may be markers of an underlying inflammatory process, such as a focus of chronic infection (e.g., in the respiratory or urinary tracts) or possibly a large amount of inflammatory atherosclerotic tissue within their vessels. Where there is infection, these proteins could serve as a marker for infection-induced atherogenesis, for instance, reflecting activation of blood monocytes by circulating bacterial endotoxin. On the other hand, the proteins may themselves play a pathogenetic role in atherosclerosis, for instance, through enhancing thrombosis as a consequence of increased levels of fibrinogen or other coagulation proteins or via the recently discovered ability of CRP to activate monocytes and enhance their endothelial adhesion (Woollard et al., 2002). In some instances, both considerations may apply.

4.2. Clinical evidence for the non-lipid-lowering effects of the statins

It is now well recognised that statins reduce vascular clinical events; until recently, this has been attributed solely to their lipid-lowering action. However, their efficacy in patients without raised cholesterol levels (Sacks et al., 1996; MRC/BHF Heart Protection Study, 2002) and 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. In the WOSCOPS study, the reduction in LDL produced would have been anticipated to give a 24% reduction in events, whereas a 35% reduction was observed (West of Scotland Coronary Prevention Study Group, 1998). Furthermore, the Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) trial showed that statin therapy reduces the recurrence rate of cardiac events following acute coronary syndrome, with reduction in angina from within 4 weeks (probably through normalisation of endothelial function) and new ischaemic events by 16 weeks following initiation (Schwartz et al., 2001); although at this time point a considerable reduction in serum cholesterol was observed, it is unlikely that any significant vascular remodelling could have occurred in so short a time.

4.2.1. Lesion size

It has been recognised from the earliest attempts at the medical therapy of atherosclerosis that it is difficult to achieve reduction in lesion size, and the changes produced are always small, no doubt reflecting the slow growth of the primary lesions. Although some regression in lesion size has been achieved with statins (de Groot et al., 1998; Herd, 1998; Taylor et al., 2002), it is not of a magnitude that is consistent with their clinical efficacy. In the FATS trial, the improvement in stenosis was less than 1%, while the reduction in coronary events was 75% (Brown et al., 1993). It was further noted in that study that the clinical improvement related almost entirely to an inhibition of the progression of mild lesions to highly occlusive forms, an event often likely to be due to thrombosis. Further evidence that statins affect thrombotic processes can be derived from the observation that withdrawal of statins increases the risk of adverse events in acute coronary syndromes (Heeschen et al., 2002). Similarly, in patients undergoing elective percutaneous angioplasty, the risk of fatality was found to be reduced with statin therapy, both at 1 month (by 47%) and at 6 months (by 33%); the early reduction suggests that statins could be influencing postoperative thrombosis (Chan et al., 2002).

4.2.2. Stroke

The epidemiology of stroke shows the influence of inflammatory factors more strongly than other vascular disease (Ridker, 2002). CRP can predict stroke independently of cholesterol in elderly patients (Rost et al., 2001), while most reports have shown little relationship between stroke and cholesterol levels. Furthermore, it has been reported that lipid levels only predict stroke if circulating inflammatory markers are elevated (Engström et al., 2002). Statins can reduce CRP independently of their effects on lipids (Ridker et al., 2001b). In a metaanalysis of three pravastatin trials, there was a reduction in total strokes by 20% (Byington et al., 2001). Thus, it appears that statins are effective in the treatment of cerebrovascular disease despite the existence of only a weak association with hypercholesterolaemia. More significantly, the occurrence of stroke associates with an acute phase response in the patient, which can be reduced by statins, and this may relate to their beneficial effects in stroke prevention.

4.3. A caveat—cholesterol lowering itself has an anti-inflammatory effect

Some caution is needed in attributing all anti-inflammatory action of statins as effects independent of cholesterol lowering, since animal studies have revealed that hypercholesterolaemia itself has an inflammatory effect on the vascular endothelium and liver (Liao et al., 1994). Indeed, cholesterol lowering and cholesterol-independent pathways influenced by statins may have similar effects, for instance, on reduction in MCP-1 expression via the oxidation sensitive NFκB pathway (Liao et al., 1994; Kothe et al., 2000).

4.4. Laboratory and clinical evidence for an anti-inflammatory effect of statins

Clinical testing of endothelial function by forearm venous occlusion plethysmography has demonstrated that statins can produce an improvement in endothelial function in hypercholesterolaemic subjects. Simvastatin treatment for 1 month gave significant improvement in endothelium-dependent vasodilatation stimulated by acetylcholine (O'Driscoll et al., 1997), thus providing evidence that nitric oxide (NO) bioavailability from the endothelium could be increased in vivo by statin treatment. Similarly, coronary artery endothelial function was improved after 5 months of lovastatin therapy in patients with coronary atherosclerosis undergoing cardiac catheterisation prior to angioplasty (Treasure et al., 1995).

There is now good evidence that statins produce histological changes characteristic of plaque stabilisation. Pravastatin increases collagen and decreases lipid, inflammatory cell infiltration, metalloproteinase enzymes, and cell death in human carotid atherosclerotic plaques obtained at endarterectomy (Crisby et al., 2001).

The significance of circulating CRP as a systemic marker of an inflammatory state and potential atherogenic factor was discussed in Section 4.1.3. Pravastatin was found to reduce mean CRP levels by 24% after 8 weeks of treatment,

an effect not related to the dose of pravastatin used nor to its effect on LDL cholesterol (Ridker et al., 1999).

4.4.1. Cardiac transplantation

An anti-inflammatory effect of pravastatin was implied by the reduction in vascular changes in the coronary arteries of a group of 97 hearts used in cardiac transplantation (Kobashigawa et al., 1995). Despite no changes in episodes of rejection in the treated half, the frequency of coronary vasculopathy was lower, and the survival at 1 year was substantially improved at 94% compared with 78%. This suggests that the statin had little effect on immune responsiveness (see Section 4.4.2.6), but the reaction in the target arterial wall was reduced.

4.4.2. Evidence in animal and in vitro models

Reduction in the oedema of a carrageenan-induced inflammatory reaction in an animal foot pad is a standard method of assessing an anti-inflammatory compound. Simvastatin was found to produce this effect in mice following oral administration (Sparrow et al., 2001). However, the extent of the anti-inflammatory effects of the statins remains controversial, as some laboratories have not had positive results in other animal models.

4.4.2.1. Leucocyte-endothelial adhesion in vivo. There is now substantial evidence from animal work that statins can inhibit leucocyte-endothelial cell interactions. This is likely to be an important anti-inflammatory mechanism, as it is the stage at which the pathophysiological control of inflammation normally occurs, and it may similarly be a rate-limiting factor in atherogenesis. Simvastatin was found to profoundly inhibit both the rolling of leucocytes on activated rat mesenteric endothelium in vivo and their subsequent static adhesion and transmigration (Pruefer et al., 1999). Likewise, rosuvastatin was found to have similar anti-adhesive effects in stimulated rodent mesenteric vessels, which were absent in mice that had had their NO synthase type 3 (NOS 3) gene deleted (Stalker et al., 2001). Clearly, NOS 3 expression and the regulation of NO production play a significant role in the effects of statins on the vessel wall, at least in these animal models.

4.4.2.2. Experimental atherosclerosis. As might be anticipated, experimental atherosclerosis can be reduced effectively by statin treatment. In a study in rabbits (Bustos et al., 1998), atherosclerosis was induced in the femoral arteries by endothelial injury and a high lipid diet for 4 weeks; this was followed by atorvastatin or vehicle administration for a further 4 weeks. Arterial macrophage infiltration was abolished by atorvastatin treatment, and the chemokine MCP-1 and the inflammatory transcription factor NFκB were diminished in the lesions. Lesion size was decreased, but it is the complete reduction in the important macrophage inflammatory component that is the significant finding in this work. A further important confirmation of the effect of statins on inflammatory components of atherosclerosis has been provided in a primate animal model (Sukhova et al., 2002). Groups of cynomolgus monkeys on a high cholesterol diet were treated with pravastatin or simvastatin, but their dietary cholesterol was adjusted to maintain equal values with a control nontreated group. After a year, the lesions did not differ in size, but the macrophage content was lowered 2.4-fold with pravastatin and 1.3-fold with simvastatin. Furthermore, the expression of the vascular cell adhesion molecule (VCAM)-1, inflammatory cytokine IL-1, and tissue factor were diminished, while the lesional content of smooth muscle cells and collagen was increased, factors leading to plaque stability. These results provide good evidence that statins selectively reduce the inflammatory mechanisms in primate atherosclerosis independently of cholesterol lowering.

4.4.2.3. Cerebral ischaemia. The importance of NO production in the vascular action of the statins was again emphasised by their effects in a model of cerebral ischaemic injury in normocholesterolaemic mice. The administration of statins resulted in improved blood flow, diminished infarct size, and improved cerebral function. However, if NOS 3 gene-deleted mice were used, the statins were without effect (Endres et al., 1998).

4.4.2.4. Ischaemia-reperfusion. In an acute rat model of cardiac ischaemia and reperfusion, simvastatin and to a lesser extent pravastatin were able to improve coronary artery flow and left ventricular function. This was likely to be the result of the decreased neutrophil infiltration found, which in turn depended on reduced adhesion due to a diminished expression of P-selectin on the endothelium and of β_2 integrins on the neutrophils (Lefer et al., 1999). Further work by the same group showed that the beneficial effects persisted to 6 months, with a decrease in infarct size by 51% with simvastatin (Jones et al., 2001).

4.4.2.5. Multiple sclerosis and experimental allergic encephalomyelitis. Multiple sclerosis is a disease with some similarities to atherosclerosis, as it consists of focal demyelinating perivascular lesions in the brain, which are infiltrated by macrophages and lymphocytes. The destructive interactions of macrophages with the lipid-rich myelin are central to the disease. Experimental allergic encephalomyelitis (EAE) is an animal model, in which demyelination is caused by the induction of autoimmunity against a major myelin constituent, myelin basic protein. The anti-inflammatory action of statins has stimulated their investigation in EAE (Zamvil & Steinman, 2002). Lovastatin was found to significantly improve EAE in rats (Stanislaus et al., 2002). Likewise, statins could prevent disease in a mouse model by inhibiting leucocyte migration into the brain (Walters et al., 2002).

4.4.2.6. Effects on immune responses. Statins inhibit the immune response-mediated proliferation of peripheral blood

cells and thus exert an immunosuppressive and anti-inflammatory effect (e.g., the production of T-lymphocyte metalloproteinase-9 is decreased); however, in man, but not in the rat, they have an immunomodulatory effect on T-lymphocytes, such that the Th1-promoting macrophage cytokine IL-12 is increased, as is the Th1 cytokine interferon (IFN)-γ, with a shift in Th1/Th2 balance towards Th1 (Neuhaus et al., 2002; Zamvil & Steinman, 2002). The significance of this change is uncertain, but the T-lymphocyte profile produced is not necessarily beneficial in either multiple sclerosis or atherosclerosis, as IFN-γ may be disease promoting in both. It seems likely that effects by statins on inflammatory mechanisms are more important than those on specific immune responses.

4.4.2.7. Effects on adhesion molecule expression and monocyte adhesion. A study by Niwa et al. (1996) showed that fluvastatin decreased the adhesion of the monocyte cell line U937 to human umbilical vein endothelial cells (HUVEC). This correlated with a specifically decreased expression of LFA-1 and ICAM-1 on the U937 cells, which was reversed by mevalonate, the initial product of HMG-CoA reductase. In another study (Weber et al., 1997), MAC-1 expression in monocytes was slightly decreased by lovastatin, and MAC-1-dependent adhesion of the monocytes to endothelial cells was decreased. Again, abolition of the effect of the statin was possible with mevalonate but not with LDL, suggesting that intermediate metabolites on the cholesterol pathway from mevalonate were involved. The role of the HMG-CoA reductase/mevalonate pathway is discussed in Section 4.5.1. Other adhesion molecules with separate pathways can also be affected, as simvastatin can decrease the expression of the endothelial adhesion molecule P-selectin following its induction by staphylococcal toxin (Pruefer et al., 2002).

Remnant lipoproteins are a form generated by the hydrolysis of chylomicrons and VLDL that are considered to be highly atherogenic. They have been found to be capable of inducing adhesion molecules on endothelial cells. They can also increase the expression of integrins on U937 cells, and atorvastatin can both decrease this induction and reduce the adhesion of the U937 cells to HUVEC (Kawakami et al., 2002).

Cerivastatin can reduce the adhesion of human monocyte cell lines to HUVEC under physiological flow conditions via intracellular pathways dependent on RhoA (discussed in Section 4.5.1); this is associated with a diminution of the expression of LFA-1, β_2 integrin β chain (CD18), and VLA-4 on the monocytes (Yoshida et al., 2001).

4.4.2.8. Serum lipids and adhesion. The effect of serum lipids on monocyte adhesion molecules has not been investigated until recently. The monocyte adhesion molecules CD11b (Weber et al., 1997) 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 hypercholesterolaemia as compared with control subjects; furthermore, the levels of each of these adhesion molecules were found to correlate with those of LDL (Serrano et al., 2001). Simvastatin was found to correct the levels of each of these towards normal. The exposure of normal leucocytes to LDL induced changes similar to those in the hypercholesterolaemic 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 hypocholesterolaemic effect and to another direct effect on the cells unrelated to lipid lowering.

4.5. Molecular pathways

4.5.1. Prenylation of G proteins and effect on integrin adhesion molecules

The ability of statins to inhibit HMG-CoA reductase not only gives rise to their cholesterol-lowering effect but also is a major contributor to their ability to mediate non-cholesterol-dependent effects. The isoprenoid derivatives, geranyl (C10) and farnesyl (C15) pyrophosphates, are intermediates on the cholesterol synthesis pathway (Fig. 1), and these residues can be covalently bound to proteins, a process termed prenylation. Signalling GTPase proteins (G proteins) are major targets. Prenylation is required for the assembly of G protein heterotrimers by chain interactions (Higgins & Casey, 1996). Furthermore, G proteins are anchored to cellular membranes through prenylation, and this anchoring is required for them to exert their biological effects. The enzymes involved in prenylation have been proposed as potential targets for therapeutic intervention (Cohen et al., 2000).

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 fibres of the cytoskeleton (reviewed by Hall, 1998) and more recently of focal adhesion sites, a cell membrane component connected to stress fibres. 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 (Flinn & Ridley, 1996). This localisation is necessary for integrin adhesion function through the increase in avidity for substrates that results from the clustering. It was found initially that, in a fibroblast cell line, the Rho-induced activation of a tyrosine kinase (TK) was involved in the formation of focal adhesions and was required to induce stress fibres; genistein, a TK inhibitor, inhibited the function of phosphorylated proteins accumulating at focal adhesions (e.g., pp125 FAK). This was associated with the inhibition of stress fibre formation by exogenous agents or by injected Rho protein (Ridley & Hall, 1994). Further evidence by the same group confirmed the essential role of Rho in the assembly of the focal adhesion sites and that it involved the phosphorylation

of their constituent proteins (Flinn & Ridley, 1996). Other G protein pathways may also be involved in focal adhesion formation, as the lipoxygenase-derived arachidonic acid metabolite 5-hydroxyeicosatetraenoic acid can activate neutrophil self-adhesion via the G protein pathway Raf-1/Mek/Erk (Capodici et al., 1998).

In the case of the β_1 integrins, which are involved in leucocyte adhesion to extracellular matrix, there is direct evidence that adhesion of U937 cells involves geranylgeranylated signalling proteins (Liu et al., 1999). Lovastatin produced inhibition of adhesion that was reversed by geranylgeraniol and not by farnesol. Additionally, a specific enzyme inhibitor of geranylgeranyl transferase was capable of inhibiting adhesion. These events were paralleled by changes in RhoA geranylgeranylation. Hence, the ability of HMG-CoA reductase activity to produce isoprenoids for RhoA geranylgeranylation appears to be necessary for normal integrin activity to occur.

Integrins undergo a molecular conformational change that results in activation. Although this conformational change can be induced in vitro by binding to Mg^{2+}, it has been suggested recently that the principal regulation of integrins in vivo is by receptor clustering into adhesion complexes (van Kooyk & Figdor, 2000). Rho is required to associate with the plasma membrane to acquire this activity; therefore, its geranylgeranylation by HMG-CoA reductase products is likely to play a crucial role.

Cerivastatin was shown by Yoshida et al. (2001) to reduce human monocyte cell line adhesion to endothelial cells under physiological flow conditions via RhoA-dependent mechanisms. The membrane translocation of RhoA was decreased, and actin polymerisation was reduced (Yoshida et al., 2001). Similarly, atorvastatin was found to reduce the adhesion of U937 cells to HUVEC and to decrease RhoA and FAK activation also in those cells (Kawakami et al., 2002). It is therefore likely that HMG-CoA reductase inhibitors can inhibit focal adhesion complex formation and thereby inhibit leucocyte adhesion.

In the absence of an activating signal, the β_2 integrin LFA-1 does not associate with lipid rafts. These are localised plasma membrane subdomains, which are rich in cholesterol and sphingolipids, and can be recognised experimentally by their affinity for cholera toxin B. After LFA-1 activation in T-lymphocytes, this molecule is mobilised 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 $\alpha_4\beta_1$ integrin (Leitinger & Hogg, 2002). Direct evidence of the involvement of these events in leucocyte-endothelial adhesion has been available from the recent work of Lum et al. (2002), who have shown that IL-8/G protein-mediated stimulation of neutrophils efficiently produces a conformational change in the CD18 (β_2) integrin chain that allows arrest from flow on an ICAM-1-coated surface. This affinity for ICAM-1 was associated with a redistribution of LFA-1, but not of the other major β_2 integrin MAC-1, into membrane patches.

The events of integrin activation described above take place in leucocytes, and the relevance to atherosclerosis lies with the monocyte. However, RhoA 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 fibres is not (Wojciak-Stothard et al., 1999). 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 signalling, though evidence from cellular adhesion studies suggests that it is mainly the monocyte that is affected in vivo.

4.5.2. Other molecular mechanisms in adhesion

A recent unexpected finding was that lovastatin, simvastatin, and other statins were capable of binding to a novel site on the I-domain of LFA-1 (Weitz-Schmidt et al., 2001). 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, and a novel high affinity statin-related compound, LFA703, was found to have powerful anti-inflammatory activity. However, this field remains controversial, as other laboratories have had difficulty in confirming the statin-LFA-1 interaction.

4.5.3. Effect on 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. Likewise, they reduced the exudate content of MCP-1 and the degree of leucocyte accumulation in a mouse air-pouch inflammation model (Romano et al., 2000a). The expression of MCP-1 in both endothelial cells and monocyte-derived macrophages of atherosclerotic plaques is believed to be important in mediating monocyte chemotaxis and hence in stimulating atherogenesis. Further parallel evidence was obtained from the suppression of IL-8 and MCP-1 production by cerivastatin in *Chlamydia*-infected human macrophages (Kothe et al., 2000).

The production of the inflammatory cytokines IL-8 and IL-6 was seen to be decreased by statins in THP-1 cells, a macrophage-like cell line (Terkeltaub et al., 1994; Ikeda & Shimada 1999). Similarly, in endothelial cells, the more lipophilic statins up-regulate PPAR-α, resulting in decreased expression of IL-1, IL-6, and cyclooxygenase-2 (Inoue et al., 2000). 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 (Kluft et al., 1999).

4.5.4. Akt activation and nitric oxide

Laufs et al. (1997) discovered that statins prevent the hypoxia-induced down-regulation of NOS 3 in human endothelial cells and that this occurs via inhibition of mevalonate synthesis. Similar effects were seen with simvastatin in toxin-stimulated rat mesenteric vessels in vivo (Pruefer et al., 2002). Furthermore, in man, increased circulating NO was found in response to fluvastatin treatment, which correlated with decreased circulating soluble P-selectin and ICAM-1 levels (Kureishi et al., 2000; Romano et al., 2000b). Kureishi et al. (2000) found that simvastatin activates the signalling molecule Akt (also known as protein kinase B) in HUVEC. This activation was shown also to require phosphatidylinositol 3-kinase (PI3-kinase, which indeed is normally the upstream activator of Akt), as it could be 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 vasculoprotective effects: inhibition of atherogenesis, inhibition of platelet activation and aggregation, attenuation of endothelial cell apoptosis, and promotion of angiogenesis. Resistance to the inhibitory effect of oxidised LDL on NOS 3 activity has also been elicited by statin treatment in vitro (Laufs & Liao, 1998). Interestingly, in that study, the statins were found to exert their effect on NOS 3 expression by increasing the stability of its mRNA. 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 (Pahan et al., 1997). This may be advantageous, as the large quantities of NO generated by NOS 2 may not be so benign as the smaller amounts generated through NOS 3.

5. Effects on thrombosis

In view of the rapid beneficial effects of statins on the outcome of atherosclerotic disease, the hypothesis has to be considered that they are working directly on the thrombotic component. Although this aspect has not received much emphasis until recently, there is now much evidence in support of it. Thrombosis on mildly damaged swine arteries at high shear rate was reduced by half with atorvastatin (Alfon et al., 1999). Likewise, blood from patients on statins showed significant reduction in platelet thrombus formation (Thompson et al., 2002), and platelet-derived thrombin generation was decreased (Puccetti et al., 2001). Both lipid-lowering and non-lipid-related effects are likely to contribute, as patients with hypercholesterolaemia have hyperreactive platelets (Fuster et al., 1992; Opper et al., 1995), which would be normalised with lipid lowering. Furthermore, multiple other routes of action have been detailed (Hussein et al., 1997). 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 (Cipollone et al., 2002).

Mechanisms involved in the anti-thrombotic 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 (Laufs et al., 2000). This decreased platelet activity may relate to the reduction of geranylation of Rap1b, a protein involved in platelet aggregation (Rosenson & Tangney, 1998; Comparato et al., 2001). 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 (Degraeve et al., 2001). Quite what influence these cells could have on thrombosis in vivo is debatable, and it would be interesting to know if endothelial cells behave similarly. Monocytes are also influenced in an anti-thrombotic direction by statins, as synthesis of plasminogen activator inhibitor (PAI)-1 is decreased, a change likely to result in enhanced fibrinolysis and thrombus dissolution (Ishibashi et al., 2002). The same result may be achieved in another route in endothelial cells, as statins cause an increase in expression of tissue plasminogen activator (Essig et al., 1998). 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 (Eto et al., 2002). 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 (Undas et al., 2001). These changes did not relate to cholesterol lowering. Finally, initiation of thrombosis may be substantially inhibited by the stabilisation of plaques by statin therapy, as discussed in Section 4.

6. Other vascular effects of statins

By contrast to their action on endothelial cells, statins have been shown to induce apoptosis in rat smooth muscle cells (Guijarro et al., 1998). This could potentially be beneficial in reducing atherosclerotic lesion size. They increase small blood vessel elasticity (Leibovitz et al., 2001) and decrease vascular responsiveness to angiotensin II (Nickenig et al., 1999). Moreover, measurable decrements in blood pressure

have been found in small numbers of normotensive (Leibovitz et al., 2001), hypertensive (Sposito et al., 1999; Borghi et al., 2000), and diabetic (Velussi et al., 1999) individuals treated with statins, and a recent study in renal transplant recipients has showed blood pressure-reducing effects of statins in this group also (Prasad et al., 2003).

7. Clinical trial data

Initial clinical trials used interventions with statins to show clinical regression of disease based on quantitative coronary angiography (Vaughan et al., 2000). Typically, after 2 years of therapy, a 0.15 mm reduction was observed in the statin-treated group, and this was taken as proof of efficacy (Ross et al., 1999). However, the true significance of these trials was that the reductions in intercurrent coronary events observed in some of the larger studies were of considerable magnitude, on the order of 30–40% (Byington et al., 1995). This finding prompted a large series of end point trials (Table 2).

The Scandinavian Simvastatin Survival Study (4S), which recruited high-risk (5% per year), high-cholesterol secondary prevention (i.e., established coronary heart disease) patients, showed that a treatment-to-target regime with 20–40 mg simvastatin per day aimed at producing a final LDL cholesterol of 3.5 mmol/L reduced all events including total mortality by 30–35% (The Scandinavian Simvastatin Survival Study [4S], 1994). The similar Cholesterol and Recurrent Events (CARE) study, recruiting patients with lower cholesterol (mean total cholesterol 5.4 mmol/L) and lower risk (2.4% per year) but with more coronary heart disease complicated by stable angina, extended these findings to lower LDL cholesterol levels by showing a 25% reduction in cardiovascular events with pravastatin 40 mg daily in such patients (Sacks et al., 1996). This was also the first statin study to show a reduction in stroke (24%). The LIPID study (The Long-Term Intervention with Pravastatin in Ischaemic Disease [LIPID] Study Group, 1998) confirmed the results of CARE while suggesting additional benefits in patients with unstable angina (Tonkin et al., 2000) and cardiac failure. A comparison of treatment regimes based on lovastatin and cholestyramine showed that reducing LDL to 2.5 mmol/L resulted in less in-graft restenosis as compared with reduction to 4.5 mmol/L in patients who had received saphenous vein coronary artery by-pass grafts (The Post Coronary Artery Bypass Graft Trial Investigators, 1997). The Lescol in Prevention Study (LIPS) has extended the proof of benefit of lipid lowering to patients undergoing coronary angioplasty using fluvastatin 80 mg daily (Serruys et al., 2002). Recent evidence for the efficacy of atorvastatin has been provided by the Greek Atorvastatin Coronary Events (GREACE) study (Athyros et al., 2002) and is likely to be confirmed by the results (soon to be published) of the lipid-lowering arm of the Anglo-Scandinavian Coronary Outcomes Study (ASCOT).

The debate has now moved on to consider whether "lower is better." The Heart Protection Study (HPS) superficially confirms this idea by showing no diminution in relative risk benefit (25%) with statin therapy in patients with low LDL cholesterol (<2.5 mmol/L) (MRC/BHF Heart Protection Study, 2002). However, a prespecified metaanalysis of the pravastatin studies (the Pravastatin Pooling Project [PPP]) has shown that, where LDL cholesterol <3 mmol/L, most of the benefit of statin therapy accrues to patients with diabetes and not to those with normoglycaemia (Sacks et al., 2002). Given the heterogeneity of the cohort recruited in HPS and the large number of male patients with diabetes and a total cholesterol <5.5 mmol/L, a similar effect cannot be excluded in the HPS study (MRC/BHF Heart Protection Study, 1999). Thus, evidence is still required from the ongoing low dose-high dose statin secondary prevention comparator trials (Treatment to New Targets [TNT], Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine [SEARCH], and Incremental Decrease in Endpoints Through

Table 2
Summary of major end point trials

Primary	Treatment	Number		Starting		Reduction		Events		
		Men	Women	LDL (mmol/L)	TG (mmol/L)	LDL (%)	TG (%)	PTCA/ CABG	MI	Death
LRC	Cholestyramine	10,627	–	5.3	1.70	8	+3	–	25	20
WHO	Clofibrate	3806	–	≈5	–	(9)	–	–	19	19
HHS	Gemfibrozil	4081	–	5.37	2.01	11	35	–	34	37
VA-HIT	Gemfibrozil	2531	–	2.90	1.81	0	25	9	22	22
4S	Simvastatin	3617	827	4.87	1.51	35	10	37	34	42
CARE	Pravastatin	3583	576	3.60	1.00	28	14	27	27	24
LIPID	Pravastatin	7498	1516	3.89	1.56	25	11	20	29	22
WOSCOPS	Pravastatin	6595	–	5.00	1.70	26	12	37	31	32
AF/TexCAPS	Lovastatin	5608	997	3.89	1.78	25	15	33	40	N/A
Post-CABG	Lovastatin± cholestyramine	1243	108	3.98	1.76	14/38	–	N/A	12.5	10
HPS	Simvastatin	15,454	5082	3.5	2.0	31	23	24	26	13
GREACE	Atorvastatin	1256	344	4.65	2.08	46	31	51	59(nonfatal)	43

Aggressive Lipid Lowering [IDEAL]) to show that further reducing LDL cholesterol to 1.5–2 mmol/L will result in a significant extra benefit in absolute risk, though it is likely that a 15–20% reduction in relative risk will be seen.

The data suggesting the benefit of statin therapy in the prevention of stroke have been confused by the lack of benefit on this end point seen in the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) study despite a reduction of 17% in cardiac end points within 3 years (Shepherd et al., 2002). The cardiac end point in PROSPER follows the general rule of statin studies: a 1% reduction in coronary events for a 1% fall in LDL over a 5 year treatment period. A significant early reduction was seen in transient ischaemic attacks in this elderly (mean age 75) population, but the lack of effect on strokes could be due to the short period of the study (3 years), as statins seem to have a slower benefit on strokes than on cardiac end points or to relatively poor blood pressure control in this study (160/90 mmHg) compared with other statin studies (Wierzbicki et al., 2003b). An increase in gastrointestinal cancers was also seen in PROSPER, but all other statin studies show no effect on cancer; indeed, a decrease in colorectal cancers was seen after 8 years in 4S (Bjerre & LeLorier, 2001).

In primary prevention, pravastatin therapy was associated with a 32% reduction in mortality in middle aged, high-risk (event rate 1.8% per year) males in the West of Scotland Coronary Prevention Study (WOSCOPS) (Shepherd et al., 1995), but this study was criticised for including a significant fraction of individuals with established atherosclerosis (angina and peripheral vascular disease). These doubts were dispelled by the Air Force/Texas Coronary Arterial Prevention Study (AF/TexCAPS), which recruited a mixed population at lower risk (1.5% per year) with low HDL cholesterol (<0.95 mmol/L) and moderate LDL cholesterol (mean 4 mmol/L) and showed a 32% reduction in first cardiac events with lovastatin therapy (Downs et al., 1998).

Thus, there is now considerable evidence that statin treatment is beneficial in both primary and secondary prevention of clinical events associated with atherosclerotic disease. Traditionally, this benefit has been ascribed to their cholesterol-lowering and other lipid-related actions. As we have seen, however, there is now a considerable body of evidence that these drugs exert other effects unrelated to lipid changes—some or all of which may contribute importantly to their beneficial effects on the cardiovascular system. The relative importance of the lipid- and non-lipid-related components remains to be fully clarified.

8. Conclusion

It is now beyond question that the statins are one of the most powerful tools available in the prevention of adverse clinical outcomes, both in patients with established atherosclerotic disease and in those with subclinical disease. Much of the benefit associated with these drugs relates to improvement in lipoprotein profile. However, it is now clear that the statins exert a host of other actions on different elements of the vasculature, which would also be expected to give rise to clinical benefit; in addition, different lines of evidence now suggest that many of these actions are indeed clinically important. The findings of the statin studies in atherosclerosis have been compiled into the National Cholesterol Education Program (Executive Summary of the Third Report of the National Cholesterol Education Program [NCEP], 2001) and Joint European (Wood et al., 1998) and Joint British Societies (Joint British Recommendations on Prevention of Coronary Heart Disease in Clinical Practice, 1998) guidelines, and the use of these drugs now forms an important element of national policy in many countries (e.g., National Service Framework for Coronary Heart Disease in the United Kingdom). Studies are under way to investigate whether the benefits can be extended to other groups with high rates of atherosclerosis, including patients with diabetes mellitus, renal disease, and senescent aortic stenosis. Niche uses for statins exist in the treatment of certain lymphomas (Matar et al., 1999), and trials are being considered in the fields of colon and breast cancer. As statins affect the activity of bone morphogenetic proteins, trials are being considered in osteoporosis (Edwards, 2002). It is likely that the uses of these drugs will undergo much further investigation in the next few years to fully define the scale of their utility in clinical practice. Additionally, further research will clarify the relative importance of the lipid- and non-lipid-related actions of statins in cardiovascular disease prevention.

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