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Review

Statins: the new aspirin?

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Abstract. 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, or statins, have been described as the principal and the most effective class of drug to reduce serum cholesterol levels. Statin therapies have been shown to reduce cardiovascular events, including myocardial infarction, stroke, and death, significantly, by altering vascular atherosclerosis development in patients with or without coronary artery disease symptoms. Extensive use of statins has led to the increase of some undesirable effects that are heavily counterbalanced by the benefits. Indeed, pleiotropic effects extend far beyond cholesterol reduction and involve non-lipid-related mechanisms that modify endothelial functions, immunoinflammatory responses, smooth muscle cell activation, proliferation and migration, atherosclerotic plaque stability, and thrombus formation. In this review, we describe in detail the targets and mechanisms of action of statins.

Key words. Statin; atherosclerosis; inflammation; cholesterol; pleiotropic effect.

Introduction

Atherosclerosis is a chronic immunoinflammatory disease, which very often begins in early childhood and can lead to severe clinical manifestations later in life. Atherosclerotic vascular lesions are characterized by accumulation of lipids, fibrous elements, and immune cell infiltrates. Weakening of the fibrous cap covering an atherosclerotic lesion causes plaque rupture and thrombosis, crucial features inducing acute clinical complications such as unstable angina (UA), myocardial infarction (MI), or cerebral stroke [1–3]. Over the past few decades, atherosclerosis has been established as the most common cause of death in Western countries, responsible for half of the morbidity and mortality. Every year, 1.5 million over 250 million Americans are hospitalized for acute coronary syndromes, either UA (50%) or MI (50%). After acute MI, patients remain at high risk for recurrent coronary events and cardiovascular mortality. Indeed, within one year after an acute MI, 25% of men and 38% of women will die from vascular diseases [4, 5], and MI will reoccur in 18% of men and 35% of women within 6 years after a clinical event. Cerebral stroke leads to serious disabilities: among stroke survivors, 50% have hemiparesis, 33% are clinically depressed, 25% are unable to walk, and 17% are aphasic [6]. Silent strokes or infarct-like lesions (ILLs) increase with age with an incidence of 22% in patients over 65 years compared to 43% in patients older than 80 years [7].

Cardiovascular risk factors are numerous and vary with age. The increased incidence of cardiovascular events in Western countries mainly results from hypercholesterolemia, hypertension, smoking, diabetes, obesity, genetic backgrounds, and prolonged life span. Recent results have shown that mental stress may also participate in the development of atherogenesis [8, 9]. 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, or statins, have been described as the principal and most effective class of drugs to reduce serum cholesterol levels, and have been shown to reduce significantly

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cardiovascular events and mortality in patients with or without coronary artery disease. Due to their broad and extensive clinical beneficial effects, some of which are unrelated to lipid-lowering effects (see below), and their extremely low incidence of side effects, statins could be perceived as the new aspirin.

Structure and properties of statins

Looking to the microbial world, researchers were hoping to find a microorganism that produced an HMG-CoA reductase inhibitor as a defense mechanism against attack by other microbes which relied on sterols as part of their biochemical make-up [10]. *Pythium ultimum* was found to produce an anti-fungal substance called citrinin [11], which irreversibly inhibits HMG-CoA reductase. A new compound known as mevastatin was isolated from a second mold, *Penicillium citrinum* [12], and investigations showed that it was capable of inhibiting lipid synthesis. By 1976, Endo and Kuroda had isolated a similar molecule, lovastatin, from *Aspergillus terreus* [12]. The new compound was slightly more effective as an HMG-CoA reductase inhibitor than mevastatin. Further development of drugs based on mevastatin and lovastatin has followed three main approaches. First, synthetic compounds, such as fluvastatin, were produced by replacing the decaling ring of the fungal compounds with an aromatic ring. Second, chemical alteration of fungal products created drugs such as simvastatin. Finally, microbial alteration of fungal compounds has lead to drugs such as pravastatin. The affinity of HMG-CoA reductase is substantially higher for the natural statins (in the case of mevastatin, 10,000 times higher) than it is for HMG-CoA; mevastatin acts as a reversible competitive inhibitor to the enzyme reaction, and less mevalonic acid is produced in its presence. On the other hand, synthetic statins model the product mevalonate. Thus, the cholesterol production pathway is broken. The introduction of a competitive inhibitor for HMG-CoA reductase results in two physiological responses. In compensation for the inhibition, cells begin to produce more HMG-CoA. The direct reduction in circulating cholesterol is therefore only small. However, the number of low-density lipoprotein (LDL) receptors on the cell surface of patocytes increases markedly [13]. As the liver is responsible for removing LDL cholesterol from plasma via the LDL receptor mechanism, blood cholesterol levels also fall dramatically.

Because of the increased incidence of cardiovascular events in western countries, statins have interested the pharmacological industry. Since the development of lovastatin and its approval in 1987, 11 other statins have been developed or are in the process of being studied. Five of these are actually commercialized: atorvastatin, fluvastatin, lovastatin, pravastatin, and simvastatin. Atorvastatin, pravastatin, and simvastatin represent almost 70% of statins used in Europe and the United States [14]. Two subtypes of statins are available on the market: the natural (fermentation-derived) and the synthetic statins (fig. 1). Molecules derived by fermentation, including lovastatin, pravastatin, and simvastatin have very similar chemical structures. Simvastatin is approximately twice as potent as pravastatin and lovastatin, whilst mevastatin is the least powerful. On the other hand, the structures of the synthetic statins, atorvastatin, cerivastatin, and fluvastatin, are very different. The described structural characteristics are closely related to the physicochemical properties of HMG-CoA reductase inhibitors. By altering the basic chemical composition of the mevastatin molecule, drug potency can be increased. Statins differ in their lipophilicity/hydrophilicity (table 1) [15], which reflects their potential to cross cellular membranes nonselectively by passive diffusion, and explain why pravastatin does not easily cross cellular membranes whereas lovastatin and simvastatin do [16]. Large clinical human studies have demonstrated that statins reduce total serum cholesterol by 15–40%, LDL cholesterol by 20–60%, triglycerides by 10–30%, and increase high-density lipoprotein (HDL) cholesterol by 5–15% [17]. LDL plasma levels are more effectively reduced by the latest developed atorvastatin compared to the older statins [18], with a incremental reduction in LDL by 6–7% when the statin dose is doubled [19]. Nevertheless, compared to other statins, atorvastatin does not increase HLD cholesterol in a dose-dependent manner [20].

Mechanism of action

By inhibiting L-mevalonic acid synthesis, statins also prevent the catabolism of other isoprenoid intermediates of the cholesterol biosynthetic pathway (fig. 2), such as farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP) [21]. Thus, since mevalonate is not the only precursor of cholesterol, statins have pleiotropic effects by concomitant regulation of numerous other mevalonate metabolites [21–24]. Indeed, isoprenoids are vital for multiple cellular functions, such as covalent attachment, and GGPP and FPP prenylation of numerous

Table 1. Comparison of the lipophilicity of statins.

Statins	Lipophilicity at pH 7.4 (log + SE)
Cerivastatin	1.69 + 0.02
Simvastatin	1.60 + 0.06
Fluvastatin	1.27 + 0.07
Atorvastatin	1.11 + 0.02
Rosuvastatin	–0.33 + 0.06
Pravastatin	–0.84 + 0.06

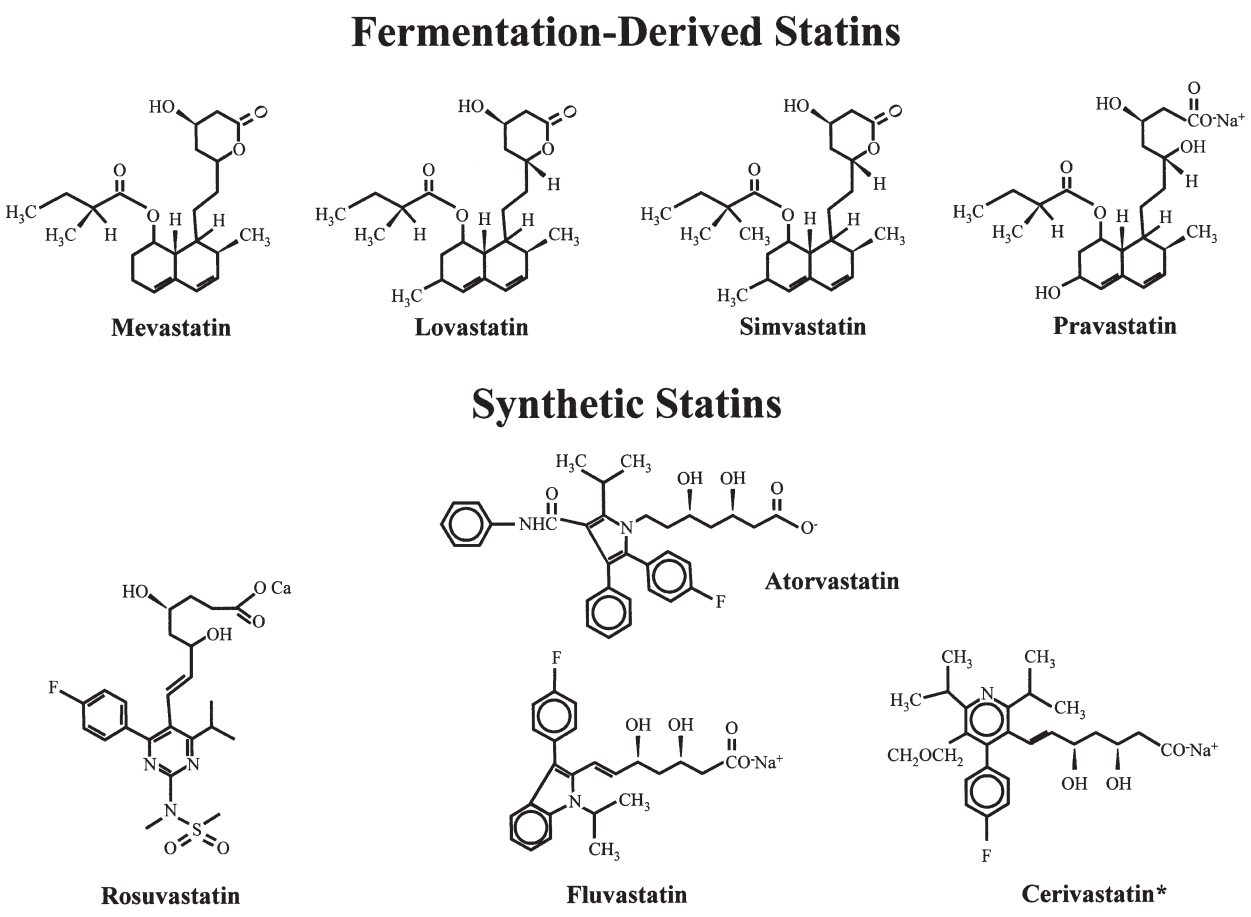

Figure 1. Chemical structures of fermentation-derived (natural) statins and synthetic statins. *Cerivastatin was removed from the market since August 2001.

proteins. Prenylation of proteins is a prerequisite for the cell membrane association of both plasma and internal membranes, and is essential for their functions [25, 26]. These prenylated proteins include the γ subunit of heterotrimeric G proteins, heme-a, nuclear lamins, and the GTP-binding proteins Ras and Ras-like proteins (Rho, Rab, Rac, Ral, and Rap) [27]. Within endothelial cells, the posttranslational modification of Ras is FPP dependent, whereas Rho is GGPP dependent [28, 29]. Proteins of the Rho family are implicated in cell shape, cytoskeleton organization, motility, secretion, proliferation, and cell signaling. In response to extracellular signals such as growth factors or during cellular proliferation, migration or mitosis, cells undergo reorganization of their actin cytoskeleton, which alters the three-dimensional colocalization of their proteins [27–30]. Thus, Rho-induced changes in the actin cytoskeleton may affect intracellular transport, membrane trafficking, mRNA stability, and gene transcription [31]. Statin inhibition of the Rho kinase pathway, a downstream Rho target, leads to the accumulation of inactivated Rho within the cell cytoplasm, which is believed to have pleiotropic effects on vascular cells, leukocytes, and bone [29, 32–35]. In addition, several studies have shown that statins decrease the expression and secretion of many different immunoinflammatory molecules, which could be prevented by the addition of L-mevalonate and, in some cases, by farnesol or geranylgeraniol [23, 29, 36–39].

By inhibiting the RhoA signal transduction pathway [40], statins are able to activate peroxisome proliferator-activated receptor (PPAR) α, β/δ, and γ in a dose-dependent manner [41], an effect which could be reversed by FPP, GPP, cholesterol, or sterol regulatory element-binding protein-1 (SREBP-1) [41]. PPARs constitute a subfamily of the nuclear receptor family [42] comprising three isotypes, PPARα, β, and γ, which are characterized by distinct tissue [43, 44] and developmental distribution patterns [45]. PPARs are ligand-activated transcription factors which, upon heterodimerization with the retinoic X receptor (RXR), bind to specific peroxisome proliferator response elements (PPREs) located in the promoter of target genes [46]. PPARs exhibit direct anti-inflammatory properties beside their crucial role in β-oxidation of fatty acids and arachidonic acid metabolites [47]. Indeed, PPARs are involved in the control of vascular inflammation and thrombogenicity related to atherosclerosis [48,

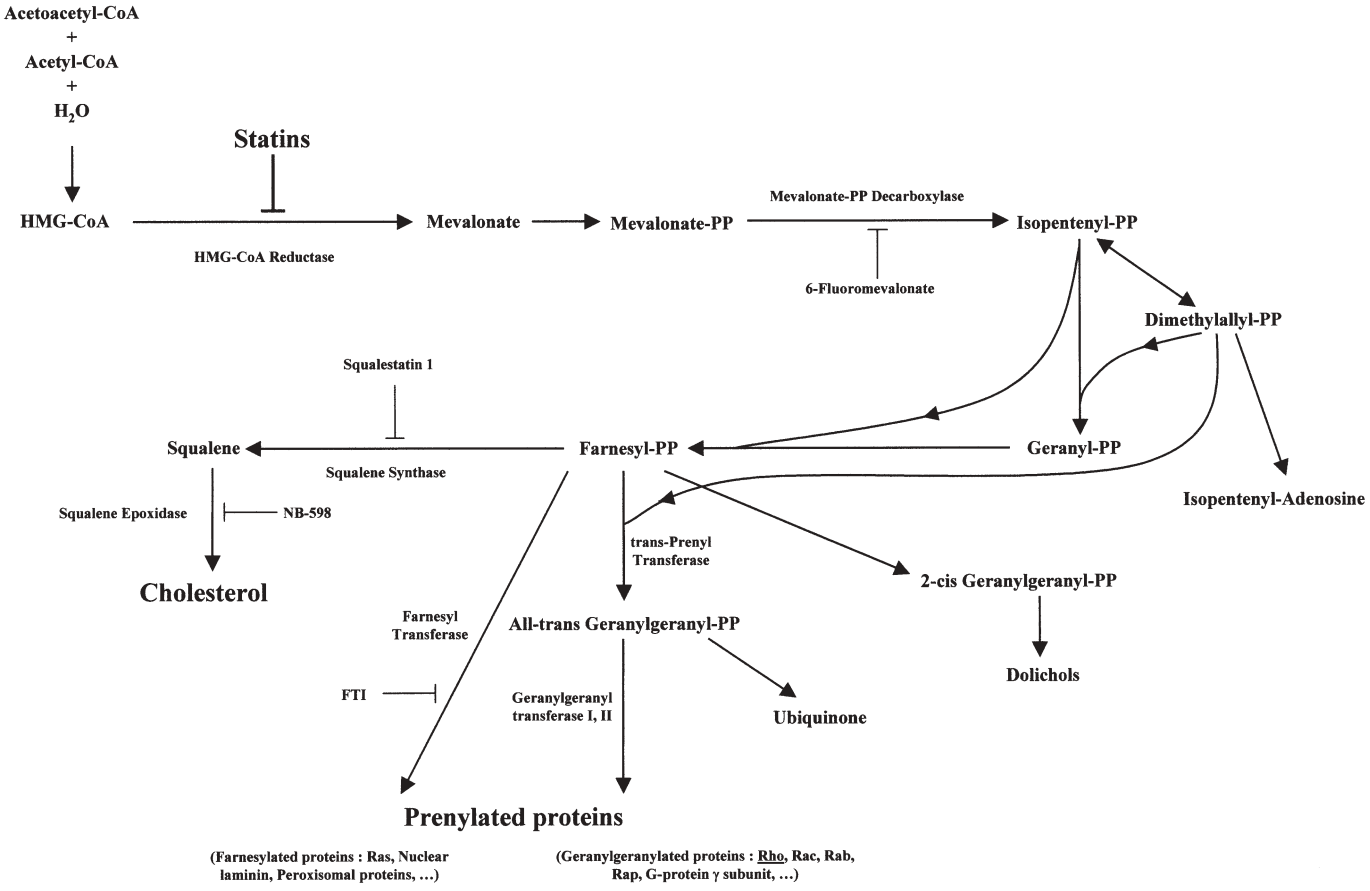

Figure 2. The metabolic pathway of mevalonate and cholesterol. The enzymatic steps and their inhibitors are indicated.

49]. PPARα has been shown to exert anti-inflammatory actions by preventing the binding of the activator protein-1 (AP-1) and the nuclear factor kappa B (NF-κB) protein to their DNA target sequences [50–52]. The activation of NF-κB and the synthesis of numerous NF-κB-dependent gene products have been linked to the activation of inflammatory cells [53] (fig. 3). Recent reports have shown that NF-κB was activated in 60% of atherosclerotic lesions of untreated mice, especially within macrophages and smooth muscle cells (SMCs), compared to only 30% for atorvastatin-treated animals [54]. In addition, SMCs activated with tumor necrosis factor alpha (TNF-α) and treated with atorvastatin showed reduced activity for NF-κB and decreased expression of the chemokine MCP-1 [54], an important chemoattractant protein crucial for the recruitment of inflammatory cells [55]. Of note is that NF-κB is one of the most pleiotropic transcription factors, and is the main nuclear factor involved in the activation of MCP-1 transcription [56, 57]. Thus, mechanisms of anti-inflammatory properties of statins may be in part mediated via PPARs (fig. 3).

Pharmacokinetic effects

Absorption

With the exception of lovastatin and simvastatin, which are administered as lactone prodrugs and must be hydrolyzed in vivo to the corresponding β-hydroxy acid to achieve pharmacologic activity, all statins are administered as the active β-hydroxy acid form.

The extent of absorption of statins varies from 30 to 98% [15, 58, 59]. All statins are absorbed rapidly following oral administration, with time to peak concentration reached within 4 h. Fluvastatin is the only statin able to achieve peak plasma concentration in the micromolar range, which may explain its direct antiatherosclerotic properties. Food intake has variable effects on the absorption of statins, with decreased bioavailability for fluvastatin, pravastatin, and atorvastatin. However, lipid-lowering effects of statins do not differ when taken with the evening meal or at bedtime. The plasma half-life is 2–3 h for all statins except atorvastatin, which has a half-life of 14–20 h [18]. Recently, fluvastatin has also become available in a slow-release form. The pharmacokinetic half-lives of statins do not correspond to the dura-

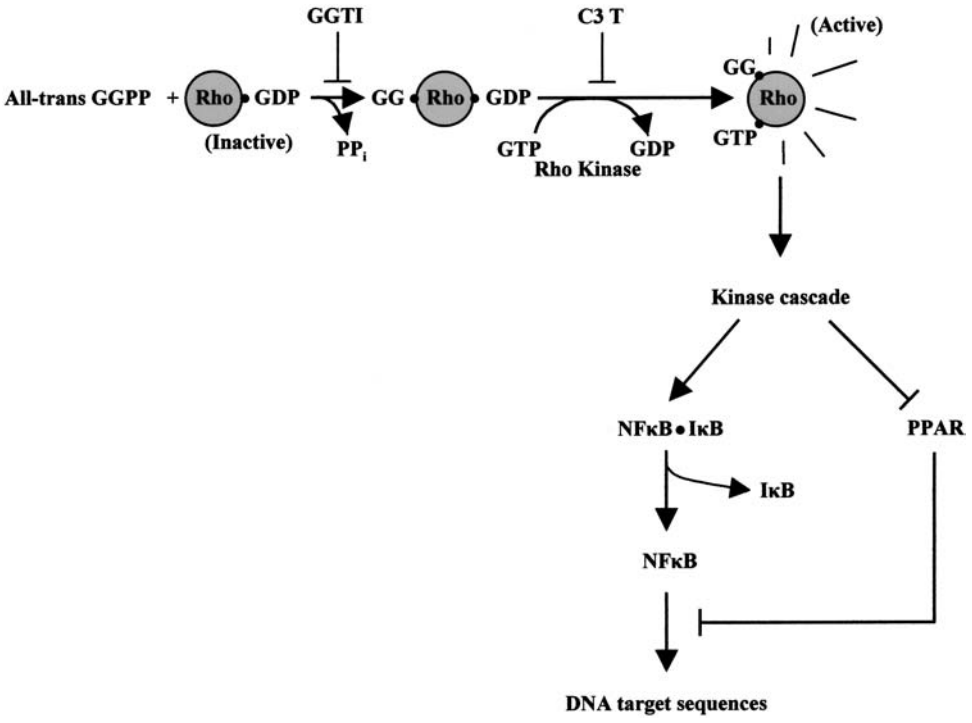

Figure 3. The RhoA prenylation pathway and its main targets.

tion of their pharmacodynamic properties. Due to its long half-life, atorvastatin can accumulate in plasma, achieving a steady-state drug concentration after multiple doses.

Distribution

Except for pravastatin, all statins are highly bound to plasma proteins (mainly albumin). Therefore, systemic exposure to unbound active drugs remains extremely low. In addition, since statins are highly extracted by the liver, drug displacement interactions are also limited. As a result of low plasma protein binding, unbound pravastatin is tenfold higher than the unbound form of other statins [60]. Nevertheless, widespread tissue distribution is prevented by the high hydrophilic properties of pravastatin. With the exception of pravastatin, all statins and their metabolites are excreted via the bile into feces. The amount of statin excreted in urine is negligible, except for pravastatin, which can reach 20% [61, 62].

Metabolism

Almost all statins are metabolized by cytochrome P450 (CYP450) enzymes (3A4, 2C9, 2D6, 1A2) [63] present in the liver, but also in the intestine. Numerous drugs that can be administered concomitantly with statins are often inhibitors of CYP 3A4 (e.g., cyclosporine A, verapamil, diltiazem, and grapefruit juice), and therefore might reduce or block the metabolism of certain statins. This inhibition of statin metabolism can raise their plasma concentration above a toxic threshold, leading to muscular lysis. Furthermore, some statins are transported to the intestinal lumen via P-glycoprotein (P-gp) [59], and most CYP 3A4 inhibitors are also P-gp inhibitors. Thus, these drug interactions often result in the coinhibition of their hepatic and intestinal CYP 3A4-dependent metabolism and of their P-gp-dependent transport [64]. For example, coadministration of a statin and cyclosporine A increases the incidence of toxicity. Among statin molecules, some can interact with other medications, depending on their hepatic and intestinal metabolism. In contrast, certain statins do not, or interact less through the CYP 3A4-dependent metabolic pathway [64]. Pravastatin has multiple metabolic pathways, thus decreasing the likelihood of any drug interactions with the CYP 3A4 enzyme. The statin fluvastatin is predominantly metabolized by the cytochrome CYP 2C9, and also by CYP 3A4 and CYP 2C8, and the use of CYP 2C9 inhibitors is rare [65]. Cerivastatin and atorvastatin are metabolized in part by CYP 3A4, but if this cytochrome is inhibited they will be eliminated via other metabolic pathways with a decreased degradation rate. The statins simvastatin and lovastatin are almost exclusively CYP 3A4 dependent. Although pharmacokinetic effects are of great importance, most of the drugs that might cause interaction problems, like erythromycin and cyclosporine, can be managed clinically. Indeed, low doses of statin used with cyclosporine A

seem to be very safe, and statin therapy can easily be interrupted for some days if needed.

Toxicity

The benefits of HMG-CoA reductase inhibitors are supported by extensive literature. However, the growing interest in statins and concomitant increase in prescription rates have led to a rise in undesirable side effects such as hepatotoxicity and myopathy. In August 2001, cerivastatin was reported to cause severe myopathy and rhabdomyolysis with an unexpectedly high frequency compared to other statins of equal or greater efficacy [66, 67]. Cerivastatin has since been removed from the market. These clinical symptoms occur in 1 out of 1000 patients, however, if the clinical events related to cerivastatin are excluded, this ratio is reduced to roughly 1 out of 10,000 patients for all statins together [14]. In changing the shape of the active molecule, the chances and severity of side effects are also altered. For example, there is an increased risk of muscle toxicity with lovastatin in comparison to pravastatin. Although the incidence seems to be dose-dependent, the explanation of myotoxicity is still unclear and controversial, and many hypotheses have been proposed.

Most of the HMG-CoA reductase inhibitors are metabolized by the liver. Damage to this organ is assessed by a persistent elevated level of its transaminases: aspartate transferase (ATS) and alanine transferase (ALT) [14]. Statin treatment commonly leads to a small increase of ALT. Levels of ALT more than three times the upper normal limit indicate a potential liver toxicity (cholestatic jaundice) in patients. Up to 5% of patients will show a slight increase in ATS or ALT at about 6 weeks after initiation of therapy [14]. Transaminase elevation up to the toxicity threshold is present in 1–2% of patients under statin treatment, and clinical symptoms are dependent on patients [68, 69]. In addition, the frequency of persistent transaminase elevation is consistent with all commercialized statins, and is dose dependent [70].

Pharmacodynamics

Although any drug should be administered with appropriate caution, it can be said that the clinical benefits of statin therapy far exceed the very low incidence of risk. Indeed, the beneficial effects of statins extend far beyond their lipid-lowering properties. The LDL cholesterol level is an important target for cardiovascular prevention and the concept of LDL plasma cholesterol level magnitude has been tightly correlated with arteriosclerosis lesion extent and clinical cardiovascular events. However, only half of individuals suffering from an elevated LDL plasma cholesterol level will die from coronary heart disease (CHD) [71, 72]. Serum total cholesterol and CHD have been correlated [72–74]; however, to improve and better define CHD risk, measurements of HDL [75], fibrinogen [76, 77], plasma viscosity [78] and C-reactive protein (CRP) [76] are very helpful. Several clinical studies have demonstrated that statin therapy reduces CRP levels independently of lipid-lowering effects (see below).

HMG-CoA reductase inhibitors may delay or improve the pathogenesis and clinical symptoms of Alzheimer's disease (AD) [79, 80]. Recent studies suggest that lipids play an important role in the development of AD [80]. Indeed, apolipoprotein (ApoE) $\varepsilon 4$, an allele of Apo E, is the major genetic risk factor for AD [81]. Furthermore, the isoform ApoE $\varepsilon 4$ correlates with an increased risk for atherosclerosis [82] and amyloid plaque formation [83]: a high cholesterol plasma concentration increases amyloid plaque formation [84, 85]. Since the cellular cholesterol level affects neuronal beta-amyloid peptide ($A\beta$) production in vitro [86], and $A\beta$ has been found to be the major constituent of amyloid plaque, the serum cholesterol concentration might be correlated with $A\beta$ protein expression. In retrospective studies, statin therapy has shown to reduce the occurrence of AD by 70% [87]. However, AD may also occur because of other biological factors, as is the case with atherosclerosis. Recent in vitro and in vivo experiments have highlighted the antiatherosclerotic effects of statins by lipid-independent mechanisms. These pleiotropic effects of statins on the development of atherosclerosis lesions are described below.

Improvement of endothelial function

Nitric oxide (NO) synthesis is a crucial mediator of vascular homeostasis and blood flow. Decrease of NO synthesis by vascular endothelial cells promotes, in part, vasoconstriction, platelet aggregation, and leukocyte recruitment and adhesion [88–93]. Knock-out mice lacking endothelial NO synthase (eNOS) revealed increased arterial blood pressure and exhibited larger cerebral infarctions after middle cerebral artery occlusion [92–94]. Moreover, cerebral blood flow is reduced and postfocal ischemia tissue damage is induced when eNOS activity is inhibited [95]. In contrast, enhanced NO production by administration of either an NO donor or the eNOS substrate, L-arginine, confers stroke protection after induction of cerebral ischemia [96–98]. Statins can directly upregulate eNOS expression in vitro under cholesterol-clamped condition [29, 99, 100]. Indeed, beneficial effects of statins are absent in eNOS-deficient mice [101, 102]. Statins increase eNOS expression by extending eNOS mRNA half-life, but not eNOS gene transcription [28]. Furthermore, statins reduce in vivo cerebral infarct size [101] as well as oxidative stress [103], and improve neurological function in normocholesterolemic mice

[101]. However, statins have additional effects on endothelial cells. Expression of the procoagulant tissue factor induced by thrombin is prevented by simvastatin treatment through inhibition of Rho/Rho kinase and activation of Akt [104]. Statins also increase the expression of tissue-type plasminogen activator [37, 105] and inhibit the expression of endothelin-1 [38], a potent vasoconstrictor and mitogenic molecule that regulates vascular tone and remodeling [106]. These findings suggest antiatherothrombotic effects of statins that might be very relevant in the prevention of acute coronary syndromes [107].

Reduction of inflammation

Endothelial dysfunction, related to vascular injury in response to cardiovascular risk factors, triggers the migration of leukocytes within the vessel wall, mainly that of monocyte/macrophage and T lymphocyte types [108]. Adhesion molecules, proinflammatory cytokines and chemokines mediate the extravasation of inflammatory cells. Within atherosclerotic sites, endothelial cells and leukocytes both increase their expression of numerous adhesion molecules and counter receptors, such as the intercellular adhesion molecule-1 (ICAM-1) [109], vascular cell adhesion molecule (VCAM-1), β_1-integrin and β_2-integrin [110], and P-selectin [102]. Blocking these adhesion molecule interactions by administration of antibodies or gene targeting (knock-out) has been found to decrease atherosclerotic lesion formation in vivo, indicating a potential therapeutic role for inhibition of leukocyte adhesion and extravasation [111, 112]. Indeed, several in vitro studies have described the beneficial effects of statins by decreasing adhesion molecules such as the monocytic CD11b [113] and the leukocyte function antigen-1 (LFA-1) [114, 115]. Of note is that the expression LFA-1 is HMG-CoA reductase independent; statins can bind directly via a novel regulatory site of β_2-integrin, which serves as a major counter receptor for ICAM-1 on leukocytes [114]. In addition, other in vitro studies have demonstrated that statins could also reduce the secretion of the proinflammatory cytokines interleukin (IL)-6 and IL-1β but not TNF-α, the chemokines IL-8, IP-10 and MCP-1, as well as the important immunoregulator CD40 [F. Mulhaupt and F. Mach, unpublished data; 54, 88, 116–122]. All these observations support recent human studies suggesting that statins reduce the number of inflammatory cells within atherosclerotic plaques [123, 124]. The inhibitory effect of statins on leukocyte recruitment was confirmed by in vitro measurements on monocytic cells (U937) pretreated with cerivastatin. This treatment reduced the adhesion of U937 cells to an activated endothelium by the downregulation of CD11a, CD8, and VLA4 [125], an effect that could be reversed by mevalonate. Furthermore, Yoshida et al. [125] demonstrated that cerivastatin also reduced F-actin polymerization via RhoA inhibition within monocytes. These results were confirmed by the preincubation of monocytic cells with C3 transferase, an exoenzyme inhibiting Rho by ADP ribosylation, which could reduce monocyte adhesion to endothelial cells [125, 126]. However, the expression level of integrins on the U937 cell surface was not significantly altered. Protein geranylgeranylation, specifically RhoA geranylgeranylation, was recently reported to be required for integrin-dependent adhesion of leukocytes [127]. Therefore, RhoA may modulate the affinity of integrins. Indeed, as mentioned in another report, RhoA is required for the clustering of adhesion molecules, such as E-selectin, ICAM-1, and VCAM-1 on the endothelial cell surface when monocytes adhere to the endothelium [126]. These data suggest that Rho is required in endothelial cells for the assembly of stable adhesions with monocytes via the clustering of monocyte-binding receptors and their association with the actin cytoskeleton [126]. Similar but complementary in vivo experiments [102] with the new statin rosuvastatin (not yet commercially available) showed important anti-inflammatory effects via inhibition of endothelial cell P-selectin expression, a protective action mediated by vascular endothelial NO. Rosuvastatin had no effect on leukocyte-endothelium interactions in eNOS-deficient mice, thus underlining the crucial anti-inflammatory role of NO. Since P-selectin stored in Weibel-Parade bodies within endothelial cells is translocated to the cell surface upon stimulation by various inflammatory mediators (such as histamine, thrombin, and oxygen-derived free radicals [128]) via a process modulated by NO [129], increased NO production by statins could explain the modulation of these leukocyte-endothelium interactions.

The reduction of inflammation by statin treatment has been demonstrated by the reduction of high-sensitivity CRP (hs-CRP), a clinical marker of inflammation produced by the liver in response to proinflammatory cytokines such as IL-6 [130, 131]. The level of CRP expression is elevated in patients with coronary artery disease, coronary ishemia, and MI compared to normal subjects [132, 133]. Statin therapy lowers CRP levels, without correlation with lipid lowering (either LDL or total cholesterol) [70, 131, 134–136]. Indeed, almost all patients who clinically benefit from statin therapy had abnormal elevated CRP values [137].

SMC proliferation

SMC proliferation is a central event in the pathogenesis of vascular lesions such as atherosclerosis, postangioplasty restenosis, or transplant arteriosclerosis. Stimulation by chemokines or growth factors, released from endothelial cells, macrophages, or T cells, causes activation, proliferation, and migration of SMCs from the media to

the intima [138, 139]. Independently of their lipid-lowering properties, statins (simvastatin, cerivastatin, and fluvastatin) have been reported to reduce in a dose-dependent manner SMC migration and proliferation in vitro [39, 140, 141]. This inhibitory effect was prevented in vitro by addition of mevalonate, all-trans farnesol (F-HO) and all-trans geranylgeraniol (GG-OH), but not by 2-cis GG-OH, squalene, or ubiquinone [140]. In the same direction, statin treatment has demonstrated a beneficial effect on the rate of restenosis as well as reducing major adverse cardiac events following percutaneous transluminal coronary angioplasty (PCI) in human studies [142–144]. Statins may affect cell growth via interference with signaling pathways that require prenylated proteins [21, 26]. Isoprenoid intermediates may also regulate platelet-derived growth factor (PDGF), which is implicated in SMC DNA synthesis [33]. Whereas statins inhibit PDGF, they also downregulate retinoblastoma gene products and cyclin-dependent kinase (cdk)-2, -4, and -6 activity [33]. In contrast, Laufs et al., [33] demonstrated that the cdk inhibitor protein $p27^{Kip1}$, which binds to and inhibits the activation of cdk-cyclin complexes, increased under statin treatment, but no changes were observed for p16 and $p21^{Waf1}$ cdk inhibitors or the p53 tumor suppressor gene [33]. This upregulation of $p27^{Kip1}$ was linked to the inhibition of Rho but not of Ras, due to the reverse effect of GGPP and not of FPP. Furthermore, addition of C3 or N19RhoA could also increase $p27^{Kip1}$ and inhibit retinoblastoma hyperphosphorylation, resulting in the release of the transcription factor E2F, which induces the expression of genes required for progression through S, G2, and M mitosis phases [145]. In addition, activation of Rho GTPase decreased $p27^{Kip1}$ and increased SMC DNA synthesis, suggesting that the downregulation of $p27^{Kip1}$ by Rho GTPase is mediated by PDGF. These findings are consistent with recent studies showing that elimination of $p27^{Kip1}$ during the G1 to S phase is required for the growth activation of FRTL-5 cells, and that GGPP but not FPP restores the inhibitory effect of statins on the degradation of $p27^{Kip1}$ and allows cdk2 activation [146]. Statins have been reported to cause a significant and dose-dependent reduction in cell proliferation (vascular SMCs in culture). This delay of cycling cells in the G1 and G2/M phases, a phenomenon reversible by mevalonate, leads to apoptosis in different SMCs of different origin [36, 147]. Statins (simvastatin, lovastatin, and fluvastatin) not only decrease SMC migration and proliferation in vitro, but also in vivo [36, 148]. Stark and colleagues [149] showed that geranylgeranylated proteins were not only required for growth but also for apoptosis protection. Indeed, recent studies have highlighted the possible implication of apoptosis in SMC proliferation within atherosclerotic lesions [150, 151].

Apoptosis

Programmed cell death of vascular SMCs has recently been identified in physiological remodeling of the vasculature, as well as in atherosclerosis and restenosis. Statins induced apoptosis of endothelial cells and SMCs in a dose-dependent manner [152, 153], an effect that could be reversed by L-mevalonate, FPP, and GPP, but not by isopentenyl adenosine, ubiquinone, or squalene. Prenyl transferase inhibitors confirmed the involvement of prenylated proteins in SMC apoptosis. Treatment with statin (atorvastatin, lovastatin, and simvastatin but not pravastatin) was associated with a decrease in the prenylation of p21-Rho B, a protein that plays an important role in the control of apoptosis [152]. Human vascular SMCs express the receptor Fas, whereas Fas ligand is expressed on endothelial cells, macrophages, and T lymphocytes, indicating that Fas/Fas ligand-mediated apoptosis may occur in vascular cells at sites of high inflammation [154]. Stimulation with the cytokines interferon (IFN)-γ, TNF-α and IL-1β induces Fas expression and thereby enhances the apoptotic effect of Fas in certain cell types [154, 155]. The elevated secretion of proinflammatory cytokines and chemokines from vascular endothelial cells, SMCs, and inflammatory cells may increase the apoptotic rate of these cells within the neointima of atherosclerotic plaques. Other pathways may induce apoptosis, such as c-myc, p53, and Bcl-2. Apoptosis is also regulated by insulin-like growth factor (IGF-1) and PDGF, which are survival factors reduced by statins in vitro [33, 156]. In contrast, apoptosis of SMCs is decreased in human carotid plaques of pravastatin-treated patients [124]. Statin treatment may reduce apoptosis by decreasing proinflammatory mediators as well as inflammatory cells within atherosclerotic lesions.

Plaque stability and thrombosis

The composition and tensile strength of an atherosclerotic plaque, rather than its size, are the most important determinants of atherosclerosis complications (fig. 4). Advanced atherosclerotic plaques are characterized by a highly thrombogenic lipid core covered by a thin fibrous cap composed of SMCs and extracellular matrix [157]. Collagen, secreted by SMCs, is the main component of the fibrous cap, and is responsible for its tensile strength [157]. In addition to collagen, paucity of vascular SMCs and prominent accumulation of inflammatory cells are the characteristic components of atherosclerotic plaques prone to rupture with further thrombus formation and consequent clinical cardiovascular events [158]. The degradation of collagen plays an important role in the development and subsequent instability of the plaque. Secretion of proteolytic enzymes, such as matrix metalloproteinases (MMPs), by SMCs and macrophages affect the fibrous content of atherosclerotic lesions, particularly

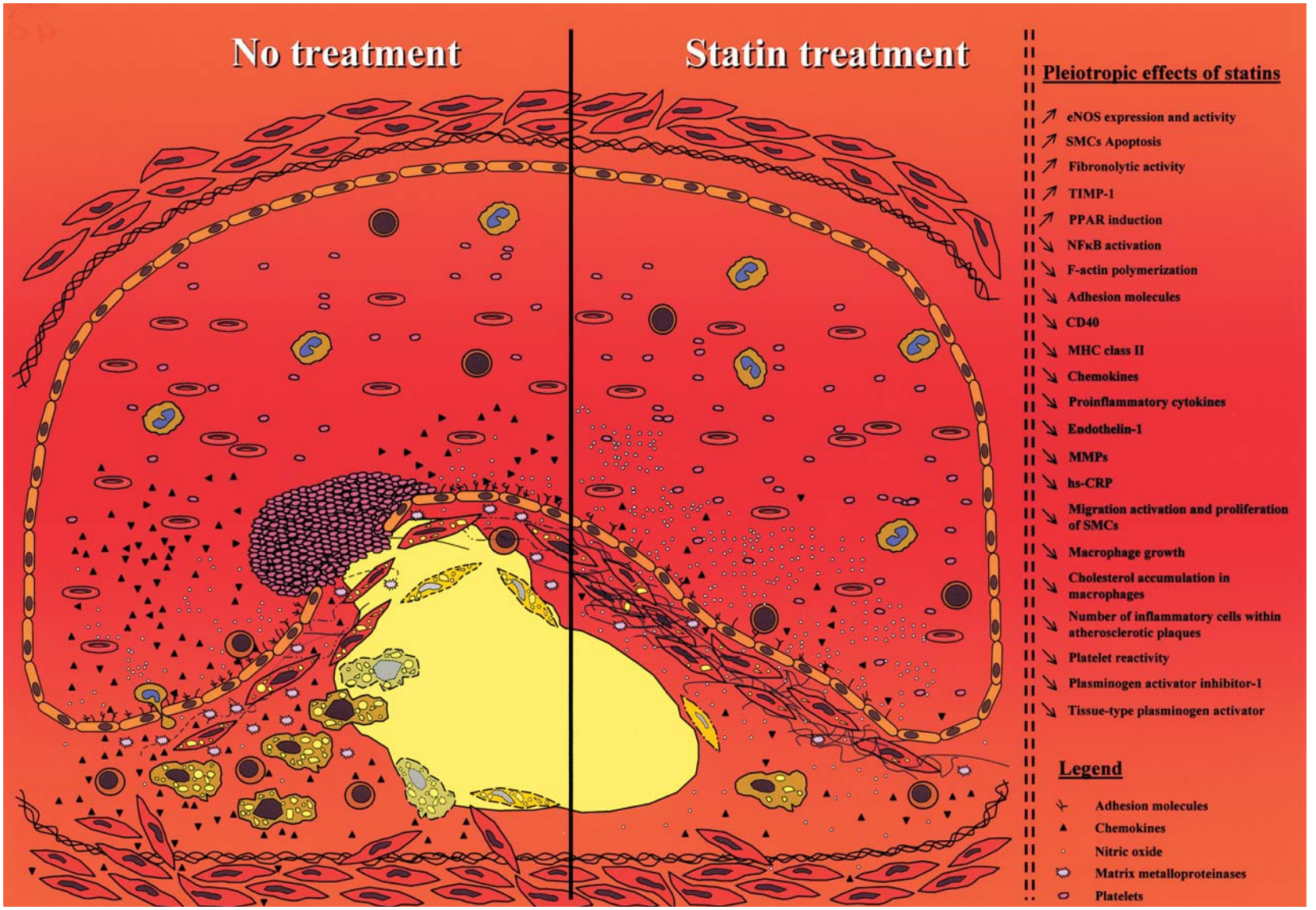

Figure 4. Schematic drawing of an advanced atheroma. Necrosis of macrophages and SMC-derived foam cells leads to the formation of a necrotic core with accumulation of extracellular cholesterol. On the left side are represented the major pathophysiological features occurring within an arterial vessel wall of a non-statin treated patient not treated with statins. Numerous inflammatory cells, poor SMCs and collagen content of the fibrous cap, degraded principally by the matrix metalloproteinases often lead to plaque rupture followed by thrombus formation. On the right side are represented the main beneficial effects of statin therapy resulting in a more stable atherosclerotic plaque.

at the shoulder region of the plaque, the margin between the lesion and the unaffected portion of the artery. Indeed, this vulnerable region is predominantly inhabited by foam cell macrophages [158]. In addition, macrophages are capable of weakening the extracellular matrix by phagocytosis [159]. Furthermore, apoptosis of SMCs and macrophages, resulting from cell-cell interactions and the local cytokine environment, involving pro- and antiapoptotic proteins that include death receptors, proto-oncogenes, and tumor suppressor genes, also appears to influence the stability of the plaque [2, 160]. Thus, fissuring, erosion, and ulceration of the fibrous cap leads to plaque rupture followed by thrombosis [161]. Plaque disruption with superimposed thrombosis is the main cause of acute coronary syndromes such as UA, MI, and sudden death [158].

Although changes in plaque size by lipid-lowering treatment may occur over an extended time and are quite minimal [162], statins may contribute to plaque stability mainly by modifying cellular composition and physiochemical properties of atherosclerotic plaques. Statins decrease monocyte/macrophage infiltration as well as the proliferation and migration of SMCs into the neointima. Statins also reduce the secretion of MMPs by SMCs [163] and macrophages [164–166]. MMP-1, MMP-3, and MMP-9 secretion could be inhibited in vitro in a dose-dependent manner in macrophages and other vascular cells [164, 167, 168]. Similar results were obtained for MMP-2 in human carotid plaques with prospective statin treatment [124]. The nuclear factor NF-κB (regulated by statins) has also been demonstrated to control the secretion of MMP-1 and MMP-3, but not the expression of MMP-9, which might be linked to another regulation factor of the GGPP pathway cascade such as I-κBα [167, 168]. In the same way, statins induce the expression and secretion of tissue inhibitor of metalloproteinase 1 (TIMP-1) by SMCs and macrophages [124]. Regarding thrombosis, statin treatment has been shown in vitro and in vivo to reduce greatly the expression of tissue factor by vascular cells [104, 166]. Major determinants of the fibrolytic balance, such as plasminogen activator inhibitor-1 (PAI-1) and the tissue-type plasminogen activator (tPA) are also reduced by statin treatments. Indeed, simvastatin has been shown to reduce the secretion of PAI-1 from endothelial cells and SMCs, while the release of tPA was increased at the same concentrations [105]. This effect was linked to geranylgeranyl-modified intermediates, since it could be mimicked by C3 exoenzyme and prevented by GGPP, but not by FPP [105]. All these recent findings indicate that beyond their lipid-lowering effects, statins may reduce important inflammatory processes implicated in atherogenesis and plaque rupture that might in part also explain their great clinical benefits in cardiovascular diseases.

Statins as immunomodulators

Although a few clinical trials have suggested a better outcome of cardiac transplantation for patients on statin therapy [169, 170], statins have never been shown to be involved in the immune response. Major histocompatibility complex MHC class II (MHC-II) molecules, expressed on the surface of specialized cells, play a direct role in the control of the immune response and thus determine rejection after organ transplantation. Whereas a limited number of specialized cell-types express MHC-II constitutively, numerous other cells become MHC-II positive upon induction by the inflammatory mediator IFN-γ [171], including human endothelial cells and monocytes. This complex regulation is under the control of the transactivator CIITA [172, 173]. In recent studies, we showed that four different statins were able to inhibit inducible MHC-II expression in primary human endothelial cells and human monocyte/macrophages via inhibition of the promotor IV of the transactivator CIITA [174, 175]. These findings were confirmed by other reports [176, 177]. Since interactions between the endothelium and immunocompetent cells are crucial in the pathogenesis of cardiac allograft vasculopathy, our findings may represent one among other important mechanisms by which statins exert their beneficial effects after cardiac transplantation [169, 170, 178].

Therapy and prevention

The development of cholesterol-lowering agents helped to elucidate the implication of LDL cholesterol in cardiovascular diseases. Extensive clinical trials have demonstrated that some agents could reduce cardiovascular events, including MI, stroke, and death, by altering vascular atherosclerosis development in patients with or without previous symptoms of vascular artery disease [70, 134, 179–185]. Bile-sequestering resins, fibrates, and nicotinic acid (NA) have been used to reduce cholesterol, but were modestly effective and only poorly tolerated with low compliance [14]. Fibrates and NA have even inherent toxicities as they amplify the risk of hepatitis and myopathy. Indeed, several studies showed that these older lipid-altering agents increase the risk of noncardiovascular death, and hence do not contribute to reducing total mortality.

Since their discovery in 1973, statins have been described as the principal and the most effective agents used to reduce serum cholesterol levels. Statins, or HMG CoA reductase inhibitors, have proven their beneficial effects in reducing total and LDL cholesterol as well as triglycerides, and increasing HDL cholesterol. Statin therapy has been shown by overwhelming clinical evidence to reduce significantly the risk of new or recurrent cardiovascular events and to improve survival of patients with pre-

vious cardiovascular disease [17]. In addition, statin treatments have demonstrated a marked reduction of ischemic strokes, the third leading cause of death in the United States after coronary disease and cancer. This effect on stroke was seen in patients whether or not they had suffered from previous vascular diseases (LIPID, CARE, WOSCOPS, 4S, HPS, and GREACE studies) [134, 144, 179, 180, 185–188]. In addition, evidence is increasing to highlight the importance of statin molecules with their beneficial effects on nonlipid mechanisms that control vascular immunoinflammatory processes.

Summary

For almost a decade, the use of statin therapy to treat dyslipidemia has demonstrated great beneficial effects, with reduction of cardiovascular morbidity and mortality in primary and secondary prevention. These large clinical benefits may not only be due to the lipid lowering effects of statins, but also to their pleiotropic effects. These new nonlipid vascular properties include: the improvement of endothelial function, anti-inflammation, anti-atherothrombosis, immunomodulation, antiproliferation, and antimigration. Together, these new findings of vascular effects of statins help us to better understand their great clinical benefits and may also provide a scientific rationale for their use in other diseases, such as rheumatoid arthritis, multiple sclerosis, Alheimer's disease and following organ transplantation.

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