Anti-inflammatory and immunomodulatory effects of statins

LUÍS MIGUEL BLANCO-COLIO, JOSÉ TUÑÓN, JOSE LUIS MARTÍN-VENTURA, and JESÚS EGIDO

Renal and Vascular Research Laboratory and Department of Cardiology, Fundación Jiménez Díaz and Autonoma University, Madrid, Spain

Antithrombotic and immunomodulatory effects of statins. 3-Hydroxy-3-methyl-gutaryl coenzyme A (HMG-CoA) reductase inhibitors or statins constitute the most powerful class of lipid-lowering drugs. Clinical trials have demonstrated a marked reduction in cardiovascular mortality in patients treated with statins. However, the benefits observed with statin therapy appear to be related, at least in part, with their cholesterol-lowering independent effects. Extensive research carried out mainly in the last decade suggests that the clinical benefits of these drugs could be related to an improvement in endothelial dysfunction, a reduction in blood thrombogenicity, anti-inflammatory properties, and, recently, immunomodulatory actions. In this sense, statins decrease T cell activation, the recruitment of monocytes and T cells into the arterial wall, and enhance the stability of atherosclerotic lesions. Many of these effects are related with the inhibition of isoprenoid synthesis, which serve as a lipid attachment for a variety of proteins implicated in intracellular signaling. In fact, small G proteins, whose proper membrane localization and function are dependent on isoprenylation, may play an important role in the lipid-lowering independent effects of HMG-CoA reductase inhibitors. This article summarizes the anti-inflammatory and immunomodulatory effects of statins and their participation in the different steps of atherosclerotic lesion formation.

Atherosclerosis is currently described as an inflammatory disease [1], given that the main components of chronic inflammation such as cell recruitment, sclerosis, cell proliferation and neovascularization are present in this process. Moreover, recruitment of inflammatory cells is involved in plaque rupture and subsequent thrombosis. Atherosclerotic lesions are formed by a lipid-rich nucleus covered by a fibrous cap. This cap confers the lesions resistance to rupture, and consists of collagen and other extracellular matrix proteins, synthetized by vascular smooth muscle cells (VSMC) [2–3]. Inflammatory cells, mainly macrophages, release metalloproteinases (MMPs) that weaken the fibrous cap making lesions vulnerable to rupture by the action of the hemodynamic forces [2–4]. Fibrous cap rupture allows the blood to contact with the atheromatous gruel, which is highly procoagulant [5, 6], triggering thrombosis. According to this process, it has been observed that atherosclerotic plaques responsible for an acute coronary event are infiltrated by macrophages, T lymphocytes and activated VSMC [7] more frequently than those that are stable [8]. Moreover, expression of MMPs has been found in human atherosclerotic plaques located mainly near macrophage infiltrates and in the shoulder region [4, 9, 10]. This explains in part why this region, where the lesions join the normal arterial wall, is the most vulnerable to rupture. Accordingly, we and others have observed that human specimens of carotid endarterectomy display an increase of macrophage infiltrate and a decrease in VSMC in this region as compared with other zones of the plaque [abstract; Martín-Ventura et al, Circulation 102(Suppl II):319, 2000] [11].

The concept of atherosclerosis as an inflammatory disorder has led to an exploration of new avenues in the pathogenesis of this disease. In this sense, the levels of several inflammatory molecules in circulating blood are increased more frequently in subjects at risk of developing an acute coronary event [12–16]. This reflects a relationship between the events that are taking place in the blood and into the vessel wall. This is not surprising since, as explained, inflammatory cells are circulating in the blood before entering the atherosclerotic lesion. In fact, using a rabbit model of atherosclerosis, we demonstrated a significant correlation between the inflammatory activity of circulating monocytes and that of the cells present in the atherosclerotic plaques [17]. Together, these data suggest that the grade of activity of inflammatory cells is determined before they enter the arterial wall. Another advance derived from the inflammatory concept of atherosclerosis is related to therapeutics. Several drugs efficient in the treatment of atherosclerosis, such as acetylsalicylic acid, angiotensin-converting enzyme inhibitors and statins, have been shown to have anti-inflammatory actions [13, 18–20]. Moreover, our data indicate that even a small amount of red wine, which
Table 1. Immunomodulatory and anti-inflammatory effects of HMG-CoA reductase inhibitors

<table>
<thead>
<tr>
<th>Anti-inflammatory effects</th>
<th>Immunosuppressive effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>↓ Adhesion molecules</td>
<td>↓ Proliferation of lymphoid cells</td>
</tr>
<tr>
<td>↓ Chemoattractant proteins</td>
<td>↓ Natural killer activity</td>
</tr>
<tr>
<td>↓ Proinflammatory transcription factors</td>
<td>↓ Major histocompatibility class II antigens</td>
</tr>
<tr>
<td>↓ Proinflammatory enzymes</td>
<td>↓ Organ rejection</td>
</tr>
<tr>
<td>↓ Inflammatory serum markers</td>
<td></td>
</tr>
</tbody>
</table>

has been suggested to have a protective role in coronary artery disease, has anti-inflammatory properties [21].

**ANTI-INFLAMMATORY EFFECTS OF STATINS**

Since the demonstration that hydroxy-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) reduce mortality and the incidence of coronary events [22], great effort has been made to elucidate the underlying mechanisms of these effects. Angiographic studies revealed that the reduction in lesion progression achieved by these drugs was too mild to fully explain them [23]. Also, the clinical benefits appear after too short an interval of treatment time to see any significant effect on plaque progression [24]. Extensive research carried out mainly in the last decade suggests that, instead of an improvement in endothelial function [25], the clinical benefit of these drugs could be related to a reduction in blood thrombogenicity [26] and to anti-inflammatory properties. Moreover, it has been shown that these actions in part could be independent of the lipid-lowering effects of statins. This review focuses on the anti-inflammatory and immunomodulatory actions of statins (Table 1).

**Endothelial dysfunction and adhesion**

One of the early stages in atherogenesis is the adhesion of monocytes to the endothelium, which acquires an activated phenotype due to various stimuli. This activation is believed to be related to a decrease in nitric oxide (NO) availability, which may be secondary to different cardiovascular risk factors (hypertension, dislipidemia, diabetes, and so forth) [25, 27–31]. This dysfunctional endothelium will express different adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1) and E-selectin, which are essential for the first steps of monocyte recruitment: rolling and adhesion to the endothelium.

In addition to the above-mentioned benefits of statins in endothelial dysfunction, different components of this family have been shown to regulate inflammatory cell adhesion. Fluvastatin treatment inhibits the expression of ICAM-1 on human monocytes [32] and cerivastatin prevents lipopolysaccharide (LPS)-induced ICAM-1 expression in endothelial cells via inhibition of Rho activity [33]. Furthermore, lovastatin decreases CD11b-dependent adhesion of monocytes stimulated or not with monocyte chemoattractant protein-1 (MCP-1) [34], and fluvastatin reduces the leukocyte-adherence responses to platelet-activating factor and to leukotriene B4 in hypercholesterolemic rats [35]. Also, statins blocked the adhesion of lymphocytes mediated by leukocyte function antigen-1 (LFA-1), αβ2 integrin expressed on leukocyte surface that binds to ICAM-1 [36]. According to these data, Seljeflot et al demonstrated recently that atorvastatin and simvastatin reduce plasma levels of ICAM-1, VCAM-1, E-selectin and P-selectin in patients with coronary artery disease [37].

**Recruitment of mononuclear cells**

After adhesion, monocytes enter the arterial wall in a process driven by chemoattractant molecules. HMG-CoA reductase inhibitors have been shown to extensively interfere with this step. In a rabbit model of atherosclerosis we demonstrated that atorvastatin decreases the expression of chemoattractant molecules MCP-1 and interleukin-8 (IL-8) [20, 38]. As a consequence, there was a reduction in macrophage infiltration and MMP-3 expression. The mechanism of the reduction of MCP-1 and IL-8 expression seemed to be due to a decrease in the activation of nuclear factor-κB (NF-κB; Fig. 1). This redox-sensitive transcription factor is involved in the transmission of various signals from the cytoplasm to the nucleus of numerous cell types [39]. It is found in the cytosol as a trimer consisting of p50 and p65 subunits bound to its inhibitor IκB. The release of IκB from the trimer results in the migration of the p50/p65 heterodimer to the nucleus and subsequent DNA binding [40]. This process activates genes involved in the immune, inflammatory or acute phase response, such as adhesion molecules, chemoattractant cytokines like MCP-1 and IL-8, proinflammatory enzymes such as cyclooxygenase-2 (COX-2), and procoagulant proteins as tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1) [41, 42]. Data strongly suggest that NF-κB could be involved in the pathogenesis of atherosclerosis [43], since it participates in dysregulation of VSMC in human atherosclerosis [44] and is present in the nuclei of macrophages and endothelial cells of human atherosclerotic lesions [45]. Moreover, Wilson et al have shown that it is present more markedly in cells from human atherosclerotic lesions that have been responsible for an acute coronary syndrome [46], supporting the hypothesis of a link between inflammation and plaque instability.

Cyclooxygenase-2 expression was reduced also by statin treatment [38]. This isoform of COX was first related to inflammation in rheumatic diseases and enhances the production of the chemoattractant prostaglandin E2 [47]. Later, it was confirmed that it behaves similarly in the arterial wall, as it appears in atherosclero-
Fig. 1. Effect of atorvastatin on NF-κB activation in atherosclerotic lesions of experimental rabbits. NF-κB was determined by Southwestern histochemistry in untreated (A), atorvastatin treated (B), and control (C) animals. Magnification ×400. Adapted from reference [20], with permission from the American College of Cardiology Foundation Journal of the American College of Cardiology, 1998. (D) Schematic representation of NF-κB activation. NF-κB activation leads to coordinated expression of different genes that encode proteins such as cytokines, chemokines, adhesion molecules and enzymes, involved in the initiation and perpetuation of the inflammatory response.

sis, but is absent in the healthy vessel [48]. In addition, COX-2 has been demonstrated to colocalize with prostaglandin E2 (PGE2) synthase, with macrophages and MMP-9 in human carotid atherosclerosis [49]. Of interest, COX-2 expression was more intense in the lesions of patients who had suffered an ipsilateral stroke [49]. These data showing the blockade of cell recruitment into the arterial wall by statins are reinforced by studies from other groups. Shiomi and Ito reported that cerivastatin treatment retarded the enlargement of plaque size and reduced macrophage accumulation in Watanabe heritable hyperlipidemic rabbits [50]. Furthermore, Aikawa et al demonstrated that cerivastatin reduces the macrophage infiltrate and the expression of MMP-1, -3 and -9 and tissue factor in the same model, and decreases macrophage proliferation and the proteolytic activity due to MMP-9 in human macrophages in vitro [51]. The same group demonstrated that pravastatin and fluvastatin reduced MMP-3 and MMP-9 expression in the absence of modifications of the macrophage infiltrate, suggesting a reduction in their expression [52]. Accordingly, Bellosta et al also demonstrated that statins inhibit the expression of MMP-9 by macrophages [53]. Then, HMG-CoA reductase inhibitors do not only decrease the macrophage infiltrate in the vascular wall, but also reduce the ability of these inflammatory cells to produce MMPs.

As seen, most of the evidence for the ability of statins...
to reduce the inflammatory infiltrate in the arterial wall comes from animal experimentation. This is logical, since animals can be randomized to statin or no treatment during the study period and afterwards be sacrificed in order to analyze the composition of atherosclerotic lesions. However, Crisby et al have demonstrated recently that there is an experimental design allowing the investigation of the effects of statins in human atherosclerosis. They studied patients with symptomatic carotid stenosis who were scheduled for a carotid endarterectomy [54]. Three months before the operation, the patients were randomized to pravastatin versus no lipid-lowering therapy and, at surgery, endarterectomy specimens were collected and analyzed. Patients receiving pravastatin had less macrophage and T cell infiltration, reduced MMP-2 expression as well as a higher immunoreactivity for tissue inhibitor of MMP-1. Probably as a result of the lower collagenolytic activity, the collagen content was higher in patients on pravastatin therapy, suggesting a stabilizing effect of atherosclerotic lesions.

**Immunomodulatory effects of statins**

The triggers of the inflammatory response observed in atherosclerosis have not been completely elucidated. It has been suggested that autoantigens expressed in the atherosclerotic plaque may induce an immune response. One of these possible antigens, heat-shock protein 70 has been demonstrated to be present around sites of necrosis and lipid accumulation, colocalizing with macrophages, in human and rabbit atheroma [72]. Other proposed antigens, such as low-density lipoprotein (LDL) and non-LDL oxidative epitopes, are also more frequently present in atherosclerotic than in normal human arteries [73]. In fact, T cells derived from human atherosclerosis respond to oxLDL by proliferation and cytokine secretion [74]. It also has been speculated that infectious agents could stimulate immunity in this disorder [75, 76]. The presence of activated T lymphocytes in the peripheral blood and coronary plaques of patients with acute coronary syndromes supports the hypothesis of an immune response in the pathogenesis of coronary atherosclerosis [7, 76–78]. According to this hypothesis, there is an increase of CD4+ and CD3+/DR+ T cells and of IL-2 and IgM in patients with unstable angina [75]. Furthermore, in this condition, monocytes present nuclear translocation of STAT-1 complexes and up-regulation of CD64 and IP-10 genes, which are known to be inducible by interferon-γ (IFN-γ) [79]. This points to a possible monocytic activation via IFN-γ produced by stimulated T lymphocytes. In accordance with this hypothesis, it has been observed that monocytes need incubation and contact with lymphocytes to express procoagulant activity [80].

Different effects of statins on lymphoid cell function have been shown. For example, suppression of proliferation or natural killer cell activity by compactin [81], lovastatin [82] and simvastatin [83] has been reported in vitro experiments. Lovastatin, by binding to LFA-1, exerts an immunomodulatory effect, because this integrin does not only play a role in leukocyte adhesion, but does also work as a T cell co-stimulator [36]. Kwak et al demonstrated that different statins inhibit the expression of major histocompatibility class II (MHC II) antigens by primary human macrophages and endothelial cells in response to IFN-γ [84]. This effect resulted from the reduced activation of the inducible promoter IV on the transactivator CIITA [85] and was limited to cells that express MHC II only in response to IFN-γ stimulation [84]. In contrast, professional antigen-presenting cells constitutively expressing MHC II, such as B lymphocytes and dendritic cells, were not inhibited, and neither was MHC I expression.
Major histocompatibility class II antigen is required for antigen presentation and T-cell activation through the T cell receptor. This T-cell receptor may trigger proliferation of other T cells, their differentiation into two different effector cell populations (T helper 1 and 2 cells; Th1 and Th2) and cytokine release. In this sense, Th1 cells secrete proinflammatory cytokines such as IFN-\(\gamma\) and TNF-\(\alpha\). In contrast, Th2 cells produce anti-inflammatory cytokines interleukin-4 (IL-4), IL-10, IL-13 and transforming growth factor-\(\beta\) (TGF-\(\beta\)). It has been suggested that the ability of statins to down-regulate the expression of MHC II may lead to decreased Th1 activation in vivo and inhibition of the pro-inflammatory cytokines release. Accordingly, statins reduce T cell proliferation and IL-2 release [84]. However, these drugs may induce opposite effects via a similar impact on Th2 proliferation and effector function or shift toward a Th1 immune response [86].

Clinical research has been carried out to examine the immunomodulatory effect of statins, and the results have been discordant. Muldom et al did not find any effect of six months of lovastatin on the number and function of circulating immune cells [87]. However, Kobashigawa et al have demonstrated that pravastatin reduces the cytotoxicity of natural killer cells and the incidence of coronary vasculopathy in patients that have undergone cardiac transplantation [88]. Moreover, although it had no effect on the incidence of mild and moderate episodes of cardiac rejection, pravastatin decreased the incidence of rejection with hemodynamic impairment and reduced mortality [88]. In a similar population, Wenke et al showed that simvastatin significantly increased survival and reduced the incidence of coronary artery disease and intimal thickness at four years of follow-up, although it did not affect the incidence of cardiac rejection [89]. Data on kidney-transplanted patients are, for the moment, less promising. Although a small study with 48 patients showed a decrease in acute rejection [90], three larger randomized trials involving a total of 570 patients failed to confirm these data [91–93]. However, in these studies the patients were followed for only three months, a short period when compared with the one to four years reported in the cardiac transplant studies [88, 89]. Thus, it would be interesting to know the effects of prolonged statin administration and a longer follow-up period in this population. The ongoing ALERT trial may shed some light on this issue. In this study, 2100 patients with functioning renal allografts and mild-to-moderate hypercholesterolemia are being randomized to fluvastatin or placebo and will be followed up to six years. Although the primary objective of this trial is the incidence of major adverse cardiac events, it will also provide information on renal function and all causes of mortality [94].

**MECHANISMS OF ANTI-INFLAMMATORY EFFECTS OF STATINS**

**Actions related to lipid lowering**

Statins were developed as lipid-lowering drugs. HMG-CoA reductase inhibition leads to a decrease in intracellular cholesterol synthesis. To compensate for the intracellular diminution in cholesterol levels, some cells, mainly hepatocytes, expose external receptors that promote uptake and clearance of cholesterol from the blood. Given the relationship between hyperlipidemia and atherosclerosis, it seems wise to consider that their hypolipidemic effect is responsible for a part of their biological actions, including the anti-inflammatory effects.

In human studies, it has been observed that hypercholesterolemia up-regulates the pro-inflammatory receptor CD40 and its ligand CD40L [95]. This system is expressed in the cells of the atherosclerotic lesions where it mediates the production of cytokines, MMPs and tissue factor [96]. Moreover, levels of soluble CD40L are increased in acute coronary syndromes [97, 98] and predict their incidence in healthy women [99]. In animal models, hyperlipidemia also enhances NF-\(\kappa\)B activation, the expression of adhesion and chemoattractant molecules, and favors macrophage infiltrate into the vessel wall [20, 35, 100]. In vitro studies have confirmed that lipids are related to inflammatory activation of the cells from the vessel wall. It has been demonstrated that oxidized LDL increases NF-\(\kappa\)B activation and MMP-9 expression in culture human monocyte-derived macrophages [101]. Also, CD40L expression and IFN-\(\gamma\) production are enhanced by lysophosphatidylcholine in human CD4+ T cells [102].

Probably the best data confirming that a part of the anti-inflammatory effects of statins are related to lipid lowering arise from animal experimentation. Aikawa et al demonstrated that reducing lipid levels only by dietary modification, in the rabbit model of atherosclerosis, decreases the expression of CD40 and CD40L colocalizing with a reduction of tissue factor immunoreactivity [103]. Also, a diminution of macrophage infiltrate, MMP-1 expression and proteolytic activity was observed in the same model [104]. Together these data underline the fact that lipid lowering is one of mechanisms by which statins decrease inflammation in atherosclerosis.

**Lipid-independent mechanisms**

A large body of data has accumulated in the last years suggesting that some effects of statins, for example, their actions on endothelial dysfunction and the anti-inflammatory effects, could be independent of their lipid-lowering ability. This was supported by clinical studies such as the WOSCOPS study, where evidence showed that patients on pravastatin had fewer coronary events than those on placebo who had similar LDL levels [105]. Moreover, in the same study, plasma lipids at baseline did not influence...
the relative risk reduction achieved by pravastatin. In addition, the fall in LDL found in the pravastatin group did not correlate with the risk reduction of coronary heart disease.

Evidence pointing to a direct anti-inflammatory action of HMG-CoA reductase inhibitors comes from both clinical and experimental studies. The reduction of CRP serum levels achieved by different statins is not related to the decrease in lipid levels [14, 57–60].

Research in animal models of atherosclerosis strongly supports the idea of a direct anti-inflammatory effect of statins. Williams et al showed a decrease in macrophage infiltration in atherosclerotic monkeys after treatment with pravastatin and diet manipulation to avoid changes in serum lipid levels [106]. More recently, simvastatin has been found to reduce inflammation in a model of carrageenan-induced footpad edema in mice [107]. Simvastatin also has been found to reduce leukocyte rolling and adherence in rats [108] and apolipoprotein E-deficient mice [109], leukocyte transmigration in rats [108] and the aorta cholesterol content in apolipoprotein E-deficient mice [107] in the absence of lipid change. In a recent work from our group, atherosclerotic rabbits were randomized to simvastatin treatment and a hyperlipidemic diet versus a normal lipidemic diet. Given that the hyperlipidemic diet counterbalanced the effect of simvastatin, the reduction of lipid levels in this group was less pronounced. However, NF-κB activity in mononuclear cells and atherosclerotic lesions was reduced more markedly in the simvastatin group [17]. Similar results were obtained when macrophage infiltration and the expression of IL-8 and MMP-3 were analyzed. The observation that simvastatin had a greater anti-inflammatory action than diet modification in spite of less lipid lowering, suggests that a part of these effects may be due to mechanisms independent of lipid modification.

Despite the information obtained in vivo, the most comprehensive information about the mechanism of the direct actions of statins results from in vitro experiments. In these studies, the effect of HMG-CoA reductase inhibition on the cell types that form the atherosclerotic lesions was tested (Fig. 2). Given that the cells were isolated from the whole organism, the possibility of a decrease in extracellular lipids by the action of these drugs on the liver disappeared, and it was possible to observe solely their action on the studied cells. It was found that the lipid-independent effects of these agents are related to the inhibition of other isoprenoid intermediates of the cholesterol biosynthetic pathway, such as farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP) [110]. FPP and GGPP are used for the post-translational modification of several important cell proteins including nuclear lamins, Heme-a, the γ subunit of heterotrimeric G proteins, and small G proteins such as Ras and Ras-like proteins (Rac, Rab and Rho; Fig. 2) [111]. The attachment of an isoprenoid residue to these proteins is necessary for their anchorage to the cell membranes and full functionality. Statins inhibit Ras and Rho isoprenylation leading to the accumulation of their inactive forms in the cytoplasm [112]. These proteins are implicated in different functions in the cells such as gene expression, organization of the actin cytoskeleton, membrane trafficking, programmed cell death, proliferation and transformation [113, 114]. Inhibition of Rho and its downstream target, Rho kinase, is a possible mechanism mediating some of the pleiotropic effects of HMG-CoA reductase inhibitors on the vascular wall, since changes in Rho can affect intracellular transport, membrane trafficking, mRNA stability, and gene transcription [115]. Accordingly, we have seen, for example, that atorvastatin directly reduces the expression of MCP-1 and IL-8 as well as NF-κB activation induced by angiotensin II (Ang II) and TNF-α in cultured monocytes and VSMC [116]. These effects have been related to the inhibition of non-sterol compounds of the mevalonate pathway, such as FPP and GGPP, which restored the inhibition of NF-κB activity induced by atorvastatin. Moreover, Manumicyn A, an isoprenylation inhibitor, abolishes NF-κB activity induced by Ang II and TNF-α [116].

Other transcription factors also seem to be regulated by statins. Treatment with different statins can modulate proinflammatory cytokines expression such as IL-1β, IL-6, and COX-2 by up-regulating the peroxisome proliferator activated receptor-α in endothelial cells [117]. Activator protein-1 (AP-1) activation is decreased by statin treatment in renal epithelial tubular cells [118]. In addition, atorvastatin and simvastatin increase the activation of the octamer transcription factor Oct-1 in mononuclear cells, which represses the transcription of proinflammatory genes such as IL-8, CD11c/CD18, VCAM1 and PECAM-1 [119].

Finally, it must be noted that HMG-CoA reductase inhibition is not the only possible mechanism through which statins exert their effects. The above-mentioned inhibitory action of lovastatin on LFA-1 is unrelated to this pathway [36]. This opens the possibility of developing new statins with selectively potentiated immunosuppressive effects.

OTHER EFFECTS OF STATINS ON THE VASCULAR WALL RELATED WITH SMALL G PROTEINS

The accumulation of VSMC in the neointima is another feature of atherosclerosis and a consequence of their migration from the media and their proliferation within the lesion [1]. Different studies have demonstrated that treatment with statins inhibits proliferation of VSMC by arresting the cell cycle transition between the G1 to S phase. The inhibition of VSMC proliferation was corre-
Fig. 2. Small G proteins and vascular wall. Inhibition of HMG-CoA reductase by statins decreases isoprenylation of small G proteins such as Ras, Rac and Rho. This effect leads to alteration of different functions in the cell.

lated with increases in the level of cyclin-dependent kinase inhibitor p27Kip1 and is related with the inhibition of RhoA isoprenylation [120]. Antiproliferative effects of statins in animal models without significant changes in serum cholesterol concentrations also have been reported [121, 122]. In addition, serum of patients treated with statins decreases proliferation of VSMC in vitro [123].

The accumulation of VSMC in the early step of atherosclerosis is the result of the migration from the media, proliferation and eventual death, including programmed cell death. In this sense, we have demonstrated that lipophilic statins induced apoptosis of VSMC in culture and this effect is related with the interference of protein isoprenylation [124]. These experiments used higher concentrations than those used in clinical practice, but it is possible that relatively low, but sustained plasma levels of statins could exert a similar effect to that seen in vitro with higher concentrations. In this regard, Buemi et al have demonstrated that there is an increment of apoptosis in cultured VSMC following the addition of the serum of fluvastatin-treated patients [125].

Furthermore, different reports have demonstrated another pleiotropic effect of statins related to the inhibition of small G proteins isoprenylation. Statins may alter the local fibrinolytic balance within the vessel wall toward increased fibrinolytic capacity, which is related to the inhibition of PAI-1 expression from VSMC and endothelial cells [126]. Ang II plays crucial roles in the pathogenesis of cardiovascular diseases, and statins down-regulate Ang II type 1 receptor in VSMC, an effect related again with the inhibition of RhoA isoprenylation (Fig. 3) [127].

Statins and postangioplasty restenosis

One of the most important challenges in the treatment of coronary artery disease is postangioplasty restenosis. This process is not identical to the evolution of spontaneous atherosclerosis, and is due to both intima thickening with VSMC proliferation, and arterial wall remodeling. In the last years, the use of stents has reduced the incidence of restenosis by controlling arterial remodeling [128, 129], and stents are now implanted electively after angioplasty in most cases.

Despite the antiproliferative effect of statins, several studies have evidenced that these drugs fail to prevent restenosis after balloon angioplasty [130–133]. However, in a subset of patients treated with stent, statins seem to be beneficial. Walter et al have evidenced recently that these drugs reduce the incidence of post-stent restenosis specifically in those patients whose serum C reactive protein levels are above 0.6 mg/dL [134]. These data
point to a role for inflammation in restenosis. Accordingly, it has been shown that patients who develop unstable angina following directional coronary atherectomy have more macrophages and T lymphocytes in the atherosclerotic lesions than those who have only stable angina or stay asymptomatic [135]. Thus, although most promising therapies, such as local delivery of antiproliferative drugs, are now introduced to control post-stent restenosis [136], statins may provide a positive effect in this situation due to their anti-inflammatory effects.

**LIMITATIONS TO THE INTERPRETATION OF THE LITERATURE**

Combining clinical with experimental data, as in this review, provides a comprehensive way to explain the benefit and mechanisms of action of any drug. However, the value and limitations of every source of knowledge must be clearly pointed out. One of the most common problems in nonclinical drug experimentation is the dose employed. Most animal studies reported here employed proportionally higher doses of statins than those used in clinical practice. Nevertheless, it must be taken into account that atherosclerosis in animals is induced by stronger stimuli than those present in human disease, such as vessel injury and/or experimental diets that raise lipid levels far greater than that seen in humans. Hence, the use of high doses of statins in these studies seems to be proportional to the aggressive disease induced. The same problem arises when interpreting in vitro studies. The concentrations used to demonstrate the biological effects of statins in cell culture, especially with regard to inhibition of Rho geranylgeranlylation, appear to be much higher than those prescribed clinically. However, it is possible that concentrations of statins needed to affect activated cells are lower than those needed to affect normal cells. In addition, one may assume that in vivo, relatively low, but sustained blood levels of statins could exert a similar effect to that seen in vitro with higher concentrations and short incubation times.

Another common question when analyzing drug actions is whether there is a “class effect” and all the members of a family share the same properties, or if there are differences among their actions. Although differences in other effects of statins have been noted, such as those on platelet aggregation, coagulation and fibrinolysis [137],
most studies using different statins show a consistent anti-inflammatory effect. The known differences among their anti-inflammatory and immunomodulatory activity are probably related to their power as, for example, in the work from Kwak et al, where atorvastatin displayed more powerful inhibitory activity of MHC-II than pravastatin and lovastatin [84].

CONCLUSIONS

Inhibitors of HMG-CoA reductase constitute a powerful class of hypolipidemic drugs currently available. Several clinical trials have demonstrated the beneficial properties of statins in primary and secondary prevention. However, the clinical effects are observed earlier than significant lesion regression could appear and these effects appear to be greater than what might be expected from changes in lipid profile alone. These findings suggest that statins have pleiotropic effects beyond cholesterol lowering (Fig. 3). In particular, in vitro and in vivo studies have demonstrated anti-inflammatory and, recently, immunomodulatory properties of statins, which may contribute to plaque stabilization and might help explain some beneficial effects observed with statins treatment. However, their relevance in humans remains to be established and further studies are needed.

ACKNOWLEDGMENTS

The authors’ studies cited here were supported by grants from Ministerio de Ciencia y Tecnología (SAF 2001-0717), Fundación Ramón Areces, Instituto Reina Sofía de Investigaciones Nefrológicas, Pfizer and Merck.

Reprint requests to Professor Jesús Egidio, Renal and Vascular Research Laboratory, Fundación Jiménez Díaz, Avenida Reyes Católicos 2, 28040 Madrid, Spain. E-mail: jegido@fjd.es

APPENDIX

Abbreviations used in this article are: Ang II, angiotension II; COX, cyclooxygenase; CRP, C-reactive protein; FPP, farnesylpyrophosphate; GGPP, geranylgeranylpyrophosphate; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; INF-γ, interferon-γ; LDL, low-density lipoprotein; LFA-1, leukocyte function antigen-1; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; MHC, major histocompatibility protein; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; NO, nitric oxide; PAL-1 plasminogen activator inhibitor-1; TGF-β, transforming growth factor-β; Th cells, T helper cells; TNF-α, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1; VSMC, vascular smooth muscle cell.

REFERENCES

7. van der Wal AC, Becker AE, van der Loos CM, Das PK: Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. Circulation 89:36–44, 1994
23. Brown BG, Fuster V: Impact of management in stabilization of coronary disease, in Atherosclerosis and Coronary Artery Disease,


31. Lewis TV, Dart AM, Chen-Dusting JP: Endothelium-dependent relaxation by acetylcholine is impaired in hyperglycemic diabetic humans with normal levels of plasma LDL cholesterol. J Am Coll Cardiol 33:805–812, 1999


