Inhibitors of HMG-CoA reductase sensitize human smooth muscle cells to Fas-ligand and cytokine-induced cell death

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Abstract

Hydroxymethylglutaryl CoA (HMG CoA) reductase inhibitors, or statins, have been shown to reduce atherosclerotic cardiovascular morbidity and mortality. Atherosclerotic plaque lesions can be chronically inflamed and vulnerable to rupture or stable and less rupture-prone. Human smooth muscle cells (SMC) are critically important in maintaining the stability of atherosclerotic plaques. This stability may be greatly influenced by pro-inflammatory mediators such as IFN-γ, TNF-α, and IL-1β and Fas ligand (FasL) that are present in human atheroma. The purpose of the present study was to examine the effect of the statins on apoptosis of SMC. We have found that SMC are normally resistant to Fas or cytokine-induced apoptosis, but can be sensitized to these agents with pharmacological concentrations of some statins. Simvastatin and lovastatin strongly sensitized the cells to apoptotic agents while atorvastatin was less effective. In contrast to the lipophilic statins, the hydrophilic statin pravastatin did not induce this sensitization of SMC to apoptosis. Treatment of SMC with either mevalonate, the product of the HMG-CoA reductase, or geranylgeranylpyrophosphate, a down stream intermediate, prevented lipophilic statin-induced sensitization to apoptosis. These results suggest that prenylation of one or more proteins is critically involved in regulating the sensitivity of SMC to apoptotic stimuli. Our data support the emerging evidence that through this pathway the various statins may have effects which are beyond a simple lowering of the levels of circulating cholesterol. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

A high level of circulating cholesterol in patients is one of the risk factors for the development of coronary atherosclerotic lesions. To lower plasma lipid levels the rate limiting step in cholesterol biosynthesis is inhibited with hydroxymethylglutaryl CoA (HMG CoA) reductase inhibitors (statins) [1–3]. In contrast to their lipid lowering mechanism, it is less clear how the different drugs may directly affect the atherosclerotic lesion and what actually triggers rupture of the atherosclerotic plaque [4]. Cardiac infarction occurs when a final thrombosis occludes the blood vessel, but this is preceded by many benign thrombotic events that probably occur throughout the lifetime of the lesion [5]. Thus, it is important to understand how the stability of the advanced, vulnerable plaque is influenced by the various cell types and soluble mediators, such as the statins.

Recent studies, both in vitro and in vivo, have demonstrated that some statins are able to induce changes in cells or tissues that are unrelated to reduction of plasma cholesterol [6–12]. Since these effects were reversed with mevalonate and other metabolites of the isoprene synthesis pathway, protein prenylation seems to play an important role [13]. Furthermore, chronic inflammation significantly contributes to the development of atherosclerotic plaques [4,14–16]. As shown by histochemical analysis, highly vulnerable and ruptured human plaques contain more macrophages and fewer smooth muscle cells (SMC) [5]. Additionally, an enhanced expression of pro-inflammatory and pro-
apoptotic mediators correlates with plaque instability [17,18]. These pro-inflammatory mediators are able to induce apoptosis of SMC in vitro [19], and SMC in the advanced plaque may undergo increased programmed cell death. One pathway that appears to be involved in apoptosis is the Fas (CD95 or Apo-1)-FasL system. Fas is a type I membrane protein belonging to the TNF receptor family and its receptor mediates a death signal after binding to its ligand, FasL. Fas expression has been found in the human atherosclerotic intima [20], indeed, most of the cells in the advanced lesion have been found to express Fas [21]. In addition, activated T-cells and monocytes are major sources for both FasL and cytokines, and these cells are present at sites of plaque rupture in human atheroma [14]. Overall, the findings from many investigators indicate that the dynamics of an atherosclerotic plaque are influenced by the different proinflammatory mediators, such as IFN-γ, TNF-α, IL-1β and FasL, that are present in the local microenvironment [14].

In the advanced, highly inflamed plaque, many different cell types, including SMC and macrophages, can undergo apoptosis. However, there is only a rudimentary understanding how plaque stability is affected through apoptosis of the various cells. Although in the human atherosclerotic plaque, both necrosis and apoptosis have been localized to sites of plaque rupture [22], abundant evidence suggests that SMC primarily die by apoptosis [23]. For example, in contrast to SMC from normal vessels, cells derived from human atherosclerotic plaques proliferate more slowly, undergo fewer proliferative cycles, and are more susceptible to spontaneous apoptotic cell death [23,24]. In addition, it has been shown that intimal lesions contain more TUNEL positive SMC than the non-atherosclerotic arterial intima [25]. Because the production of plaque stabilizing extracellular matrix proteins in the advanced lesion is considered to be beneficial and is primarily dependent on SMC in the intima, depletion of SMC in the advanced plaque is thought to enhance plaque thinning [5,21,23–28]. However, SMC may play different roles in different pathological contexts. Overall, the pro-inflammatory environment of a plaque may increase the susceptibility of SMC to cell death, and the concomitant loss of synthesis of extracellular matrix proteins would then lead to an increase in plaque instability [5,29].

The effects of the different statins in the pro-inflammatory or pro-apoptotic environment of the plaque is not well understood, but could have important implications in cardiovascular disease. In this report we demonstrate that lipophilic statins at in vitro concentrations that are potentially pharmacologically relevant, directly sensitize SMC to both FasL and cytokine induced apoptosis.

2. Experimental procedures

2.1. Reagents

Simvastatin, lovastatin and pravastatin were synthesized by Bristol-Myers Squibb and atorvastatin was a generous gift from Parke-Davis. Simvastatin, lovastatin and mevalonate were activated to their active open-ring forms as described [30]. Geranylgeranylpyrophosphate and farnesylpyrophosphate were obtained from Alexis Biochemicals and were used at 20 μmol/l. Recombinant soluble human Fas-ligand (sFasL) and human Fas-Ig fusion proteins were produced and purified as described previously [31]. The sFasL fusion protein contains the extracellular domain of human FasL and the extracellular region of mouse CD8. Analysis of the purified fusion protein by gel filtration revealed that it is a mixture of dimers and trimers. As such this protein very effectively stimulates apoptosis in Jurkat cells and additional crosslinking of the fusion protein with an antihuman CD8 mAb did not further enhance activity. Recombinant human TNF-α, IFN-γ, and IL-1β were obtained from R&D Systems. sFasL was used at 100 ng/ml and Fas-Ig at 50 μg/ml. To relate our results to a previous study on apoptosis of SMC [32], the cytokines were used at 400 U/ml for TNF-α and IFN-γ, and 100 U/ml for IL-1β. In experiments where all three cytokines were used together at these concentrations, this is called the ‘cytokine cocktail’. Alamar blue was purchased from Biosource, and the ApopTag Direct fluorescein in situ apoptosis detection kit was obtained from Oncor.

2.2. Cell culture and viability

Human aortic smooth muscle cells and human coronary artery endothelial cells (EC) were obtained from Clonetics and were cultured in growth medium from Clonetics with 10% FBS. Cells were seeded at 80% confluence and grown to confluence over a period of 5 days. The cells were not used beyond seven passages. SMC were plated in 96-well plates and cultured with the additions as noted in the text. At the end of the treatment, the medium was removed and replaced with fresh medium including 10% Alamar blue. The cells were then incubated for a further 3–4 h and the absorbance was measured at two wavelengths, 570 and 590 nm, in an ELISA plate reader (Molecular Devices Spectra Max 340) and the OD value at 590 nm was subtracted from that at 570 nm. Samples were assayed with eight replicates from each treatment, and the mean OD value calculated.

After reaching confluency, the SMC were incubated with statins for 24 h. These were then followed by the indicated concentration of cytokines either alone or in combination for an additional 48–72 h. Alternatively,
Statin treated cells were incubated with sFasL (100 ng/ml) for 24 h. When added, statins were present throughout the subsequent culture of the cells. After these incubations, the cells were harvested and viability was determined as outlined. Where appropriate, Fas-Ig, mevalonate (500 μmol/l), farnesylpyrophosphate (20 μmol/l) or geranylgeranylpyrophosphate (20 μmol/l), were added to the cultures at the same time as addition of the statins.

2.3. Staining of cells for apoptosis: TUNEL analysis

After the specified treatments, SMC were harvested by collecting both the floating cells by centrifugation and the adherent cells by trypsinization and the populations pooled. Cells were mounted on coverslips, fixed with formalin and the DNA labeled with fluorescein using terminal deoxyribonucleotide transferase according to the manufacturer’s protocol. Per image, 100 nuclei were counted, and the percentage of TUNEL-positive cells to total number of cells was determined. For each treatment, four different images were analyzed. Error bars indicate the standard deviations.

2.4. Analysis by staining with propidium iodide

SMC were harvested from the tissue culture plates by adding EDTA to the whole culture to a final concentration of 5 mmol/l; plates were incubated for 5 min at 37°C to allow full detachment of SMC. The cells and media were removed, the plates were washed once with RPMI containing 5 mmol/l EDTA and this wash was combined with the original media. Cells were spun down and then permeabilized with buffer containing sodium citrate (0.3%), Triton X-100 (0.01%) and propidium iodide (50 μg/ml). The samples were then analyzed by FACS on a Becton Dickinson FACScan.

3. Results

3.1. Morphological changes in statin-sensitized SMC indicative of apoptosis upon treatment with sFasL or cytokines

An earlier report on SMC showed that statins at high concentration could induce apoptosis [33], hence initial experiments were carried out to determine whether the statins at low micromolar concentrations would induce visible changes of SMC in culture. Furthermore, since lipophilic statins have been shown to inhibit the proliferation of various types of exponentially growing cells [10,34,35], all experiments were performed with confluent cells. Incubation of SMC with simvastatin (3 μmol/l) for 48 or 72 h, induced a slight increase in the number of rounded cells that were detached from the substratum (Fig. 1d) but most of the cells were still adherent. Incubation of SMC without statin pre-treatment, but with a cocktail of IFN-γ, TNF-α, and IL-1β for 72 h (Fig. 1b), or with sFasL for 24 h (Fig. 1c), did not induce any significant change in the morphology of the cells, in agreement with an earlier report [36]. In contrast, preincubation of the SMC with simvastatin (3 μmol/l) for 24 h followed by stimulation of the cells with the cytokine cocktail for 72 h (Fig. 1e) or sFasL for 24 h (Fig. 1f) resulted in the detachment of many cells from the substratum and prominent shrinkage of the SMC, indicative of cells undergoing apoptosis. Similar changes were seen upon treatment of SMC with lovastatin (3 μmol/l) and atorvastatin (3 μmol/l), (data not shown). In contrast, pravastatin (20 μmol/l) had no effect on its own (Fig. 1g) and did not sensitize SMC to cytokines (Fig. 1h) or sFasL (Fig. 1i).

3.2. Lipophilic statins sensitize SMC to cytokine and sFasL-induced apoptosis

The response of SMC to sFasL following statin treatment strongly suggested that the cells were undergoing apoptosis. To examine this in more detail, cells
Fig. 2. Induction of TUNEL staining in lipophilic statin-treated cells following stimulation with sFasL or cytokines. Cells were seeded at 96 000 cells/well in six-well plates and allowed to reach confluence. They were then treated with 3 μmol/l simvastatin, 6 μmol/l atorvastatin, or 5 μmol/l pravastatin for 24 h, followed by stimulation with sFasL for a further 24 h. Alternatively cells were treated with 2 μmol/l simvastatin, 2 μmol/l atorvastatin, or 5 μmol/l pravastatin for 24 h, followed by treatment with IFN-γ alone or the cytokine cocktail for a further 48 h. Cells were fixed with formalin and then stained in situ with fluorescein. Nuclear staining was examined with a fluorescence microscope. (A) Representative TUNEL staining from SMC treated with simvastatin (panel a) or pravastatin (panel b) alone and simvastatin + sFasL (panel c) or pravastatin + sFasL (panel d). Fluorescence and corresponding phase images are shown (original magnification × 200). (B) Non-adherent and adherent cells were collected and processed for TUNEL. A total of 100 cells were counted per image and the percentage of TUNEL-positive cells was calculated. For each treatment four different images were analyzed. The mean values (± S.D.) are shown.

were pretreated with statins for 24 h, and then stimulated with various combinations of cytokines for an additional 48 h or with sFasL for an additional 24 h. The cells were then analyzed either by FACS staining of nuclei with propidium iodide (Fig. 3) or by TUNEL staining of a pool of both the adherent and floating cells (Fig. 2A and B). Treatment with simvastatin or atorvastatin alone for a total of 48 h induced a low but detectable level of TUNEL positive cells which increased slightly over the next 24 h (Fig. 2A and B). Cells that were treated only with cytokines or sFasL were negative for TUNEL staining (Fig. 2B). Addition of sFasL to the simvastatin pretreated SMC cultures markedly increased (ninefold) the number of TUNEL positive cells (Fig. 2A and B). IFN-γ alone (fourfold) and the cocktail of cytokines (fourfold) also increased the number of simvastatin-treated cells that stained positive. Similar results were seen in cells that had been treated with atorvastatin prior to challenge with sFasL (Fig. 2B); the increases following addition of either sFasL or IFN-γ were not quite so marked and the combination of cytokines was slightly more effective than in the simvastatin sensitized cells (three-, three- and sevenfold, respectively). In striking contrast, pravastatin alone had no effect on cell death, and no increase in the TUNEL staining was observed following...
the addition of the cytokine cocktail or sFasL to pravastatin-pretreated SMC (Fig. 2A and B).

To gain further evidence that SMC were dying by an apoptotic pathway, the ratio of hypodiploid DNA was quantified by propidium iodide staining of cell nuclei (Fig. 3). Cells were treated with statins for 24 h and then stimulated with sFasL for 24 h before they were harvested for analysis by FACS. Simvastatin (2 μmol/l) alone increased the content of hypodiploid DNA, whereas pravastatin (5 μmol/l) was without significant effect. sFasL (100 ng/ml) on its own gave rise to a minor increase in the fragmentation of the DNA, whereas the combination of sFasL with simvastatin pretreatment markedly increased the fragmented DNA. Lovastatin (3 μmol/l), and to a lesser extent atorvastatin (5 μmol/l), likewise increased the fragmentation of DNA following the addition of sFasL to the SMC cultures (data not shown). In addition, SMC treated with simvastatin (3 μmol/l) and then challenged with sFasL or the cocktail of cytokines showed DNA laddering characteristic of programmed cell death (data not shown).

Fig. 3. Lipophilic statins increase propidium iodide staining of SMC treated with sFasL. SMC were seeded in six-well plates at 96,000 cells/well and allowed to grow to confluency. Cells were then treated with 2 μmol/l simvastatin or 5 μmol/l pravastatin for 24 h prior to stimulation with sFasL for an additional 24 h. Cells were then permeabilized, and the nuclei stained with propidium iodide (PI) and analyzed by flow cytometry. The histograms are from a single experiment which is representative of three independent experiments.
3.3. The sensitization to apoptosis by statins is dose- and cell-type-dependent

To assess the concentration-dependence of the various statins in the sensitization of SMC to sFasL or cytokine-induced apoptosis, SMC were incubated with a range of concentrations of the statins for 24 h and then stimulated with the cytokine cocktail for an additional 72 h or with sFasL for 48 h. Cell death was estimated with the dye Alamar blue. Pravastatin, at concentrations up to 20 μmol/l, had no effect on cell viability and did not sensitize the SMC to either sFasL or the cocktail of cytokines (Fig. 4A and B). In contrast, simvastatin, lovastatin and atorvastatin alone reduced SMC viability by a small but reproducible degree. Addition of the cytokine cocktail or sFasL to these statin-treated cultures markedly reduced cell viability. The effects of simvastatin, atorvastatin or lovastatin on sFasL-induced loss of cell viability could be seen at concentrations down to 150 nmol/l of the statin. Increasing the concentrations of the cytokine cocktail or sFasL above those indicated did not give rise to any additional loss in cell viability (data not shown).

It was of interest to examine if the sensitization of statins was dependent on the cell type. EC were incubated with 2 μmol/l simvastatin or pravastatin for 24 h and then stimulated with the cytokine cocktail or FasL for an additional 24 h (Fig. 4C). Cell death was assessed by Alamar blue staining. In contrast to what we observed with the SMC, the cytokine cocktail alone induced cell death. This was strongly enhanced by further treatment with simvastatin. Further, FasL had no cell death promoting activity in the simvastatin-treated cells.

3.4. Role of Fas in statin-sensitized apoptosis

In order to determine if the enhanced apoptotic sensitivity of statin treated cells was mediated predominantly through the Fas pathway, SMC were incubated with various statins in the absence or presence of 50 μg/ml Fas-Ig. Under these conditions Fas-Ig did not protect the cells from loss of viability due to statin treatment alone (Fig. 5A). In addition, no changes in the levels of cell surface Fas or sFasL could be observed following FACS analysis of statin treated cells (data not shown). Fas-Ig was able to block fully the additional cell death that was induced upon addition of sFasL to the statin-treated cell cultures (Fig. 5A) and restored the level of cell viability back to that of cells treated with the statins in the absence of exogenous sFasL.

To determine the role of the Fas pathway in the IFN-γ induction of apoptosis in simvastatin-treated SMC and to characterize whether the sFasL and IFN-γ apoptotic pathways were synergistic or overlapping, cells were sensitized with simvastatin and then treated with a combination of sFasL and IFN-γ. sFasL and IFN-γ, either alone or in combination, had little effect on cell viability in the absence of statin treatment (Fig. 5B). However, both sFasL and IFN-γ induced a significant decrease in viability of statin treated cells. The combination of the two agents significantly enhanced SMC death beyond that maximally observed with either alone. Fas-Ig very effectively reversed the loss in SMC viability in simvastatin-treated cells following treatment with sFasL alone, but did not influence the loss in viability that occurred upon stimulation of statin-treated cells with IFN-γ alone (Fig. 5B). In addition, Fas-Ig partially reversed the loss in cell viability that occurred following stimulation of simvastatin treated cells with the combination of sFasL and IFN-γ. The extent of the inhibition of cell death appeared to be equivalent to that contributed by sFasL. These results suggest that the two pathways leading to apoptosis are distinct but may either additively or synergistically contribute to the overall SMC death.

3.5. Role of intermediates in the mevalonate pathway in statin-sensitized apoptosis

Inhibition of HMG CoA reductase within the cell decreases the production of several intermediates in the mevalonate pathway [13], including the generation of farnesylpyrophosphate (FPP), and geranylgeranylpyrophosphate (GGPP), the substrates for farnesyltransferase and geranylgeranyltransferase, respectively. The reversibility of the statin-mediated sensitization of SMC to apoptosis was examined by incubation of the cells with simvastatin in the presence of mevalonate, FPP or GGPP for 24 h prior to stimulation with the cytokine cocktail or sFasL. Both mevalonate and GGPP completely blocked the ability of simvastatin to sensitize SMC to either sFasL or cytokine-induced loss of cell viability (Fig. 6). In contrast, FPP gave very little protection suggesting that geranylgeranylation mediates the protection from apoptosis. Similarly both mevalonate and GGPP, but not FPP, protected SMC from apoptosis induced by treatment of the cells with atorvastatin or lovastatin (data not shown).

4. Discussion

The role of the HMG-CoA reductase inhibitors in regulating cholesterol metabolism, particularly in the liver, has been extensively studied. The statins are all very effective at inhibiting sterol synthesis in hepatocytes. However, the effects of the statins on tissues outside the liver, and on responses other than cholesterol metabolism, are much less well understood. In particular, the potential to differentiate the statins on
non-lipid lowering effects on extrahepatic tissues is emerging [37]. Distinctions may arise from the ability of the non-hepatic cells to selectively distinguish and transport the inhibitors. For example, pravastatin is hydrophilic and highly hepatoselective while the lipophilic statins are much more widely taken up by a
broad range of tissues and cells by passive diffusion [38,39].

Previous studies have shown that several of the lipophilic statins can induce apoptosis in SMC [33] or block activation of T-cells through the T-cell receptor [40]. Many of these studies have been performed at high concentrations of drugs, in some experiments at concentrations that were 100- to 200-fold above those that are achieved in the plasma [33] and in species other than human [35]. Thus, the relevance to the physiological situation is unclear.

In this study we have examined the effect of the different statins on human SMC physiology at low doses. At low concentrations, only the lipophilic statins had a low but detectable effect on apoptosis and cell viability of the SMC. Simvastatin was the most potent, followed by lovastatin and atorvastatin. On the other hand, the hydrophilic pravastatin did not induce any detectable cell death at concentrations up to 20 μmol/l.

Since some of the statins are subject to drug interactions with CYP3A4 inhibitors, which can lead to increases in plasma levels by as much as 19-fold to a serum concentration of 2 μmol/l [41], the levels studied are especially relevant. Similar levels are reached with grapefruit juice [42]. Even though in the experiments reported here, the effects were seen at submicromolar concentrations of the statins, it is unclear whether the levels of statins achieved in vivo in the tissues would reach those that appear to be necessary to induce sensitization in vitro. However, tissue levels may be higher and duration of exposure longer than in the plasma [43] and repeated exposure may alter the sensitivity of the cells.

The in vitro studies reported here show that human SMC, without further sensitization, are resistant to apoptosis following treatment with cytokines and/or sFasL. Although this differs from what was reported earlier by another group [32], it is consistent with the results from Jovinge and co-workers who showed that the cytokines alone were insufficient to induce an apop-

Fig. 5. Role of Fas in FasL and cytokine-induced apoptosis. SMC were seeded in 96-well plates at 5000 cells/well and grown to confluence prior to initiation of treatments. (A) Cells were incubated with 2 μmol/l of either simvastatin, atorvastatin, or lovastatin together with Fas-lig for 24 h, prior to the addition of sFasL. The 10 μmol/l pravastatin was not different from control (data not shown). Cells were then incubated for a further 48 h and viability determined by the addition of Alamar blue. The mean value of the optical density (± S.D.) derived from eight replicate wells per sample was determined. The values are representative of three independent experiments.

Fig. 6. Effect of mevalonate pathway metabolites on statin-induced sensitization of SMC apoptosis. SMC were seeded at 5000 cells/well in 96-well plates and grown to confluency. SMC were cultured with 3 μmol/l simvastatin in the presence and absence of 500 μmol/l mevalonate (M), geranylgeranylpyrophosphate (GGPP) or farnesylpyrophosphate (FPP) for 24 h. The cytokine cocktail or sFasL was added to the wells and the cells incubated for an additional 48 h. The viability of the cells was then determined with Alamar blue. The optical density was determined 5 h after the addition of Alamam blue. The mean value of the optical density (± S.D.) derived from eight replicate wells per sample was determined. The values are representative of three independent experiments.
STATINS: A PARTIAL LIPID-EMULSIFYING AGENT TO RESTORE THE SENSITIVITY OF MAMMALIAN SMOOTH MUSCLE CELLS TO DEATH INDUCING SIGNALING


Cardioprotective effects of statins have been shown to protect SMC against apoptosis. However, the target proteins are as yet undefined. Recently it has been shown that both retrograde and anterograde transport (e.g. of Golgi glycosyltransferases) between the Golgi apparatus and the endoplasmatic reticulum involves prenylated proteins [49], which could be blocked by lovastatin. In one study, activation of p53 transiently increased transport of Ras from the Golgi to the cell surface and also enhanced sensitivity of cells to sFasL by the induction of Fas-FADD binding [50]. In contrast, in our studies the statins did not increase cell surface expression of Fas (data not shown). Furthermore, Fas-Ig, an inhibitor of the Fas-FasL interaction, did not prevent the small but significant level of apoptosis induced by statins alone or the much greater cytokine-induced apoptosis in statin-sensitized cells. Thus, it seems unlikely that increased cell surface expression of Fas can solely account for the statin induced sensitization of SMC to apoptosis. Rather, geranylgeranylated proteins may be involved in regulating the functional assembly of an intracellular death inducing signaling complex.

The in vivo effect of the different statins on the apoptosis of SMC and other cells or tissues may be difficult to demonstrate. However, several studies have shown that in the absence of changes in lipid serum levels, lipophilic statins inhibit SMC growth in vivo [6,51–54]. Also, Corsini et al. found ex vivo evidence that plasma from fluvastatin-treated patients inhibited SMC proliferation in contrast to that from pravastatin-treated patients [34]. Additionally, SMC in the atherosclerotic intima seem to be much more susceptible to apoptosis [55,56].

In summary, our results demonstrate that low concentrations of lipophilic statins sensitize SMC to apoptosis. One hypothesis predicts that at sites of plaque thinning, death of SMC will decrease plaque vulnerability, rupture and possibly a fatal thrombosis [5]. Thus, under suitable conditions (e.g. local inflammation), by diminishing the number of SMC, the lipophilic statins could enhance plaque thinning. On the contrary, in restenosis this effect could be beneficial. The effect in vivo may vary according to the stage of the disease. Furthermore, lipophilic statins may well sensitize other cells within the plaque or at completely different locations to apoptosis. The physiological significance of this sensitization, not only to cardiovascular disease, but also to other disease states such as inflammatory disorders remains to be determined.

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