Unraveling Pleiotropic Effects of Statins on Plaque Rupture

Wulf Palinski, Claudio Napoli

Several large-scale clinical trials have conclusively shown that statins markedly reduce clinical endpoints of atherosclerosis. A plethora of studies has also reported effects of statins other than cholesterol-lowering. These include effects on endothelial function, such as NO production and NO-mediated vascular relaxation, the recruitment of monocytes and T cells into the arterial intima, their subsequent activation and expression of proinflammatory factors, the proliferation of vascular smooth muscle cells (VSMCs), and other events that result in arterial remodeling. However, to date only a minority of these “pleiotropic” effects of statins have been demonstrated to be truly cholesterol-independent, i.e., reversible by geranylgeranyl-pyrophosphate (GGPP), but not by cholesterol. (GGPP and cholesterol represent separate branches of the mevalonate pathway downstream of the step blocked by HMG-CoA reductase inhibitors.) For example, the elegant work of Liao and colleagues established that the modulation of NO is due to the inhibition of GGPP that in turn affects the bioavailability of regulatory proteins, such as Ras and Rho. It has also been established that statins may inhibit atherogenesis by reducing the formation of superoxide and other oxygen radicals that modulate many intracellular signaling pathways. Finally, statins may affect the consequences of plaque rupture by modulating thrombosis and fibrinolysis. In fact, statins decrease the expression of tissue factor in lesions, reduce platelet activation, and improve fibrinolytic activity through preservation of endothelial function, but it is unclear whether these effects are common to the entire class of statins, because some compounds seem to exert opposite effects. Despite the increasing evidence obtained in vitro and in experimental models, the clinical relevance of pleiotropic effects of statins is still debated.

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The question whether specific pleiotropic effects contribute to the overall clinical benefits of statins is not an academic one. Although neither the indication of statins for atherosclerosis-related diseases nor their dosage recommendations will be affected by the answer, both an expansion of their indication and the development of statin-analogues optimized for specific pleiotropic effects can be envisaged, if it can be shown that cholesterol-independent effects occur in vivo and reduce disease severity or manifestation. For example, the observation of anti-inflammatory effects and T cell-inhibition in vitro and the reduction of graft atherosclerosis and mortality after heart transplantation have raised the hope that statins could be indicated for a number of immune diseases associated with chronic inflammation even under normocholesterolemic conditions. Compounds optimized for the competitive inhibition of the leukocyte function antigen 1 (LF-1) have also been developed. In addition to prompting the development of statin analogues, evidence for a biological role of specific mechanisms may also provide an antiatherogenic indication for other, unrelated drugs targeting the same mechanism, e.g., NO donors.

Unfortunately, neither in vitro experiments nor clinical trials are likely to establish the impact of selective pleiotropic effects of statins on atherogenesis or its clinical sequelae. Cell culture experiments do not reflect the complex interactions between the arterial wall and circulating leukocytes, platelets, and elements of the plasmatic coagulation system, nor can they mimic the influence of multiple organ systems that govern lipid metabolism, immune responses, or regulation of blood pressure. The main difficulty in the interpretation of clinical trials is the powerful cholesterol-lowering effect of statins, because genuine pleiotropic effects and cholesterol-dependent ones frequently affect the same pathogenic mechanisms. Experimental models in which genetic, dietary, and other variables can be tightly controlled provide the only ethically acceptable way to determine the impact of selective pleiotropic effects, even though few of these models are truly representative of human pathology. The limitations imposed by imperfect animal models are even greater when it comes to assessing pleiotropic effects of statins on plaque vulnerability, plaque rupture, and atherothrombotic events.

In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Bea and colleagues report that simvastatin promotes plaque stability in apolipoprotein E-deficient (apoE−/−) mice, independently of cholesterol lowering. In this model, cholesterol-lowering effects can be presumed minimal, because hypercholesterolemia is primarily due to increases in chylomicron remnants and VLDL, which cannot be cleared by hepatic LDL receptors or LRP via high-affinity binding to apoE. The absence of significant cholesterol-lowering by statins in this model has been experimentally validated before and used to demonstrate cholesterol-independent anti-inflammatory effects of simvastatin. Surprisingly, in the present longitudinal experiment spanning a much longer period (24 weeks), a significant temporary increase in plasma cholesterol was observed, the reason for which remains unknown. Although the extent of atherosclerosis increased over time in both groups and the higher plasma cholesterol level in the treatment group was associated with a consistent trend toward larger lesions, the fre-
### Pleiotropic Effect of Statins Potentially Affecting Plaque Rupture: Current Evidence for Their In Vivo Occurrence

<table>
<thead>
<tr>
<th>Effect of Statins on</th>
<th>Cholesterol Independent?</th>
<th>In Vitro</th>
<th>In Vivo</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endothelial function / vasotonus</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Inhibition of endothelin 1 expression[^26]</td>
<td>Yes</td>
<td></td>
<td></td>
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<tr>
<td>Increased expression and activity of endothelial nitric oxide (NO) synthase[^37,38]</td>
<td></td>
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<tr>
<td>Stroke protection mediated by endothelial NO synthase[^39]</td>
<td>Yes</td>
<td>Yes</td>
<td>Mouse</td>
<td></td>
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<tr>
<td>Preserved coronary endothelial function and coronary adventitial vasa vasorum[^40,41]</td>
<td>Yes</td>
<td></td>
<td>Pig</td>
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<tr>
<td>Preserves myocardial perfusion and coronary microvascular permeability[^42]</td>
<td>Yes</td>
<td></td>
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<tr>
<td><strong>Macrophage and T cell recruitment / modulation of immune functions</strong></td>
<td></td>
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<tr>
<td>Decreased leukocyte-endothelial interactions[^43]</td>
<td>Yes</td>
<td></td>
<td>Rat</td>
<td></td>
</tr>
<tr>
<td>Decreased CD11b expression and CD11b-dependent endothelial adhesion of human monocytes[^44]</td>
<td>Yes</td>
<td>No</td>
<td>Human</td>
<td></td>
</tr>
<tr>
<td>Inhibition of endothelial cell and monocyte MCP-1 synthesis and leukocyte recruitment into air pouch[^45]</td>
<td>Yes</td>
<td>Yes</td>
<td>Mouse</td>
<td></td>
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<tr>
<td>Decreased integrin-dependent leukocyte adhesion[^46]</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Inhibition of T cell activation through inhibition of MHC-II expression on APCs[^47]</td>
<td>?</td>
<td></td>
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<tr>
<td>Inhibition of leukocyte adhesion, T cell stimulation, and peritoneal inflammation by inhibition of LF-1 (CD11a/CD18)[^48]</td>
<td>Yes</td>
<td>Yes</td>
<td>Mouse</td>
<td></td>
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<tr>
<td><strong>Proinflammatory factors</strong></td>
<td></td>
<td></td>
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<tr>
<td>Inhibition of natural killer cell cytotoxicity by compactin[^49]</td>
<td>Yes</td>
<td></td>
<td></td>
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<tr>
<td>Inhibition of macrophage NO synthase and cytokines TNF-α, IL-1β, IL-6[^50]</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Inhibition of human B-lymphocyte activation[^51]</td>
<td>?</td>
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<td></td>
<td></td>
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<tr>
<td>Reduced neointimal inflammation[^52]</td>
<td>No</td>
<td>No</td>
<td>Rabbit</td>
<td></td>
</tr>
<tr>
<td>Anti-inflammatory and anti-atherosclerotic effects[^53]</td>
<td>?</td>
<td>Yes</td>
<td>Mouse</td>
<td></td>
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<tr>
<td>Reduction of IL-1β, IL-6, COX-2, and PPARα mRNA in endothelial cells[^54]</td>
<td>?</td>
<td></td>
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<tr>
<td>Reduced IL-6 synthesis in VSMCs[^55]</td>
<td>Yes</td>
<td></td>
<td></td>
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<tr>
<td>Reduced MMP-9 secretion by macrophages[^56]</td>
<td>?</td>
<td></td>
<td></td>
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<tr>
<td>Suppression of growth of macrophages expressing matrix metalloproteinases and tissue factor[^57]</td>
<td>?</td>
<td>No</td>
<td>Rabbit</td>
<td></td>
</tr>
<tr>
<td><strong>SMC proliferation / apoptosis of arterial cells / remodeling of the arterial wall</strong></td>
<td></td>
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<tr>
<td>Decreased VSMC proliferation[^58]</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Increased apoptosis of VSMCs[^59]</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Increased SMC and collagen, and decreased metalloproteinases in atheroma[^60]</td>
<td>No</td>
<td>No</td>
<td>Rabbit</td>
<td></td>
</tr>
<tr>
<td>Decreased metalloproteinase and increased collagen expression in plaques[^63]</td>
<td>No</td>
<td></td>
<td>Human</td>
<td></td>
</tr>
<tr>
<td>Decreased secretion of matrix metalloproteinase 9 and THP-1 cell migration[^64]</td>
<td>Yes</td>
<td></td>
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<tr>
<td><strong>Generation of oxygen radicals and modulation of oxidation-sensitive signaling</strong></td>
<td></td>
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<tr>
<td>Decreased LDL oxidation by macrophages[^65]</td>
<td>?</td>
<td>Yes</td>
<td>Yes/Rat</td>
<td>Mouse/Rat</td>
</tr>
<tr>
<td>Decreased SMC superoxide formation and reduced cardiac hypertrophy[^66]</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td></td>
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<tr>
<td>Reduced production of reactive oxygen species and improved endothelial dysfunction in normocholesterolemic hypertension[^67]</td>
<td>No</td>
<td>No</td>
<td>Rat</td>
<td></td>
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<tr>
<td><strong>Thrombosis / fibrinolysis</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Increased fibrinolytic activity in endothelial cells[^68]</td>
<td>Yes</td>
<td>Yes†</td>
<td>Rat</td>
<td></td>
</tr>
<tr>
<td>Decreased platelet activation and reduced cerebral ischemia[^69]</td>
<td>Yes</td>
<td></td>
<td>Mouse</td>
<td></td>
</tr>
<tr>
<td>Inhibition of tissue factor expression in macrophages[^70]</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Reduced tissue factor expression in carotid lesions[^71]</td>
<td>Yes</td>
<td>Yes</td>
<td>Rabbit</td>
<td></td>
</tr>
<tr>
<td>Reduced expression of cyclooxygenase-2 in the intima and in cultured VSMCs[^72]</td>
<td>Yes</td>
<td>No</td>
<td>Rabbit</td>
<td></td>
</tr>
<tr>
<td>NB: Opposite effects of some statins on thrombosis and fibrinolysis have been reported that cast doubts on the fact that these effects are common to the whole class of drugs[^22]</td>
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</tbody>
</table>

[^2]: No indicates a significant decrease in plasma cholesterol in in vivo studies rather than evidence for cholesterol-dependent mechanisms.
[^3]: ? Not established or both cholesterol-dependent and -independent mechanisms.
[^4]: *Opposite effects reported.
[^5]: †After 2 days of treatment.
functions are also increasingly noted in mice, and differences in immune-related consequences. Pronounced sex differences in immune-related consequences include the progression to atheroma with a single fibrous cap and the presence of activated macrophages secreting metalloproteinases that may waken the cap in rupture-prone areas. However, the manifestations of plaque disruption in humans differ from those in mice. In humans, the most frequent event associated with clinical sequelae is a rupture of the fibrous cap leading to the formation of thrombi that cause partial or complete occlusion of the lumen and/or downstream embolization. Some thrombi may also be formed as a result of plaque erosion in humans. In contrast, in apoE−/− and LDLR−/− mice, plaque instability mainly takes the form of erosion and intraplaque bleeding without thrombus formation. Plaque ruptures associated with thrombosis occur, but seem to be far less frequent than in humans. Rupture of human plaques, even if clinically silent, results in the transition to larger, complicated plaques. Remodeling of murine arteries following deep erosion or rupture is poorly understood. Repeated erosions have been postulated to cause accelerated lesion growth and may be responsible for the prevalence of lesions with multiple cap-like structures in mice, although similar lesions have also been observed in humans. Recent data in apoE−/−×SR-BI−/− mice suggest that a much greater incidence of murine plaque instability and shifts toward rupture/thrombosis are achievable in murine models. This should be helpful in determining to what extent the apparent differences in plaque instability and remodeling reflect fundamental differences in pathogenic mechanisms.

Increased frequency of their rupture by exogenous interventions. Induced myocardial infarction, albeit without signs of plaque rupture, have also been described. One may challenge such manipulations as not being representative of pathogenic mechanisms leading to the vulnerable plaque in humans or its rupture. Most importantly, the crucial clinical consequences—occlusive thrombi resulting in infarction and death—have not been seen in these mice. However, better mouse models that may overcome these important objections are on the horizon. Crosses between apoE−/− mice and mice deficient for the scavenger receptor BI (SR-BI−/−) developed by Krieger’s group are characterized by very early and extensive atherogenesis, even when fed normal chow. Their coronary atherosclerosis is comparable to that in 2-year-old apoE mice and shows extensive fibrin deposits indicative of plaque hemorrhage and coagulation. Most importantly, this is associated with multiple spontaneous infarctions with characteristic ECG abnormalities, enlarged hearts, defects of myocardial function (reduced ejection fraction and contractility), tissue necrosis, and death. This should finally put to rest the notion that mice cannot be models of plaque rupture, just as the development of the apoE mouse put to rest the
plaques.34 However, other protective effects of statins on alloproteinase expression, and cell death in human carotid by the observation that pravastatin treatment increases collagen.35 The presumed action of pravastatin is the reduction of cholesterol levels, although the impact of individual enzymes cannot be ruled out. Clearly, much more work will be necessary to establish the physiological significance of this discrepancy, the implications in terms of the accumulation of statins or active metabolites in cellular membranes and lipid-rich tissues in general remain a concern. Another caveat regards the temporary rise in cholesterol. The presumption is that statins inhibit HMG-CoA reductase and consequently the synthesis of both cholesterol and isoprenoids in mice, as they do in humans, and that the lack of reduction of murine plasma cholesterol levels is solely attributable to the prevalence of lipoprotein particles lacking the ligand for hepatic LDL receptors. If, however, the rise in cholesterol was not coincidental and the effect of statins on the mevanolate pathway were different from that in humans, the relevance of the model and the present findings would be questionable.

As indicated in the Table, in vivo evidence for genuine pleiotropic effects of statins is sparse. The present article adds reduced calcification, but offers no additional insights into the mechanisms actually responsible for plaque instability. Cholesterol-independent effects of statins on lesion composition that could contribute to weakening of the cap have previously been established in monkeys.29 Macrophage-secreted metalloproteinases are thought to be an important cause of fibrous cap weakening,30 although the impact of individual enzymes has not been established.31–33 This assumption is strengthened by the observation that pravastatin treatment increases collagen content and decreases lipid content, inflammation, metalloproteinase expression, and cell death in human carotid plaques.34 However, other protective effects of statins on macrophages, T cells, and inflammatory conditions prevailing in plaques and potentially contributing to plaque rupture35 cannot be ruled out. Clearly, much more work will be necessary to establish that specific pleiotropic effects of statins contribute to reduced plaque vulnerability and rupture in humans.34–41

References


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