Beneficial Cardiovascular Pleiotropic Effects of Statins

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Abstract—Pleiotropic effects of a drug are actions other than those for which the agent was specifically developed. These effects may be related or unrelated to the primary mechanism of action of the drug, and they are usually unanticipated. Pleiotropic effects may be undesirable (such as side effects or toxicity), neutral, or, as is especially the case with HMG-CoA reductase inhibitors (statins), beneficial. Pleiotropic effects of statins include improvement of endothelial dysfunction, increased nitric oxide bioavailability, antioxidant properties, inhibition of inflammatory responses, and stabilization of atherosclerotic plaques. These and several other emergent properties could act in concert with the potent low-density lipoprotein cholesterol-lowering effects of statins to exert early as well as lasting cardiovascular protective effects. Understanding the pleiotropic effects of statins is important to optimize their use in treatment and prevention of cardiovascular disease. (Circulation. 2004;109[suppl III]:III-39–III-43.)

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As HMG-CoA reductase inhibitors (statins) became more widely used in greater numbers of patients, their effects beyond lipid lowering began to emerge. Such pleiotropic effects include improvement of endothelial dysfunction, increased nitric oxide bioavailability, antioxidant effects, anti-inflammatory properties, and stabilization of atherosclerotic plaques. Additional effects of growing interest include the ability to recruit endothelial progenitor cells (EPCs), a putative immunosuppressive activity, and inhibition of cardiac hypertrophy. Research indicates that some of the pleiotropic effects of statins may be unrelated to the cholesterol-lowering properties of the drugs. Others may even be fully dissociated from inhibition of HMG-CoA reductase, and many take place at very low drug concentrations. This review focuses on effects that have special cardiovascular relevance. Understanding the full spectrum of benefits associated with statin therapy may allow better therapeutic application and foster the early use of statins in acute coronary syndromes.

Improvement of Endothelial Dysfunction

Endothelial injury contributes to the initiation of the atherogenic process. Endothelial dysfunction, an early manifestation of such injury, is associated with a paradoxical vasoconstriction to acetylcholine due to impaired synthesis, release, and activity of endothelium-derived nitric oxide (NO). Abnormal endothelium-dependent vasomotor responses predict the long-term progression of atherosclerosis and associated coronary events, as well as events shortly after vascular surgery.1,2 It is therefore not surprising that the well-established ability of statins to improve endothelial dysfunction, a class effect, has received much attention in recent years.

Normalization of Vasomotion

Short-term treatment with statins has been shown to improve endothelial dysfunction and increase myocardial perfusion. In hypercholesterolemic patients with perfusion abnormalities, for instance, treatment with fluvastatin (40 to 80 mg/d) for 6 to 12 weeks significantly increased myocardial perfusion in ischemic segments (30%; P<0.001), and the change from baseline was significantly greater than that observed in normal segments (5%; P<0.005).3 In subjects with moderately elevated cholesterol levels (6.2 to 7.5 mmol/L), treatment with simvastatin 20 mg/d, compared with placebo, significantly (P<0.0005) increased the vasodilatory response to acetylcholine as determined by forearm blood flow as early as 4 weeks after initiation of therapy.4 After an additional 3 months of treatment, improvement in the simvastatin group was significantly (P<0.005) greater than that observed at 4 weeks.

A recent study5 compared atorvastatin 10 mg/d plus dietary therapy with dietary therapy alone in postmenopausal women with hypercholesterolemia. Significant improvement in brachial artery vasoreactivity was observed as early as 2 weeks after beginning atorvastatin compared with dietary therapy alone (P<0.001), as well as at 4 and 8 weeks.5 Only a weak correlation was observed between level of cholesterol reduction with atorvastatin and the improvement in vasoreactivity.5 In fact, a small study in healthy normocholesterolemic young men indicated improved endothelial function within 24 hours of treatment with atorvastatin 80 mg and rapid impairment on statin withdrawal after 30 days.6 The effect occurred before levels of serum cholesterol and high sensitivity C-reactive protein (hsCRP) were decreased after 2 days of treatment. These findings support the view that statins may exert...
beneficial effects on endothelial dysfunction that are independent of the degree of plasma cholesterol lowering.

Long-term statin therapy also improves endothelial function in patients with atherosclerosis. Diet alone, a low-density lipoprotein (LDL)-lowering regimen (lovastatin and cholestyramine), and an LDL-lowering plus antioxidant regimen (lovastatin and probucol) were tested for 1 year on acetylcholine-induced vasoconstriction in epicardial coronary arteries. The greatest improvement in the vasoconstrictor response was seen in the LDL-lowering antioxidant group ($P<0.01$).

Whether statin therapy has a similar beneficial effect on endothelium-dependent vasodilatation in diabetes mellitus is under investigation. Recent studies with simvastatin and atorvastatin have shown no impact of statin therapy on endothelium-dependent vasodilatation in type 2 diabetes. However, another study with atorvastatin in type 2 diabetes demonstrated a significant improvement in endothelium-dependent vasodilatation. A similar finding was reported with atorvastatin in young patients with type 1 diabetes and normal cholesterol levels. The conflicting results obtained in these studies may have been due to differences in the statin dosage, study design, patient selection, concomitant medication, and technology used to measure endothelial function.

**Increased Bioavailability of Nitric Oxide**

Statins improve endothelial dysfunction in part by lowering LDL-cholesterol; more specifically, they have been shown to prevent downregulation of endothelial nitric oxide synthase (eNOS), the enzyme that catalyzes the formation of NO from L-arginine, by native LDL. Downregulation of eNOS may be mediated by the ability of LDL to increase levels of caveolin-1, a major inhibitor of eNOS activity. Statins also directly enhance constitutive eNOS activity, thereby increasing the bioavailability of NO. Several mechanisms may be involved, including a reduction of caveolin-1 abundance and an increase in Hsp90, which acts as a molecular chaperone to facilitate long-term activation of eNOS. Other mechanisms include stabilization of eNOS messenger RNA and decreased production of reactive oxygen species that inactivate NO. Statins also interfere with the prenylation of Rho GTPase by geranylgeranyl pyrophosphate (GGPP), preventing its translocation to the cell membrane where it negatively regulates eNOS activity.

The PI3-kinase/Akt pathway is also involved in NO regulation. Statins were found to activate the serine/threonine kinase Akt (protein kinase B) in endothelial cells, thereby enhancing the phosphorylation of the endogenous Akt substrate eNOS and producing an increase in NO.

**Antioxidant Effects**

The failure of antioxidants to prevent coronary artery disease in recent trials does not invalidate the oxidation theory of atherosclerosis. The lack of benefit may have been due to inadequate dosing, treatment length, or type of antioxidant. In addition, emphasis may need to be shifted toward acute events and early effects or more consideration given to the interplay of oxidative stress and inflammation in atherogenesis. In view of the central role played by oxidized LDL in atherogenesis, the established antioxidant effect of statin therapy is of major interest.

In addition to reversing the inhibitory effect of oxidized LDL on eNOS, statins also have direct antioxidant effects on LDL in vitro and ex vivo. Hydroxyl metabolites of atorvastatin, but not the parent compound, inhibit oxidation of both LDL and very-low-density lipoprotein as well as high-density lipoprotein. In addition, the hydroxy metabolites, representing 70% of active atorvastatin in plasma, demonstrate free radical-scavenging abilities that may contribute to inhibition of lipoprotein oxidation. Statins may also indirectly affect normal oxidative mechanisms by curbing the ability of macrophages to oxidize lipoproteins. Statins have also been shown to decrease the activity of macrophage CD36, a recognized receptor for oxidized LDL. The mechanism of this effect is under investigation.

Oxidized LDL particles are negatively charged. Possible causes of electronegative LDL include glycation and abnormal sialic acid content. Regardless of the source, electronegative LDL is likely to be cytotoxic. In patients with familial hypercholesterolemia, treatment with simvastatin 40 mg/d significantly decreased the proportion of electronegative LDL at 3 months (29%, $P<0.0002$) and 6 months (21%, $P<0.0001$). During 6 months of simvastatin therapy, the amount of cholesterol transported in electronegative LDL continually declined, achieving an overall reduction of 60%. Changes in the lipid parameters, however, were evident much earlier, at 1 month. These findings suggest that long-term statin therapy in humans may lead to a progressive reduction in the atherogenic potential associated with electronegative LDL.

**Antiinflammatory Effects**

Over the past decade, the importance of inflammation in the development of atherosclerosis has become clear. Elevated levels of markers for inflammation such as CRP, interleukin-6, intercellular adhesion molecule-1 (ICAM-1), and serum amyloid A (SAA) have been associated with increased risk for first and recurrent cardiovascular events. CRP levels, especially, appear to be among the most powerful predictors of future events.

**Reduction of Serum CRP**

There is now compelling evidence that statin therapy may attenuate the effect of inflammation on risk of cardiovascular events. Among 708 postinfarction patients in the Cholesterol and Recurrent Events (CARE) trial, subjects with elevated levels of CRP and SAA (>90th percentile) had a higher risk and benefited more from therapy with pravastatin 40 mg/d than those without elevated levels of these inflammatory markers. The relative risk of a recurrent coronary event was reduced by 54% and 25% in the 2 groups, respectively, compared with placebo. At baseline, both subsets had nearly identical plasma lipid and lipoprotein profiles. Long-term therapy with pravastatin in the CARE trial also reduced levels of CRP in postinfarction patients. Although baseline median CRP levels for active treatment and placebo were similar, the median level after 5 years was 21.6% lower in the pravastatin group than in the placebo group ($P=0.007$).
The change in CRP levels associated with pravastatin treatment was not correlated with the reduction in LDL-cholesterol levels. The latter finding has been confirmed in the recent 24-week prospective Pravastatin Inflammation/CRP Evaluation (PRINCE) trial.30 A 6-week triple crossover trial compared the effect of pravastatin, simvastatin, and atorvastatin therapy on hs-CRP levels in patients with combined hyperlipidemia.31 All 3 drugs, at doses reported to have equivalent effects on LDL-cholesterol, significantly reduced median hs-CRP levels (pravastatin by 20%, simvastatin by 23%, and atorvastatin by 28%; Figure), and these reductions were not correlated with reductions in LDL-cholesterol. This study is in contrast to the negative result of a similar comparison using a parallel design and a 3-month exposure with a smaller number of subjects in each subset.32 In the Atorvastatin versus Simvastatin on Atherosclerosis Progression (ASAP) study, aggressive statin therapy (atorvastatin 80 mg) reduced CRP levels to a greater extent than conventional therapy (simvastatin 40 mg).33 Moreover, a significant correlation was found in univariate analysis between the decrease in CRP and the reduction of intima media thickness (IMT) of carotid artery segments.34

Reduction of Adhesion Molecules
Adhesion molecules and chemoattractants play an important role in the inflammatory process.36 They mediate adhesion and transmigration of leukocytes to the subendothelium as part of the atherogenic process. One may measure soluble forms of adhesion molecules in plasma or study their interaction in vitro with inflammatory cell surface integrins. Statins appear to reduce adhesion and chemotactic molecules and to inhibit integrin activity. However, studies have yielded inconsistent results. Aggressive lipid-lowering therapy with simvastatin and atorvastatin was associated with a decrease in soluble E-selectin but not soluble vascular cell adhesion molecule (VCAM) or soluble ICAM in an early uncontrolled study.37 Recently, atorvastatin and simvastatin were found to lower significantly soluble E-selectin, P-selectin, and ICAM-1, but simvastatin increased soluble VCAM-1.38 Another recent comparison of atorvastatin and simvastatin at high doses, however, showed only small and inconsistent effects of both drugs on ICAM-1 levels.39 Flu- mustatin therapy in patients with hypercholesterolemia reduced circulating levels of P-selectin and ICAM-1; this effect appeared to be independent of the lipid-lowering effect.40 An important recent study demonstrated that a modified statin with no inhibitory effect on HMG-CoA reductase could have a potent and selective direct antiinflammatory effect.40 This finding proves that a statin pleiotropic effect may be fully dissociated from the inhibition of cholesterol synthesis.

Plaque Stabilization
Several mechanisms could account for the plaque-stabilizing effect of statins that was elegantly demonstrated in animal models.41 Reduction of LDL-cholesterol may contribute to the downsizing of the lipid core.42 Statins inhibit uptake of oxidized LDL by CD36,33 scavenger receptor A,44 and lectin-like oxidized LDL (LOX-1) receptor45 and inhibit macrophage oxidative properties.24 These effects of statins could theoretically contribute to reduced foam cell formation. Elevated plasma levels of several markers of the inflammatory cascade have been shown to predict future risk of plaque rupture. These markers include P-selectin, interleukin-6, tumor necrosis factor-α, soluble ICAM-1, and CRP.36 The beneficial effect of statins on the inflammatory process has been discussed above. Weakening of the fibrous cap in unstable plaques is associated with increased production of matrix metalloproteinases (MMPs) by macrophages. In cultured macrophage, fluvastatin decreased the activity of MMP-9 by 20% to 40%.46 In a study in humans,47 pravastatin treatment changed the composition of carotid artery plaques in a manner that favored stabilization. Patients with carotid artery stenosis received either pravastatin 40 mg/d or no therapy for 3 months before carotid endarterectomy. Plaques removed from the statin-treated group were composed of significantly less lipid and oxidized LDL, fewer macrophages, and fewer T cells. They had higher collagen content and demonstrated less MMP-2 immunoreactivity than control plaques. In addition, apoptosis was significantly reduced and immunoreactivity to tissue inhibitor of metalloproteinase-1 (a potent inhibitor of MMP-1 and MMP-9) was significantly increased in the pravastatin group compared with controls.47

Additional Effects
Stimulation of Endothelial Progenitor Cell Recruitment
Endothelial progenitor cells play an important role in the repair of ischemic injury.49 Data from in vitro and in vivo studies indicate that statins are at least as effective as vascular endothelial growth factor, a key cytokine in the regulation of neovascularization, in augmenting EPC differentiation.48 Evidence suggests that statins enhance the level of circulating EPCs and promote their mobilization to ischemic areas.49 In
patients with documented stable coronary artery disease, treatment with atorvastatin 40 mg/d for 4 weeks was associated with a 1.5-fold increase in the number of circulating EPCs at week 1, which increased to 3-fold over the 4-week period. Atorvastatin treatment stimulated the differentiation of a subset of endothelial precursor cells into EPCs rather than augmenting the total number of circulating hematopoietic stem cells. In addition, atorvastatin significantly enhanced the migration of EPCs in response to vascular endothelial growth factor.

The practical significance of these observations may seem remote at this time but appear promising when taken in the context of a recent study in a mouse model of myocardial infarction. Investigators found that EPCs mobilized by stem cell factor and by granulocyte colony stimulating factor homed to and partly repaired the infarcted heart, reducing mortality by 68% and improving myocardial function.

Immunomodulation

Immune mechanisms are important in atherogenesis. Increasing evidence suggests that statins may act as immunomodulators and that use of statins may have applicability in organ transplantation and other conditions requiring immunosuppression. Pravastatin treatment, added to standard antirejection medications (cyclosporine, prednisone, and azathioprine) after cardiac transplant, has been reported to significantly decrease the frequency of rejection (3 versus 14; \( P = 0.005 \)) and increase survival (94% versus 78%; \( P = 0.025 \)) at 12 months compared with controls. In cardiac transplant patients, simvastatin treatment in combination with antirejection medications and a lipid-lowering diet significantly increased survival and decreased the incidence of accelerated graft disease (a serious late complication of cardiac transplantation) compared with diet alone over a 4-year period. The possibility of intrinsic immunosuppressive activity of statins has also been raised. Recently, atorvastatin and lovastatin (10 \( \mu \text{mol/L} \)) and, to a lesser extent, pravastatin (20 \( \mu \text{mol/L} \)) were found to decrease interferon-\( \gamma \)-induced major histocompatibility complex-II (MHC-II) in human endothelial cells and macrophages. This effect was reversed by mevalonate and was attributed to the inhibitory effect of statins on promoter IV of MHC-II transactivating factor, leading to suppression of T-lymphocyte activation. This finding could explain in part the improved survival observed with statins in heart transplant recipients if T helper cell-I immune responses were suppressed in the process.

Inhibition of Myocardial Hypertrophy

Left ventricular hypertrophy is a risk factor for coronary artery disease and congestive heart failure. Rat cardiomyocyte hypertrophy induced in vitro by angiotensin II was abolished by simvastatin. Cardiac hypertrophy in vivo, induced in rats either by angiotensin II infusion (presence of hypertension) or by transaortic constriction (absence of hypertension), was also inhibited by simvastatin (2 mg/kg/d for 4 weeks). These findings add to the evidence that statins exert a protective effect on organs, including the kidney and pancreas, in addition to the vascular wall and the heart.

Conclusions

Although the possibility that statins might have pleiotropic effects was met at first with a healthy skepticism, the vast amount of knowledge accrued over the past few years has moved these effects into the spotlight. Many of the statin pleiotropic effects operate independently of LDL-cholesterol reduction, correlate poorly or not at all with LDL-cholesterol changes, take place rapidly, and are rapidly reversible on discontinuation of the drug. Direct effects in the absence of LDL or total cholesterol modification have been shown both in vitro and in vivo. The pleiotropic effects of statins and other drugs are under continued investigation to fully establish their role in the prevention of cardiovascular events. The results of several ongoing clinical trials aimed more specifically at pleiotropic effects should enlighten us on their relative clinical relevance and importance.

References


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