Effects of Statins in Reducing Thrombotic Risk and Modulating Plaque Vulnerability

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Summary: A large body of evidence demonstrates that statin therapy reduces risk for coronary events. This benefit probably stems in large part from lipid lowering, but lipid-independent cellular effects may also contribute. Statin therapy may lower the risk of thrombosis by reducing tissue factor expression and increasing plasminogen activators while reducing plasminogen-activator inhibitor. The statins may also improve endothelial function and reduce inflammatory response by increasing nitric oxide activity; all statins tested can decrease levels of C-reactive protein, a systemic marker of inflammation. Indeed, limiting inflammation may prove an important mechanism of statins' clinical benefits due to both lipid lowering and direct cellular effects. For example, attenuation of inflammation probably promotes maintained integrity of the fibrous cap of the atherosclerotic lesion by inhibiting processes that degrade the collagenous structure of the cap. Rupture of the fibrous cap causes most fatal coronary thrombosis.

Key words: inflammation, thrombosis, statins

Introduction

Evidence from clinical trials demonstrates that lipid lowering with statin treatment reduces the incidence of coronary events and stroke in a broad spectrum of individuals. A number of mechanisms may account for or contribute to the protective effects of statins. Drugs in this class may reduce plaque thrombogenicity, lessen vasospasm, decrease inflammation, and stabilize the fibrous cap of the atherosclerotic lesion.¹

Reducing Thrombogenicity

Physical disruption of the atherosclerotic plaque precipitates most fatal cases of coronary thrombosis. Once a plaque ruptures and a thrombus begins to form, a robust endogenous fibrinolytic system may favor rapid clot dissolution, or if an unfavorable balance in local and systemic hemostatic factors prevails, an occlusive thrombus may result. The procoagulant, prothrombotic, and antifibrinolytic factors involved in determining the consequences of a given plaque disruption include tissue factor, plasminogen activator inhibitor-1 (PAI-1), fibrinogen, and platelet-activating factor; the anticoagulant, antithrombotic, and profibrinolytic factors include plasminogen activators, thrombomodulin, and prostacyclin. One potential mechanism for disruption of hemostatic balance, overexpression of the potent procoagulant tissue factor by macrophage foam cells in the atherosclerotic plaque lipid core, may result from inflammatory signals, namely the proinflammatory cytokine CD40 ligand.²

Statin drugs may lower thrombogenicity by exerting anticoagulant/profibrinolytic effects. Aikawa *et al.* have shown that dietary lipid lowering reduces tissue factor expression in rabbit atheroma.³ In this study, rabbits with atheromas induced by balloon injury and high cholesterol diet (baseline group) were either switched to a purified-chow diet (low-cholesterol group) or maintained on a high-cholesterol diet (high-cholesterol group) for 16 months. Immunolocalization of tissue factor quantified by computer-assisted color image analysis showed strong expression of tissue factor in atheroma macrophages in the baseline and high-cholesterol groups and substantial reduction of tissue-factor protein expression in the low-cholesterol group (Fig. 1). This reduction corresponded to decreased activity of tissue factor assessed by *in situ* binding of tissue factor to factors VIIa and X; levels of tissue factor mRNA also decreased after dietary lipid lowering.

Other studies have found that statins can increase endothelial cell expression of an enzyme that favors fibrinolysis, tissue plasminogen activator.⁴ These *in vitro* studies demonstrated that endogenous tissue plasminogen activator levels increased in a lipid-independent manner, while levels of antifibrinolytic PAI-1 declined. Therefore, statins may act to reduce thrombus

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FIG. 1 Staining for tissue factor protein in rabbit atheromas after 16 months of high-cholesterol diet (A) and 16 months of low-cholesterol diet (B) following induction of the atheroma with balloon injury and 4 months of an atherogenic diet. Reproduced from Ref. No. 3 with permission.

accumulation in both a lipid-dependent manner (decreased tissue-factor activity) and a lipid-independent manner (decreased PAI-1 levels and enhanced plasminogen activator effect). Decreases in fibrinogen, an acute phase reactant, may also limit thrombus formation.

Opposing Vasospasm

A variety of studies indicate that lipid lowering (e.g., with statins and other drugs, plasmapheresis, or other means) can improve endothelial vasodilator function. Studies *in vitro* have shown that statins increase production of the potent vasodilator nitric oxide. Endres *et al.* showed that statin treatment significantly increased activity of endothelial nitric oxide synthase (eNOS), an enzyme responsible for nitric oxide production, in aortas of normocholesterolemic mice (Fig. 2).⁵

FIG. 2 Calcium-dependent nitric oxide synthase (Ca⁺⁺-dep NOS) catalytic activity in aortas from mice treated with simvastatin 0.2, 2, or 20 mg/kg for 14 days, compared with vehicle (V) treatment. Reproduced from Ref. No. 5 with permission.

In these animals, after ischemia and reperfusion of the middle cerebral artery territory, stroke size diminished, cerebral blood flow increased, and neurologic function improved as a result of statin pretreatment. Mice deficient in eNOS showed no improvement in cerebral blood flow or neuroprotective statin effect.

In addition to exerting a vasodilator effect, nitric oxide can impede platelet aggregation. It also limits activation of nuclear factor kappa B, a transcription factor involved in expression of genes encoding a number of proinflammatory functions of vascular wall cells and infiltrating leukocytes. Thus, nitric oxide has a direct anti-inflammatory effect.¹

Decreasing Inflammation

C-reactive protein (CRP) sensitively indicates systemic inflammation. Apparently well individuals at risk for cardiovascular events tend to have levels of CRP in the upper end of the normal range. Treatment with statins may reduce CRP levels in a manner independent of the magnitude of low-density lipoprotein lowering in an individual. In an analysis of 472 patients who remained free of recurrent coronary heart disease (CHD) events at 5 years in the Cholesterol and Recurrent Events (CARE) trial, a secondary prevention study involving a patient population with average cholesterol levels, pravastatin recipients exhibited a median decrease of 17.4% in CRP, compared with a median increase of 4.2% in placebo recipients, during the 5-year trial period.⁶

Analysis of changes in CRP among 5,719 participants who remained event free at 1 year in the primary prevention Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS, which involved a population with average cholesterol and modestly reduced high-density lipoprotein cholesterol levels) showed a 14.8% decrease in CRP in the lovastatin group, compared with no change in the placebo group.⁷ A study of the effects of cerivastatin over 8 weeks in 785 patients with primary hypercholesterolemia yielded similar findings.⁸ Likewise, in the Pravastatin Inflammation/CRP Evaluation (PRINCE) study, which included a CHD-free cohort (n = 1,702) and a cohort of patients with a history of CHD (n = 1,182),⁹ CRP levels fell a median of 14.7% from baseline in 12 weeks and 16.9% from baseline at 24 weeks among participants with no prior history of CHD. In patients with prior CHD, CRP levels were reduced 14.3% from baseline at 12 weeks and 13.1% from baseline at 24 weeks.⁹

Stabilizing the Fibrous Cap

Inflammation, particularly as it affects the stability of the atherosclerotic plaque, appears to link atherosclerosis to coronary events. Indeed, the finding that lipid-lowering treatment generally produces only slight improvements in the fixed stenoses in coronary arteries suggests that the reduced rates of myocardial infarction observed with lipid reduction involve qualitative changes in plaque biology rather than regression

per se. We have hypothesized that lipid lowering acts as an anti-inflammatory intervention to improve plaque stability.

We have proposed the hypothesis that the fibrous cap of the atherosclerotic lesion undergoes dynamic remodeling and exhibits considerable metabolic activity.^{1, 10} Interstitial collagen fibrils strengthen the fibrous cap and render it resistant to rupture. The balance among inflammatory mediators controls the level of collagen in the arterial extracellular matrix. Inflammation in the matrix, which may in part reflect effects of hyperlipidemia, results in reduced synthesis of collagen. The proinflammatory cytokine gamma interferon, for example, inhibits interstitial collagen synthesis by smooth muscle cells, the primary source of this extracellular matrix protein in the arterial wall. Other proinflammatory cytokines induce enzymes that can degrade collagen—e.g., matrix metalloproteinases, including collagenases and gelatinases. Inflammation reduces the ability of smooth muscle cells to repair and maintain the collagen component of the fibrous cap as well, since inflammatory factors overexpressed in plaques can trigger apoptosis; sites of fatal thrombosis where plaques have ruptured typically feature few smooth muscle cells. In summary, these inflammatory processes can weaken the ordinarily stable fibrous cap, rendering it prone to disruption and hence thrombosis.

Statin or other lipid-lowering therapy can stabilize the fibrous cap by reducing inflammation. In one experimental study, atheromas were induced in rabbits by balloon injury and atherogenic diet for 4 months, at which time one group (baseline) was sacrificed, while another group consumed a high-cholesterol diet for 16 months and another group consumed a low-cholesterol diet for 8 or 16 months. Measurement of macrophage and collagenase content by quantitative image analysis of standardized sections of immunostained aortas showed that atheromas in the baseline and hyperlipemic groups featured high macrophage content and high levels of collagenase; in contrast, atheromas in the lipid-lowering group showed reduction in both macrophage and collagenase content (Fig. 3).¹¹ Measurement of the amount of interstitial collagen content by Sirius red staining showed that most of the intact fibrillar collagen in the aortas of hyperlipemic animals resided in the vessel adventitia, with only a filamentous network in the intima. In contrast, animals receiving the low-cholesterol diet had a marked reinforcement of the collagenous structure of the atheroma fibrous cap (Fig. 4). If applicable to humans, these findings suggest that lipid lowering stabilizes vulnerable plaques by reducing expression of the enzymes that degrade the arterial extracellular matrix and may reduce susceptibility of atheromas to disruption and thrombosis by promoting collagen accumulation in the fibrous cap. The recent observations of Crisby *et al.* indeed suggest similar changes in carotid atheroma of patients treated with pravastatin in a nonrandomized series.¹²

Conclusion

Statins may improve outcomes and limit complications of atherosclerosis by a variety of mechanisms, including reduc-

ing thrombogenicity, opposing vasospasm, decreasing inflammation, and stabilizing the atherosclerotic lesion fibrous cap. Recent experimental investigations have shown that lipid lowering reduces other markers of endothelial activation, including expression of the leukocyte-receptor vascular cell adhesion molecule-1.¹³

A decrease in inflammatory responses as a result of lipid lowering may underlie many of the beneficial effects of statins in atherosclerotic disease. However, statins may also exert effects independent of systemic lipid lowering, including the increased levels of eNOS and expression of plasminogen activator and decreased expression of PAI-1. Inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase

FIG. 3 Macrophage (A) and collagenase (B) content of rabbit atherosclerotic lesions induced by balloon injury and 4 months of a high-cholesterol diet. After 16 additional months of high-cholesterol diet (top), macrophages continue to exhibit strong expression of collagenase. After a switch to and maintenance of low-cholesterol diet for 16 months (bottom), only scant macrophages and collagenase remain. Arrows indicate the internal elastic lamina. Reproduced from Ref. No. 11 with permission.

FIG. 4 Interstitial collagen content in the rabbit aortic intima is shown by Sirius red staining. A typical lesion in the high-cholesterol group (A) shows little intimal collagen, whereas a representative lesion in the low-cholesterol group (B) shows an abundance of interstitial collagen in the intima. Reproduced from Ref. No. 11 with permission.

by statins reduces formation of the polyisoprenoids farnesyl pyrophosphate and geranyl pyrophosphate, intermediates in the pathway of cholesterol synthesis. These polyisoprenoids can derivatize proteins and alter their functions. Inhibition by statins of the prenylation of the polyisoprenoids with intracellular signaling molecules including small G proteins may in this manner produce direct effects on cell function independent of lipid lowering. In summary, the well-characterized reduction in cardiovascular risk associated with statin therapy may arise from both direct effects on lipid lowering and lipid-independent effects.

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