Statins:

Are There Benefits Beyond Cholesterol Lowering?

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Objectives:

At the end of this presentation, the participant will be able to:

1. Appreciate the non-cholesterol effects of statins
2. Understand the role of nitric oxide in cardiovascular disease
3. Understand the mechanism by which statins protect against stroke

Summary:

The 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors, or statins, are potent inhibitors of cholesterol synthesis and large clinical trials have demonstrated that these agents reduce cholesterol and the incidence of cardiovascular diseases. Recent evidence, however, suggests that the beneficial effects of statins may extend beyond their effects on serum cholesterol levels. Because statins also inhibit the synthesis of isoprenoid intermediates in the cholesterol biosynthetic pathway, they may have pleiotropic effects on vascular wall cells. In particular, the small GTP-binding protein, Rho, whose membrane localization and activity are affected by post-translational isoprenylation, may play an important role in mediating the direct vascular effects of statins.

Recent large clinical trials have demonstrated that a class of cholesterol-lowering agents called statins decrease the incidence of myocardial infarctions and ischemic strokes in hypercholesterolemic and atherosclerotic individuals (Scandinavian Simvastatin Study Group, 1994; Packard, 1998; Sacks et al., 1996). These agents inhibit an early step in cholesterol biosynthesis by blocking the conversion of HMG-CoA to mevalonate (Fig. 1). Because serum cholesterol level is strongly associated with coronary atherosclerotic disease (Klag et al., 1993), it has been generally assumed that cholesterol reduction by statins is the predominant, if not the only mechanism, underlying their beneficial effects in cardiovascular diseases. However, subgroup analyses of large clinical trials have challenged this notion and suggest that the beneficial effects of statins may extend to mechanisms beyond cholesterol reduction (Massy et al., 1996; Blum, 1994); possibly involving direct effects on the vascular wall.

For example, subgroup analysis of the WOSCOP and CARE trials indicate that despite comparable serum cholesterol levels, statin-treated individuals have significantly lower risks for coronary heart disease compared to age-matched placebo-controlled individuals (Shepherd et al., 1995; Sacks et al., 1996; Massy et al., 1996). Furthermore, meta-analyses of past clinical trials suggest that the risk of myocardial infarctions in individuals treated with statins is significantly lower compared to individuals treated with other cholesterol-lowering agents or modalities despite comparable reduction in serum cholesterol levels in both groups (Brown et al., 1993; Pekkanen et al., 1990). Taken together, these

findings suggest that some of the beneficial effects of statins may be due to their cholesterol-independent effects on vascular wall.

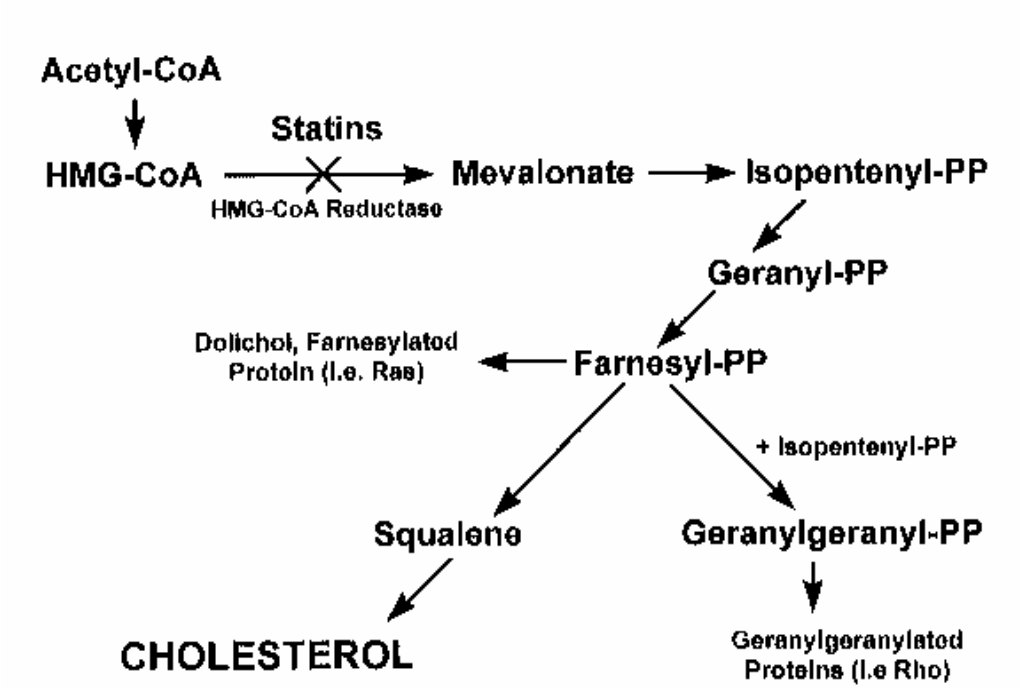


FIGURE 1 ; **Pathway for Cholesterol Biosynthesis.** Inhibition of HMG-CoA reductase by statins decreases the synthesis of isoprenoids and cholesterol.

Statins and Endothelial Function

Hypercholesterolemia impairs endothelial function and endothelial dysfunction is one of the earliest markers of arteriosclerosis, occurring even in the absence of angiographic evidence of disease (Liao, 1998; Libby et al., 1997). The vascular endothelium serves as an important autocrine/paracrine organ that regulates vascular wall contractile state and cellular composition. An important characteristic of endothelial dysfunction is the impaired synthesis, release and activity of endothelial-derived nitric oxide (NO). Endothelial NO has been shown to inhibit several components of the atherogenic process. For example, endothelium-derived NO mediates vascular relaxation and inhibits platelet aggregation, vascular smooth muscle proliferation and endothelial-leukocyte interactions. Furthermore, inactivation of NO by superoxide anion ($O_2 \cdot^-$) limits the bioavailability of NO and leads to nitrate tolerance, vasoconstriction, and hypertension (Harrison, 1997; Munzel et al., 1995).

Plasma LDL apheresis improves endothelium-dependent vasodilatation (Tamai et al., 1997) suggesting that statins could restore endothelial function by lowering serum cholesterol levels. However, in some studies, restoration of endothelial function occurs before significant reduction in serum cholesterol levels (Anderson et al., 1995; O'Driscoll et al., 1997; Treasure et al., 1995) suggesting that there are additional effects on endothelial function beyond that of cholesterol reduction. Indeed, statins upregulate endothelial nitric oxide synthase (eNOS) expression and activity and reverse the

downregulation of eNOS expression by hypoxia and oxidized low-density lipoprotein (ox-LDL) under cholesterol-clamped conditions (Laufs et al., 1997; Laufs et al., 1998a). Therefore, it is possible that statins may have other beneficial effects, irrespectively of serum cholesterol levels, in conditions associated with endothelial dysfunction such as atherosclerosis, pulmonary hypertension, and congestive heart failure.

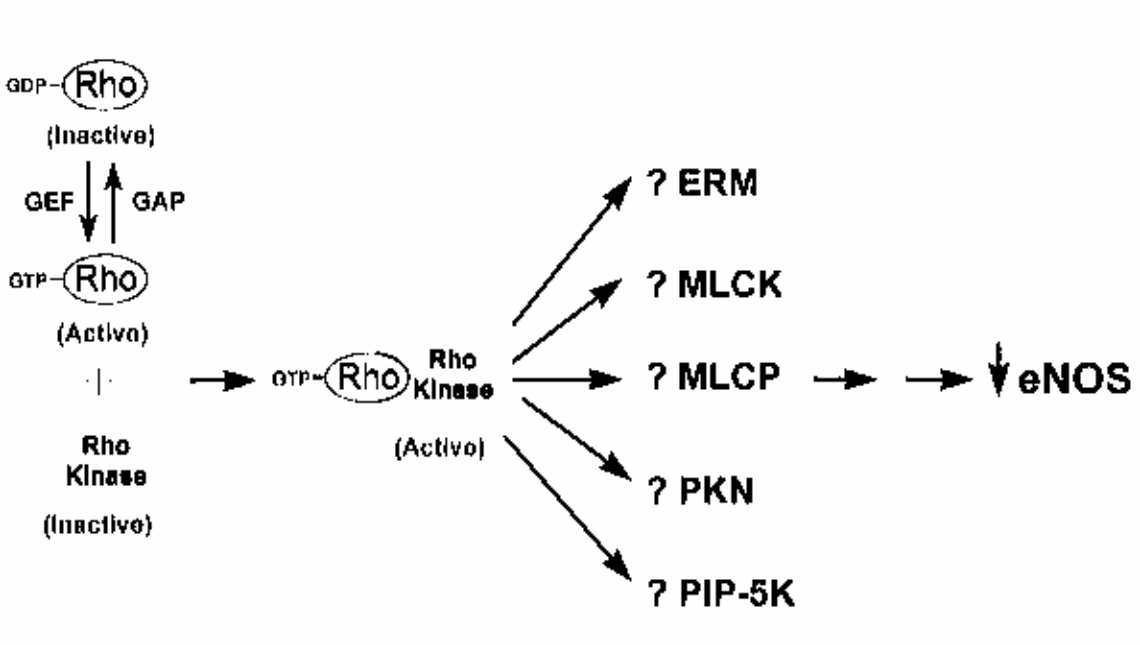
It is also important to emphasize that commercially-available statins were initially selected on the basis of their predominant uptake by the liver since this is where greater than 65% of cholesterol biosynthesis takes place in the body. However, the effect of statins on eNOS expression is probably due to a direct non-cholesterol effect on vascular endothelial cells. This may be important when one considers that the statins differ with regard to their lipid solubility; and hence, their differential abilities to penetrate vascular wall cells. For example, the more lipid soluble statins such as simvastatin and lovastatin would be expected to penetrate endothelial cells more than that of the more hydrophilic statin, pravastatin.

Statins and eNOS

By inhibiting L-mevalonate synthesis, statins also prevent the synthesis of other important isoprenoid intermediates of the cholesterol biosynthetic pathway, such as farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP) (Goldstein and Brown, 1990). These intermediates serve as important lipid attachments for the post-translational modification of variety of proteins, including the α subunit of heterotrimeric G-proteins, Heme-a, nuclear lamins, and small GTP-binding protein Ras, and Ras-like proteins, such as Rho, Rab, Rac, Ral or Rap (Van and D'Souza-Schorey, 1997). Thus, protein isoprenylation permits the covalent attachment, subcellular localization, and intracellular trafficking of membrane-associated proteins such as Rho. Members of the Ras and Rho GTPase family are major substrates for post-translational modification using isoprenoids (Van and D'Souza-Schorey, 1997; Aktories, 1997; Hall, 1998). Both Ras and Rho are small GTP-binding proteins which cycle between the inactive GDP-bound state and active GTP-bound state. In endothelial cells, Ras translocation from the cytoplasm to the plasma membrane is dependent upon farnesylation while Rho translocation is dependent upon geranylgeranylation (Laufs and Liao, 1998b). Statins inhibit both Ras and Rho isoprenylation and lead to accumulation of inactive Ras and Rho in the cytoplasm.

While the effects of statins on Ras and Rho isoprenylation are reversed in the presence of FPP and GGPP, respectively, the effects of statins on eNOS expression is only reversed with GGPP and not by FPP or LDL-cholesterol (Laufs and Liao, 1998b). These findings are consistent with a non-cholesterol-lowering effect of statins and suggest that inhibition of Rho by statins upregulate eNOS expression. Indeed, statins upregulate eNOS expression by prolonging eNOS mRNA half-life but not eNOS gene transcription. Since hypoxia, oxidized LDL, and cytokines such as TNF-α decrease eNOS expression by reducing eNOS mRNA stability, the ability of statins to prolong eNOS half-life may make them effective agents in counteracting conditions which downregulate eNOS expression. Statins prevent the downregulation of eNOS by oxidized LDL and TNF-α and under hypoxic conditions (Laufs et al., 1997; Laufs et al., 1998a).

Because Rho is major target of geranylgeranylation, inhibition of Rho and its downstream target, Rho kinase, is a likely mechanism by which statins upregulate eNOS expression (Fig. 2). The evidence that Rho negatively regulates eNOS expression comes from three sets of experiments. First, direct inhibition of Rho by *Clostridium botulinum* C3 transferase increases eNOS expression independent of isoprenylation. The C3 transferase ADP-ribosylates asparagine-41 of Rho and renders it biologically inactive in the GDP-bound state (Aktories, 1997). Second, inhibition of Rho by overexpression of a



dominant-negative RhoA mutant, N19RhoA, also increases eNOS expression. Finally, direct activation of Rho by *Escherichia coli* cytotoxic necrotizing factor (CNF)-1 leads to a decrease in eNOS expression (Aktories, 1997). These results, therefore, identify Rho as a negative regulator of eNOS expression.

FIGURE 2: **Regulation of eNOS by Rho GTPase.** Rho activates Rho kinase which leads to the downregulation of endothelial nitric oxide synthase (eNOS). The downstream targets of Rho kinase include ezrin-moesin-radixin (ERM), myosin light chain kinase (MLCK), the myosin binding subunit of myosin light chain phosphatase (MLCP), protein kinase N (PKN), and phosphatidylinositol 5-kinase (PIP-5K).

Statins and Ischemic Stroke

An intriguing result of large clinical trials with statins is the reduction in ischemic stroke (Crouse et al., 1998). Although myocardial infarction is closely associated with serum cholesterol levels, neither the Framingham Study nor the Multiple Risk Factor Intervention Trial (MRFIT) demonstrated significant correlation between ischemic stroke and serum cholesterol levels (MRFIT Investigators, 1982; Sytkowski et al., 1990). Thus, the findings of these large statin trials raise the interesting question of how a class of cholesterol-lowering agents can reduce ischemic stroke when ischemic stroke is not related to cholesterol levels. It appears likely that there are pleiotropic effects of statins which are beneficial for

ischemic stroke. Some of these beneficial effects may be attributed to the effects of statins on endothelial function and the vascular wall.

Cerebral vascular tone and blood flow are regulated by endothelium-derived NO (Dalkara et al., 1994). Mutant mice lacking eNOS (eNOS-/-) are relatively hypertensive and develop greater proliferative and inflammatory response to vascular injury (Huang et al., 1995). Indeed, eNOS-/- mice develop larger cerebral infarcts following cerebrovascular occlusion (Huang et al., 1996). Thus, the beneficial effects of statins in ischemic stroke may be due, in part, to their ability to upregulate eNOS expression and activity. Indeed, mice which were prophylactically treated with statins for up to 2 weeks, have 25-30% higher cerebral blood flow and 50% smaller cerebral infarct sizes following cerebrovascular occlusion (Endres et al., 1998; Laufs et al. 2000). Furthermore, no increase in cerebral blood flow or neuroprotection was observed in eNOS-/- mice treated with statins indicating that the upregulation of eNOS accounts for most, if not all, of the neuroprotective effects of these agents. Interestingly, treatment with statins did not affect blood pressure or heart rate before, during, or after cerebrovascular ischemia and did not alter serum cholesterol levels in mice, consistent with the previous finding that rodents are relatively resistant to changes in steady-state cholesterol levels by statins (Endres et al., 1998).

In addition to increases in cerebral blood flow, other beneficial effects of statins are likely to occur. For example, Lefer *et al*. reported that statins attenuate P-selectin expression and leukocyte adhesion via increases in NO production in a model of cardiac ischemia and reperfusion (Lefer et al., 1999). Others have reported that statins upregulate tissue-type plasminogen activator (t-PA) and downregulate plasminogen activator inhibitor (PAI)-1 expression through a similar mechanism involving inhibition of Rho geranylgeranylation (Essig et al., 1998). Thus, the absence of neuroprotection in eNOS-deficient mice emphasize the importance of endothelium-derived NO in not only augmenting cerebral blood flow, but also, potentially, in limiting the impact of platelet and white blood cell accumulation on tissue viability following ischemia. We speculate that statins may have contributed to the decrease in the incidence of ischemic strokes in clinical trials, in part, by reducing cerebral infarcts size to levels which are clinically unappreciated. Furthermore, because statins increase cerebral blood flow, these agents may also serve as an useful adjunctive therapeutic modality for increasing the delivery of other co-administered drugs to the CNS.

Summary

Statins exert many pleiotropic effects in addition to the lowering of serum cholesterol levels. Most of these effects are mediated by statin's inhibitory effect on isoprenoid synthesis. In particular, inhibition of Rho in vascular wall cells by statins leads to increased expression of atheroprotective genes and inhibition of vascular smooth muscle cell proliferation (Fig. 3). Thus, targeting Rho may have therapeutic benefits in the treatment of cardiovascular diseases.

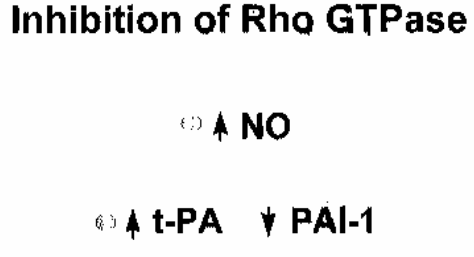


FIGURE 3: **Non-cholesterol Effects of Statins.** Inhibition of Rho in endothelial cells lead to the upregulation of nitric oxide (NO) and tissue-type plasminogen activator (t-PA), and downregulation of plasminogen activator inhibitor (PAI)-1.

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