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Pleiotropic effects of statins: basic research and clinical perspectives

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Abstract

Statins are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, which are widely used to lower serum cholesterol levels in the primary and secondary prevention of cardiovascular disease. Recent experimental and clinical evidence suggest that the beneficial effects of statins may extend beyond their cholesterol lowering effects, to include so-called pleiotropic effects. These cholesterol-independent effects include improving endothelial function, attenuating vascular and myocardial remodeling, inhibiting vascular inflammation and oxidation, and stabilizing atherosclerotic plaques. The mechanism underlying some of these pleiotropic effects is the inhibition of isoprenoid synthesis by statins, which leads to the inhibition of intracellular signaling molecules Rho, Rac and Cdc42. In particular, inhibition of Rho and one of its downstream targets, Rho kinase (ROCK), may be a predominant mechanism contributing to the pleiotropic effects of statins. In this review, we provide an update on the non-cholesterol-dependent statin effects in the cardiovascular system and highlight some of the recent findings from bench to bedside to support the concept of statin pleiotropy.

Keywords

Statin; cholesterol; pleiotropic effects; rho kinase; vascular

Statins or 3-hydroxy-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors are potent inhibitors of cholesterol biosynthesis and are established therapies for the primary and secondary prevention of coronary artery disease. Because serum cholesterol level is strongly associated with coronary heart disease, it has been generally assumed that the beneficial effects underlying statin therapy are entirely due to cholesterol reduction. However, clinical studies suggest that the overall benefits observed with statins may not be mediated solely by their lipid-lowering properties, but possibly through cholesterol-independent or pleiotropic effects [1–3].

PHARMACOKINETIC PROPERTIES OF STATINS

Statins were initially isolated and identified as secondary metabolites of fungi [4, 5]. They inhibit the rate-limiting step of cholesterol biosynthesis, the conversion of HMG-CoA to L-mevalonic acid, through binding to HMG-CoA reductase's active site and blocking the substrate product transition state of the enzyme [6]. This leads to decreased hepatic cholesterol synthesis, upregulation of low-density lipoprotein (LDL) receptor, and increased clearance of plasma LDL-cholesterol. In addition, by inhibiting HMG-CoA reductase, statins could also inhibit the synthesis of important isoprenoid intermediates, such as

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farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP) that lie downstream from L-mevalonic acid [7]. These intermediates serve as important lipid attachments for the post-translational modification of intracellular proteins such as nuclear lamins, Ras, Rho, Rac and Rap [8]. Thus, it is possible that, in addition to cholesterol lowering, the inhibition of these intracellular isoprenoid-dependent proteins may contribute to some of the biological effects of statins (Figure 1).

Because statins differ in their lipophilicity, half-life, and potency [9], they possess different potencies for extra-hepatic HMG-CoA reductase inhibition. These differences in tissue permeability and metabolism may account for some of the observed differences in their peripheral side effects [10], but at the same time, enabling them to have more pleiotropic effects. Lipophilic statins such as simvastatin and fluvastatin are considered more likely to enter vascular cells by passive diffusion than hydrophilic statins such as pravastatin and rosuvastatin, which are primarily targeted to the liver. However, the observation that hydrophilic statins have similar pleiotropic effects as lipophilic statins puts into question whether there are really any cholesterol-independent effects of statins. Indeed, recent evidence suggests that some of the cholesterol-independent effects of these agents may be mediated by inhibition of hepatic HMG-CoA reductase leading subsequent reduction in circulating isoprenoid levels [11]. This hypothesis may help explain why hydrophilic statins such as pravastatin and rosuvastatin are still able to exert cholesterol-independent benefits on the vascular wall without directly entering vascular wall cells. In this respect, the word “pleiotropic” may not reflect the hepatic versus non-hepatic effects of these agents.

DIVERSE TARGET POINTS FOR STATIN ACTIONS

Statin and Rho/ROCK

Rho kinases (ROCKs) are protein serine/threonine kinases of ~160 kDa that are downstream effectors of the small GTPase Rho. A critical step for intracellular trafficking and function of these proteins is their post-translational modification through isoprenylation [12], which can be inhibited by statins [13]. By inhibiting mevalonate synthesis, statins prevent membrane targeting of Rho and its subsequent activation of ROCKs (Figure 2). Indeed, statins, at clinically relevant concentrations that are used to reduce LDL-cholesterol, have been shown to inhibit Rho isoprenylation [14] and ROCK activity in humans [15]. It is therefore interesting to speculate whether some of the clinical benefits of statin therapy could be mediated by inhibition of the Rho/ROCK pathways.

Statins and Rac

Rac is a 20–39 kDa monomeric G-protein and also a member of the small GTPase subfamily. The major Rac signaling pathway includes remodeling of the actin cytoskeleton and generation of reactive oxygen species (ROS). Since the development of myocardial hypertrophy is exhibited by ventricular remodeling and increased oxidative stress, Rac may be an important mediator of cardiac hypertrophy [16]. Furthermore, results from animal and human studies suggest that some of the pleiotropic effects of statins may be mediated through inhibition of Rac1. For example, in a mouse model, simvastatin prevented angiotensin II (Ang II) or pressure overload induced hypertrophy through inhibition of Rac1-mediated NADPH oxidase activity in vascular smooth muscle and heart [17, 18]. This finding is further supported by analysis of failing human heart tissues, where increased ROS generation is associated with increased Rac1 activity, both of which are attenuated by statin treatment [19]. Indeed, a recent study indicates that statins at the clinical dosage used among Asians, mainly exhibits it pleiotropic effect through the inhibition of Rac1[20].

Statins and the Peroxisome Proliferator-Activated Receptor

Statins may also exert pleiotropic effects through activation of peroxisome proliferator-activated receptors (PPARs). In macrophages, statins induce PPAR-gamma transcription activity, inhibit LPS-induced TNF-alpha and MCP-1 expression, and repress the transcriptional activity of nuclear transcription factor and activator protein (AP1) through PPAR-alpha and PPAR-gamma [21]. These findings are supported by data demonstrating that statins stabilize atherosclerotic plaques through the activation of PPAR-gamma and that combined administration of simvastatin with PPAR-gamma agonists elicit additive effects on atherosclerotic plaque regression [22–24].

CELLULAR EFFECTS OF STATINS

Statins and the endothelium

Several clinical studies have shown that statins can improve endothelial function in patients with hypercholesterolemia and atherosclerosis through cholesterol-dependent and -independent pathways [25–27]. Because endothelium-derived nitric oxide (NO) is an important mediator of endothelial function, there are several different mechanisms by which statins could upregulate endothelial NO synthase (eNOS) (Figure 3).

The first pathway involves the Rho/ROCK signaling, through which statins increase the stability of eNOS mRNA, leading to increased eNOS expression [28, 29]. *In-vitro* studies show that direct inhibition of Rho by *Clostridium botulinum* C3 transferase or overexpression of a dominant-negative mutant of RhoA increases eNOS expression, confirming that RhoA negatively regulates eNOS expression and activity [29]. Furthermore, inhibition of ROCK by the ROCK inhibitors, fasudil or Y27632, also leads to increased eNOS expression and activity [15, 30, 31].

A second important mechanism by which statins activate eNOS is mediated through the serine-threonine protein kinase Akt. Statins rapidly promote the activation of Akt in endothelial cells leading to eNOS phosphorylation and increased angiogenesis [32]. Because this process is inhibited by the phosphatidylinositol-3 kinase (PI3K) inhibitors, wortmannin and LY294002, these findings indicate that statins activate Akt by upregulating PI3K signaling [32]. Interestingly, inhibition of the Rho/ROCK pathway also leads to the rapid activation of PI3/Akt pathway and cardioprotection, suggesting a similar mechanism by which statins upregulate eNOS expression [31].

A third mechanism, through which statins regulate eNOS activity, is through their effects on caveolin-1. Caveolin-1 is an integral membrane protein that binds to eNOS in caveolae and thereby inhibit NO production directly [33]. Its allosteric competitor calmodulin (CaM) promotes the calcium-dependent activation of eNOS through binding to the CaM-binding motif, and therefore can displace an adjacent auto-inhibitory loop on eNOS [34, 35]. *In-vitro* data with atorvastatin show that caveolin-1 abundance is reduced after statin treatment, leading to restoration of eNOS activity in endothelial cells. This effect is completely reversed by the addition of mevalonate [36].

Finally, statins may exert beneficial non-cholesterol effects through enhancing the mobilization of EPCs [37, 38]. Indeed, statins, by stimulating the PI3K/Akt pathway, induce angiogenesis by promoting the mobilization, proliferation, migration, and survival of circulating EPCs [39, 40]. Interestingly, this effect is observed at lower concentrations of statins only, while higher concentrations of statins elicit anti-angiogenic effects [41].

Statins and vascular smooth muscle

Vascular smooth muscle cells (VSMC) contribute to vascular proliferative diseases and recent studies have shown that statins can attenuate cytokine-mediated VSMC proliferation in coronary artery smooth muscle cells and also inhibit pathological proliferation such as that observed in transplant-associated arteriopathy [42, 43]. The ability of statins to inhibit cell proliferation through an isoprenoid-dependent way is demonstrated in fibroblasts where G1 cell cycle arrest induced by lovastatin is reversed by the addition of mevalonate or GGPP [44]. Furthermore, DNA synthesis in VSMC induced by platelet-derived growth factor (PDGF) is reversed by isoprenoid, but not cholesterol [45]. It appears that inhibition of Rho may be the predominant effect of statins on VSMC proliferation as the inhibition is reversed by GGPP, but not by farnesyl pyrophosphate or LDL cholesterol [46]. Indeed, direct inhibition of Rho by *Clostridium botulinum* C3 transferase or by a dominant-negative Rho mutant increases $p27^{Kip1}$ and inhibits SMC proliferation after PDGF stimulation [46]. Similarly, another study showed that atorvastatin inhibited serotonin-induced mitogenesis and migration through inhibition of GTP-RhoA formation in pulmonary artery smooth muscle cells [47]. This effect is also reversed by GGPP, but not FPP. Taken together, these findings suggest that Rho/ROCK pathway mediates SMC proliferation and that inhibition of Rho isoprenylation by statins may be the predominant mechanism by which statins inhibit vascular SMC proliferation.

Statins and the myocardium

Cardiac hypertrophy is mediated, in part by myocardial oxidative stress. Because Rac1 is required for NADPH oxidase activity, it is likely that statins inhibit cardiac hypertrophy through an antioxidant mechanism involving inhibition of Rac1 geranylgeranylation. Indeed, statins inhibit AngII-induced oxidative stress and cardiac hypertrophy in rodents [17, 48]. Data from *in-vivo* studies also demonstrate a protective function for statins against ischemic myocardial injury [49, 50]. Several follow-up studies confirmed these findings in normocholesterolemic as well as hypercholesterolemic animal models [51, 52]. One of the main contributors to this protective effect of statins is the increase in NO bioavailability, resulting in increased vasodilation and facilitating regional myocardial blood flow under hypoxic conditions[53, 54]. Furthermore, statin-induced NO production inhibits the upregulation of adhesion molecules involved in leukocyte-endothelial cell interactions [55]. Finally, statins could also preserve mitochondrial membrane potential in response to oxidative stress in cardiac myocytes in a NO-dependent manner [56].

Statins and platelets

A critical role in the manifestation of acute coronary syndromes is mediated by platelets. Circulating platelets are associated with mural thrombus formation at the site of plaque rupture and vascular injury [57]. Hypercholesterolemia has been associated with increases in platelet reactivity [58]. Although statins could decrease platelet reactivity through the reduction of serum cholesterol levels, recent studies suggest that some of the effects may be non-cholesterol-dependent. For example, in the recent JUPITER trial, treatment with rosuvastatin was associated with decreased venous thromboembolism [59], an effect, which is probably unrelated to cholesterol reduction since hypercholesterolemia is not a risk factor for venous thromboembolism.

One of the non-cholesterol-dependent mechanism includes the statin-mediated upregulation of eNOS in platelets leading to decreased platelet activation [60]. This is demonstrated for atorvastatin in a mouse model [60], as well as for fluvastatin in a clinical study with hypercholesterolemic patients [61]. Furthermore, statins inhibit tissue factor expression by macrophages and thereby reduce the thrombotic potential of the vascular wall [62].

Interestingly, a recent study suggests that PPARs may also mediate some of the antiplatelet actions of statins and fibrates [63].

DIRECT NON-LDL EFFECTS OF STATINS IN CARDIOVASCULAR DISEASE

Statins and atherosclerosis

Atherosclerosis is a complex inflammatory process that is characterized by the cross-talk between excessive inflammation and lipid accumulation [64]. Statins have been found to modulate immune activation and to exert anti-inflammatory effects on the vascular wall by decreasing the number of inflammatory cells in atherosclerotic plaques [65]. The mechanism is due, in part, to the immunomodulatory ability of statins to decrease the expression of endothelial adhesion molecules, such as ICAM-1, VCAM-1 and E-selectin directly [66, 67]. Furthermore, statins attenuate P-selectin expression and leukocyte adhesion by increasing endothelial NO production [68]. Interestingly, this cholesterol-independent effect of statins was absent in eNOS-deficient mice, suggesting that eNOS mediates some of the vascular protective effects of statins [69].

T-lymphocytes also play an important role in the pathogenesis of atherosclerosis. The activation of T-lymphocytes and the control of the immune response are mediated by the major histocompatibility complex class II (MHC-II) and CD40/CD40L. Statins inhibit MHC-II expression on endothelial cells and monocyte-macrophages *via* inhibition of the promoter IV of the transactivator CIITA and thereby repress MHC-II mediated T-cell activation [70]. In addition, statins decrease INF-gamma induced CD40 expression and CD40-related activation of vascular cells [71, 72]. Interestingly, statins could also modulate LFA, a major counter receptor for ICAM-1 on leukocytes, through a non-Rho/ROCK pathway [73]. By binding directly to its regulatory site in the beta-2 integrin, statin inhibit T-cell activation and suppress the inflammatory response independent of HMG-CoA reductase inhibition and small GTPases [73, 74].

Statins and stroke

Although the correlation between elevated cholesterol level and cerebrovascular disease remains controversial [75, 76], statins have become one of the most important therapies for stroke prevention and treatment. In humans, statins may prevent ischemic strokes through their vascular effects. Because studies with other lipid-modifying drugs have not been successful in reducing the incidence of stroke, these findings suggest that statins may protect against stroke through non-cholesterol-dependent mechanisms [77, 78].

One possible target for statin in stroke prevention could be the effects of statin on eNOS. Following cerebrovascular occlusion, $eNOS^{-/-}$ mice develop greater proliferative, inflammatory response to vascular injury and larger cerebral infarcts [79, 80]. However, mice pre-treated with statins, show higher cerebral blood flow and smaller cerebral infarct sizes following cerebrovascular occlusion [81]. In contrast, 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 of the neuroprotective effects of these agents [80].

EVIDENCE OF STATIN PLEIOTROPY IN CLINICAL TRIALS

Traditionally, it has been assumed that the beneficial effects underlying statin therapy are predominantly due to cholesterol reduction. However, several clinical trials have suggested that their benefits may extend beyond cholesterol, supporting the notion that the clinical benefits of statin therapy may be independent of LDL-cholesterol reduction. Recently, the JUPITER (Justification for the Use of statin in Primary prevention: an Intervention Trial Evaluating Rosuvastatin) trial showed that statins could be beneficial in the primary

prevention of cardiovascular disease in patients with elevated hsCRP, but relatively low cholesterol levels and other cardiovascular risks [82]. This study evaluated the effect of rosuvastatin (20 mg/day) in reducing the rate of cardiovascular events among apparently healthy subjects with LDL-cholesterol levels of less than 130mg/dl (3.4 mmol/L), but hsCRP levels of greater than 2 mg/L [83]. Subanalysis of this trial show that asymptomatic individuals randomly allocated to rosuvastatin benefited particularly if low concentrations of both LDL-cholesterol and C-reactive proteins were achieved [84]. These finding supports the importance of inflammatory components in mediating cardiovascular diseases, but also suggests a non-cholesterol-dependent effect of statins since the reduction in hsCRP by rosuvastatin was not related to the reduction in LDL-cholesterol. Interestingly, recent findings from the COSMOS (Multicenter coronary atherosclerosis study measuring effects of Rosuvastatin using intravascular ultrasound in Japanese subjects) trial confirm these data. Using intravascular ultrasound (IVUS) to investigate the effect of rosuvastatin on coronary atherosclerosis in Japanese subjects with ischemic heart diseases, the authors found that rosuvastatin treatment significantly reduced intracoronary plaque volume and increased in lumen volume. However, there was no significant correlation between the reduction in plaque volume and the reduction in plasma LDL, supporting the idea of effects beyond cholesterol lowering [85].

In addition, two clinical trials with ezetimibe further support the notion of statin pleiotropy in humans. Ezetimibe which acts by decreasing cholesterol absorption in the intestine is used alone or in combination with statin therapy to enhance lipid lowering [86, 87]. Interestingly, several clinical studies have demonstrated beneficial effects of statins over ezetimibe despite comparable reduction in LDL-cholesterol [27, 88]. In the ENHANCE (Ezetimibe and Simvastatin in Hypercholesterolemia Enhances Atherosclerosis Regression) study – a double blind, randomized trial comparing ezetimibe/simvastatin (10 mg/80 mg) to the highest recommended dose of 80 mg simvastatin, ezetimibe/simvastatin combination did not reduce the intima-media thickness of the carotid artery wall in patients with familial hypercholesterolemia, despite significant incremental reductions in levels of both LDL-cholesterol and C-reactive protein [89]. In addition, our group has recently compared high-dose statin monotherapy with equivalent cholesterol-lowering efficacy of the same statin at a lower dose plus ezetimibe. Two recent studies demonstrated that high dose statins alone improve flow-mediated dilation (FMD) more than dual therapy with low-dose statin and ezitimibe despite comparable reductions in LDL-cholesterol [25, 90].

FUTURE DIRECTIONS

Because of the potential for pleiotropic effects, statin therapy is currently being considered for medical conditions beyond cardiovascular disease. Statin pleiotropy, however, is still an evolving concept and clinical studies have yet to show how much of the benefits of statins are non-LDL-dependent. Part of the frustration is due to the lack of a definitive biomarker for statin pleiotropy. With the molecular insights obtained from basic studies regarding isoprenoid synthesis and inhibition of the Rho/ROCK and Rac pathways, it may be possible in the near future to actually define the clinical benefits of statin therapy attributed to non-LDL effects. For example, measurement of leukocyte ROCK activity is currently being utilized to identify patients at cardiovascular risks and to gauge statin efficacy on vascular function that is non-LDL-dependent [25, 91, 92]. Whether ROCK activity could also independently predict cardiovascular outcomes of statin therapy beyond cholesterol reduction remains to be determined.

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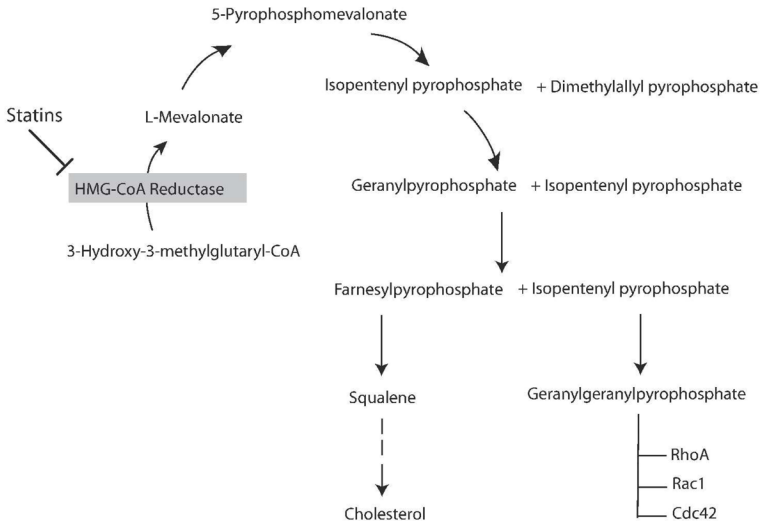

Figure 1. Biological actions of isoprenoids

Statins inhibit HMG-COA reductase acitivity and decreases the isoprenylation of intracellular signaling molecules, such as RhoA, Rac1 and Cdc42.

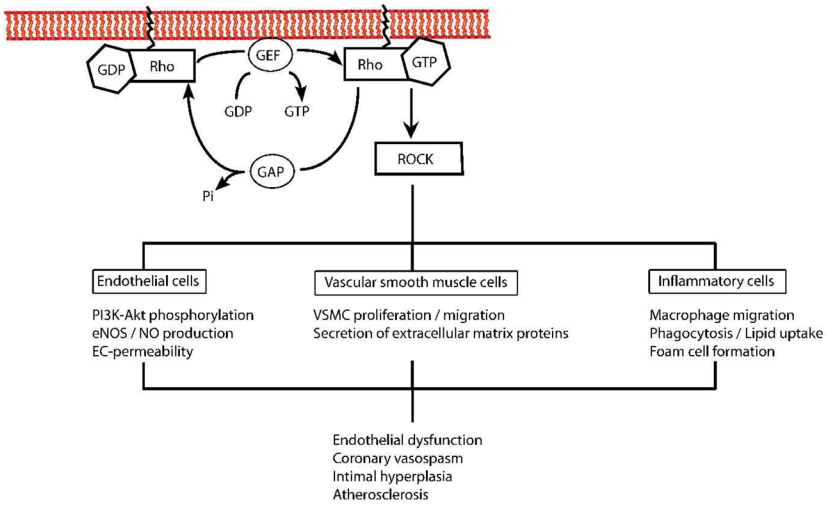

Figure 2. Regulation of the Rho GTPase cycle

Rho protein cycle between an inactive GDP-bound and an active GTP-bound state. Inhibition of the mevalonate synthesis by statins prevents memrbane targeting of Rho and its subsequent activation of ROCK. The inhibition of the Rho/ROCK pathway may mediate some of the pleiotropic effects of statins on the vascular wall.

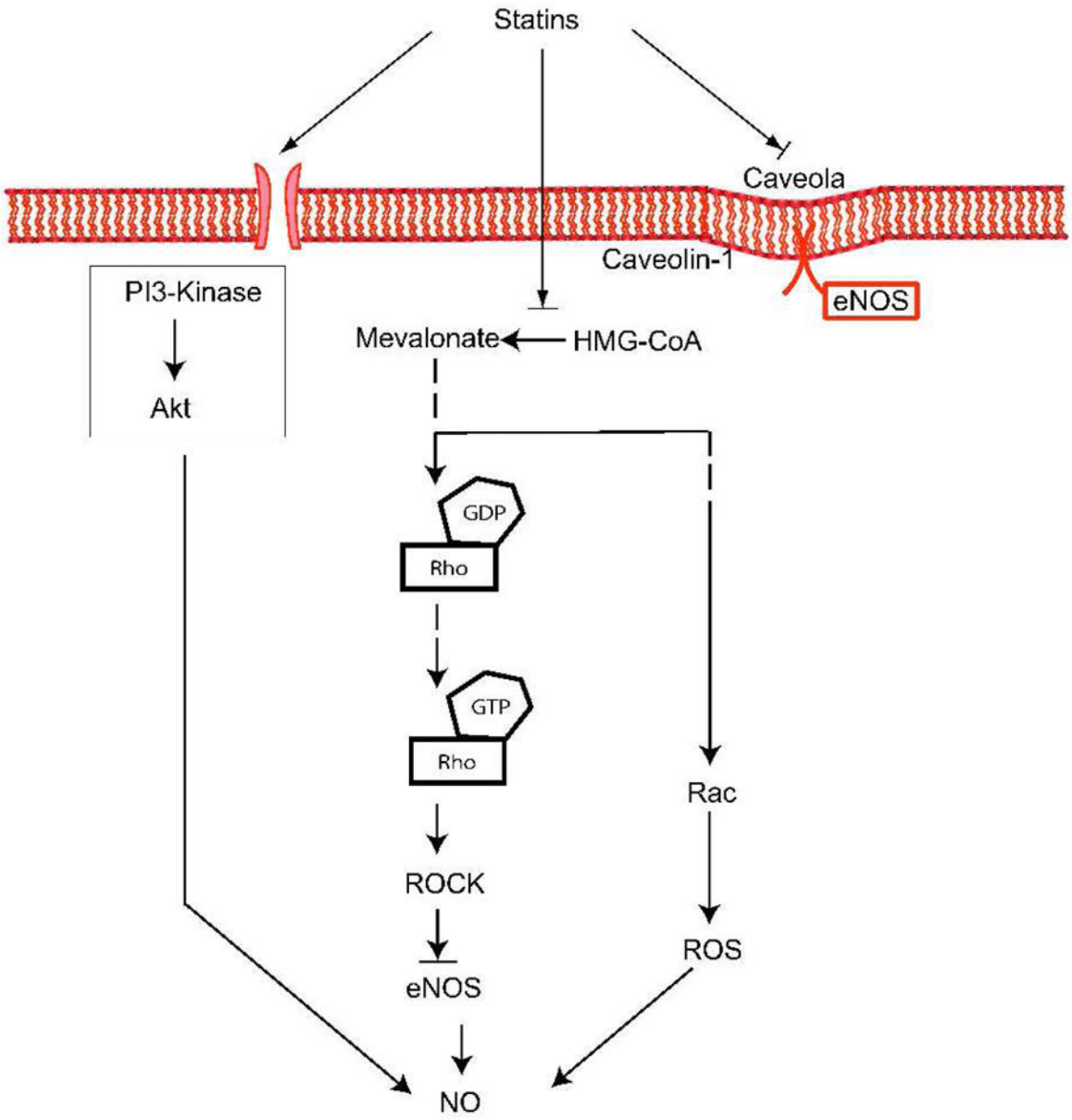

Figure 3. Upregulation of eNOS by statins

Statins modulate eNOS expression through three major mechanisms: 1) Increased eNOS mRNA stability through inhibition of Rho isoprenylation. 2) Increased eNOS phosphorylation through PI3K-dependent signaling. 3) Restoration of eNOS activity through reduction of caveolin-1 abundance.