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Pleiotropic Effects of Statins on the Cardiovascular System

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Abstract

The 3-hydroxy-methylglutaryl coenzyme A reductase inhibitors (statins), have been used for thirty years to prevent coronary artery disease and stroke. Their primary mechanism of action is the lowering of serum cholesterol through inhibiting hepatic cholesterol biosynthesis thereby upregulating the hepatic low-density lipoprotein (LDL) receptors and increasing the clearance of LDL-cholesterol (LDL-C). Statins may exert cardiovascular protective effects that are independent of LDL-C lowering called “pleiotropic” effects. Because statins inhibit the production of isoprenoid intermediates in the cholesterol biosynthetic pathway, the post-translational prenylation of small guanosine triphosphate binding proteins such as Rho and Rac, and their downstream effectors such as Rho kinase and nicotinamide adenine dinucleotide phosphate oxidases are also inhibited. In cell culture and animal studies, these effects alter the expression of endothelial nitric oxide synthase, the stability of atherosclerotic plaques, the production of pro-inflammatory cytokines and reactive oxygen species, the reactivity of platelets, and the development of cardiac hypertrophy and fibrosis. The relative contributions of statin pleiotropy to clinical outcomes, however, remain a matter of debate and are hard to quantify since the degree of isoprenoid inhibition by statins correlates to some extent with the amount of LDL-C reduction. This review examines some of the currently proposed molecular mechanisms for statin pleiotropy and discusses whether they could have any clinical relevance in cardiovascular disease.

Introduction

Cardiovascular diseases remain the leading cause of death worldwide.[1] The development of coronary atherosclerosis involves a complex interplay between metabolic and inflammatory processes.[2] Mechanistic and genetic evidence shows that apolipoprotein B (ApoB) containing lipoproteins, specifically low-density lipoprotein cholesterol (LDL-C) is causal for atherogenesis.[3] Statins or 3-hydroxy-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, decrease cholesterol biosynthesis and decrease serum LDL-C and triglyceride levels [4] Landmark clinical trials have demonstrated the efficacy of statins for both primary and secondary prevention of coronary heart disease (CHD).[5-17] It has been proposed that

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statins exert both LDL-C-dependent and LDL-C-independent (or pleiotropic) effects.[18] Clinical studies show statin benefits in diseases that are not clearly related to LDL-C (Table 1), but some of the outcomes may be due to direct cholesterol lowering.[19-30] Decreased gallstone formation could be due to decreased hepatic cholesterol formation, decreased cholesterol reduces platelet aggregation and could lead to less deep vein thrombosis, and decreased cholesterol could affect the progression of renal disease by decreasing renal artery atherosclerosis.[20,25,31] The clinical significance of the pleiotropic effects of statins in the cardiovascular system remains controversial given the overwhelming benefits of cholesterol reduction in preventing cardiovascular events.

Pharmacokinetic Properties of Statins

HMG-CoA reductase produces mevalonate and is the rate limiting enzyme for cholesterol biosynthesis in the liver, and it is competitively and reversibly inhibited by statins through their lactone ring and side chains that help them bind to the enzyme's active site (Figure 1).[32] Statins were initially identified as metabolites of fungi, have been on the market since 1987, and each vary in their lipophilicity, elimination half lives, and potency (figure 2).[32-34] Inhibition of cholesterol synthesis leads to decreased cholesterol production and upregulation of the LDL receptor.[4]

The lipophilic statins cross cell membranes largely by passive diffusion, while pravastatin and rosuvastatin require activated carrier-mediated transport with organic anion transporting polypeptide (OATP) 1B1 and are more selective for hepatic tissues.[35-37] Similar transporters exist in other tissues, such as OATP 1A4 and OATP 2B1 although their efficacy in transporting hydrophilic statins is unknown.[38-40] The concentrations of statins and mevalonate in different cell types are incompletely understood. It is unclear if the pleiotropic effects of statins are due to the hepatic or non-hepatic effects of isoprenoid inhibition.

It is unclear whether statins exert effects independent of mevalonate synthesis inhibition. One paper reported that statins could bind to an allosteric site within the β2 integrin leukocyte function-associated antigen-1 (LFA-1).[41] LFA-1 is involved in leukocyte trafficking and T cell activation and binds intercellular adhesion molecule-1 (ICAM-1).[42] ICAM-1 is crucial for the adhesion of monocytes to the endothelium and it is a biomarker for coronary events that is reduced by atorvastatin [43] However, to date, no consistent mevalonate-independent effects of any statin have been reported.

Evidence of Statin Pleiotropy in Clinical Trials

The concept of anti-inflammatory pleiotropic effects of statins has been tested for perioperative risk reduction. Several studies provide evidence for beneficial effects of statins on atrial fibrillation (AF) and outcomes after cardiac surgery [44-47] In contrast, in a study with 1922 patients in sinus rhythm who underwent elective cardiac surgery and received perioperative rosuvastatin 20 mg or placebo, statin therapy did not prevent postoperative AF or myocardial damage.[48] Similarly, in a large trial among patients undergoing cardiac surgery, atorvastatin treatment did not reduce the risk of acute kidney injury.[49] Cardiac surgery is pro-inflammatory and rosuvastatin reduced C-reactive protein (CRP) in one study, but subgroup analyses are not available from either study by CRP level. Although these

clinical studies do not show benefits of statin therapy, they do not exclude whether statin pleiotropy exists, but rather that statins are not beneficial in these diseases.

Additional considerations regarding the pleiotropic effects of statins come from their effects on CRP. JUPITER was a primary prevention trial between rosuvastatin and placebo for 17,802 patients with a LDL-C of < 130 mg/dL and a CRP ≥ 2.0 mg/L.[12] Rosuvastatin reduced LDL-C by 50%, CRP by 37%, and the primary endpoint by 44%.[12] Plotting the expected benefit from JUPITER based on LDL-C lowering on the Cholesterol Treatment Trialists' (CTT) Collaboration regression line, suggests that the realized benefit may be greater then the expected benefit based on LDL-C reduction alone (Figure 3). In contrast, the recent HOPE-3 study was a primary prevention trial with rosuvastatin 10 mg that did not have LDL-C or CRP as inclusion criteria and rosuvastatin reduced LDL-C by 26.5% and the co-primary outcomes by 24% and 25%.[50] The benefit of rosuvastatin occurred in both high and normal CRP groups, and while rosuvastatin did lower CRP, the HOPE-3 study suggests that the benefit of statins may be primarily due to LDL-C lowering.[50] In the A-Z trial, patients with acute coronary syndrome (ACS) received either simvastatin 40 mg for 1 month followed by titration to 80 mg versus placebo for 4 months and then simvastatin 20 mg. High dose simvastatin lowered LDL-C more effectively, but there was no difference in CRP levels at 30 days and the trial did not achieve its pre-specified endpoint [51] From month 4 onwards, there was a reduction of CRP in the high intensity simvastatin group, and the trend to benefit was stronger after 4 months then earlier.[51] Both groups had relatively low CRP (2.5 mg/L versus 2.4 mg/L) at 1 month, which may explain the lack of effect.[51] The MIRACL trial was a ACS trial comparing atorvastatin 80 mg with placebo and atorvastatin lowered the primary endpoint in patients with both high and normal LDL-C and lowered CRP by 83%.[14,52] While these data on CRP suggest that statins reduce inflammation, there are no data showing the change in CRP correlates with efficacy and it is unclear whether the benefit seen with CRP reduction on medication is due to statin therapy or a lower baseline CRP, indicating a lower risk cohort..

It is difficult to separate the LDL-C lowering benefit of statins from their potential pleiotropic effects in clinical trials given the strong association between elevated cholesterol and CHD.[53] Lowering ApoB containing lipoproteins, therefore, represents the most important mechanism of statins. Nevertheless, there is cumulative evidence for the existence of pleiotropic effects in humans but the contribution in addition to LDL-C lowering remains unknown for two reasons:[54]

- **a.** Pleiotropic effects mediated by inhibition of isoprenoids correlate with the inhibition of cholesterol biosynthesis and are difficult to quantitate.
- **b.** Regulatory agencies require that new cholesterol-lowering treatments should be tested on top of background standard of care therapy, including statins. This does not allow quantitating potential cholesterol-independent effects of statins because potential pleiotropic statin effects are present in both treatment arms.

Non-statin LDL-C lowering therapies could reduce the risk of CHD, thus confirming the cholesterol hypothesis. A recent meta-analysis suggests that non-statin therapies that increase the LDL receptor have the same benefits as statins and fall on the CTT regression

line.[55,56] Non-statin trials often take longer to show a benefit then statin trials. In the Lipid Research Clinic-Coronary Primary Prevention Trial (LRC-CPPT) with cholestyramine, the Program on the Surgical Control of Hyperlipidemias (POSCH) with partial ileal loop bypass surgery, and the IMPROVE-IT trial with ezetimibe in addition to simvastatin, benefits occurred after 7.4, 9.7, and 7.0 years, respectively whereas most of the statin trials showed benefits within 5 years (Table 2).[56-58] Interpreting the time to benefit data is difficult because of the low event rates early in the trials prevent the separation of survival curves and both LRC-CPPT and POSCH were primary prevention trials with lower event rates but many of the statin trials are secondary prevention trials and the IMPROVE-IT trial was conducted until a pre-specified total event number was reached. Guidelines have generally recommended lifestyle changes and statins as first-line pharmacotherapy, with or without LDL-C targets. At present, non-statins are indicated only as adjunctive therapy for patients who are unable to reach their lipid goals despite optimal statin therapy.

Studies using “clamped” cholesterol designs, e.g. comparing the effect of statin-mediated LDL-C lowering with equal LDL-C lowering mediated by another intervention (e.g. diet) have reported pleiotropic effects of statins in animals including cynomolgus monkeys.[59] Non-statins such as the inhibitor of Niemann-Pick C1 like protein, ezetimibe, have been used in humans for this purpose. Ezetimibe lowers LDL-C by 15-20% and can only be compared with less potent statins, which makes vascular effects more difficult to observe. Ezetimibe reduces cholesterol absorption in the small intestine and animal data suggests an increase in the LDL-C receptor, but human data is inconsistent.[60,61] Nevertheless, multiple small studies have attempted to determine if statins have pleiotropic effects compared to ezetimibe. These studies are characterized by surrogate endpoints. For example, a randomized study of heart failure patients with simvastatin 10 mg or ezetimibe 10 mg found a 15% reduction in LDL-C for both groups, but only simvastatin improved radial artery flow-dependent vasodilation, increased functionally-active endothelial progenitor cells (EPCs), and increased superoxide dismutase.[62] Several studies randomized healthy volunteers or patients with CHD to protocols comparing high dose statins to a combination of lower dose statins and ezetimibe and reported greater improvement in endothelial function and vascular inflammation with high dose statins, despite comparable lowering of LDL-C in both groups.[63-66] Other studies did not find differences between groups, suggesting the absence of statin pleiotropy.[67-69] Additional evidence for pleiotropy stems from studies that report effects of statins that were observed before serum LDL-C was lowered. [70] All of these studies were relatively small, were performed in heterogeneous patient populations with different outcome measures and duration of therapy, and showed conflicting results. These studies provide interesting data but do not provide definitive clinical evidence of statin pleiotropy.

The notion of whether statin pleiotropy has clinical relevance in terms of cardiovascular risk reduction may benefit from ongoing trials with the proprotein convertase subtilisin kexin 9 inhibitors (PCSK9i) that lower LDL-C levels by about 60% alone and could be compared with a high dose statin in terms of equivalency in LDL-C reduction.[71,72] High dose statin therapy has demonstrated impressive benefits on plaque reduction that has been postulated to be due to their anti-inflammatory effects in addition to their intensive LDL-C lowering effects.[73,74] The GLAGOV (NCT01813422) results have been recently announced and will

be published later this year and demonstrate that the PCSK9i reduce plaque size on top of optimal statin therapy demonstrating that plaque regression may not be due primarily to pleiotropic effects of statins. The large FOURIER (NCT01764633), ODYSEEY Outcomes (NCT01663402) and SIPRE1,2 (NCT01975376, NCT01975389) are event driven trials that test the effects of PCSK9i on cardiovascular outcomes and are expected to result in the next few years. It would be interesting to see if the outcomes benefits with PCSK9i are equivalent to outcome trials with high dose statins when comparable LDL-C lowering is considered.

PCSK9i lower LDL-C by a mechanism similar to statins because they increase the LDL receptor-mediated hepatic uptake of ApoB-containing lipoproteins. However, they do not inhibit the mevalonate pathway, and would not have similar pleiotropic effects stemming from Rho GTPase inhibition. Despite their potent LDL-C lowering effects, PCSK9i do not reduce serum markers of inflammation such as CRP, interleukins (IL) or tumor necrosis factor alpha (TNFα).[75] These observations do not exclude anti-inflammatory effects on circulating monocytes or on vascular cells. Together with CANTOS (NCT01327846), a study testing the inhibition of the pro-inflammatory cytokine IL-1β and CIRT (NCT01594333) examining methotrexate, the PCSK9i outcome trials will provide important information on the relevance of the reduction of systemic inflammatory markers for clinical outcomes.

DIVERSE TARGET POINTS FOR STATIN ACTIONS

Statins and Isoprenylated Proteins

By inhibiting mevalonic acid synthesis, statins prevent the synthesis of isoprenoid intermediates farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP).[76] FPP and GGPP serve as lipid attachments for the post-translational modification of heterotrimeric G-proteins, including Ras and Rho.[77] Ras and Rho regulate cell proliferation, differentiation, apoptosis, and the cytoskeleton.[78] In endothelial cells (ECs), Ras translocation is dependent on farnesylation, whereas Rho translocation is dependent on geranylgeranylation.[79,80] While the inhibition of isoprenoid intermediate synthesis is central to the possible pleiotropic effects of statins, it is unclear if the primary LDL-C lowering benefit of statins is due to reduced cholesterol production and reduced mevalonic acid production or upregulation of the LDL receptor.[81] There is a relative paucity of human studies regarding the levels of FPP and GGPP with chronic statin therapy.

Statins and Rho/Rho Kinase

Rho kinases (ROCKs) are protein serine/threonine kinases of 160 kDa that contribute to the downstream effects of Rho GTPases.[82] ROCK shifts to an active open conformation when RhoA binds to ROCK (Figure 4).[82] ROCKs regulate actin cytoskeletal changes through effects on myosin light chain phosphorylation. This affects focal adhesion complex formation, smooth muscle contraction, cell migration, and gene expression.[83]

In human aortic ECs, simvastatin prevented tissue factor induction by thrombin in a Rho/ROCK-dependent manner.[84] In animal models, inhibition of ROCK has cardiovascular effects similar to statins, the ROCK inhibitors (fasudil and Y27632), limit cardiac fibrosis,

hypertrophy, and pathologic remodeling in response to angiotensin II (Ang II) and N^G-nitro-L-arginine methyl ester, transverse aortic constriction (TAC), and myocardial infarction (MI).[85-88] Increased leukocyte ROCK activity is observed in patients with hypertension, pulmonary hypertension, metabolic syndrome, dyslipidemia, coronary artery disease (CAD), coronary vasospasm, left ventricular hypertrophy (LVH), and in heart failure with decreased systolic function.[63,89-99] Statins reduce ROCK activity (Table 3).[100-107] Statins have demonstrated inhibition of leukocyte ROCK activity in humans independent of LDL reduction.[108] ROCK inhibition is a candidate for mediating statin pleiotropy because of ROCK's effects on the cardiovascular system, ROCK activity is a biomarker of cardiovascular disease, and ROCK inhibition by statins occurs through cholesterol-independent mechanisms.

Statins and Rac

Rac is a 20-39 kDa monomeric G-protein and a member of the Rho GTPase subfamily.[109] Rac1 modulates phosphorylation of intercellular proteins occludin, vascular endothelial cadherin, and β-catenin, which are critical for tight junction and adherence junction integrity.[110] Rac1 activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and produces reactive oxygen species (ROS) leading to LVH.[111] ROS could also modify LDL to oxidized LDL which is atherogenic and mediates foam cell formation.[112] Elevated Rac1 and NADPH oxidase activity is seen in rats undergoing TAC, vascular smooth cells (SMC) stimulated by Ang II, in saphenous vein grafts and internal mammary artery after coronary artery bypass, in ischemic and non-ischemic cardiomyopathy (NICM), and AF and is attenuated by statins.[54,113-118] The inhibition of Rac1 links statins to reduced ROS and NADPH oxidase activity, and may explain some of the pleiotropic actions of statins.

Statins and the Peroxisome Proliferator-Activated Receptor (PPAR)

Statins have been shown to activate PPARs.[119] Statins acutely decrease lipopolysaccharide (LPS) related inflammation in wild type mice but not in PPARα-null mice, independent of cholesterol lowering mechanisms.[120] Statins increase PPAR-γ activity and inhibit LPS induced TNFα and monocyte chemotactic protein 1 (MCP-1) activity.[119,121] The administration of simvastatin in combination with PPAR-γ agonists elicits additive beneficial vascular effects.[122] Atorvastatin reduces advanced glycation end products in rats and attenuates fibroblast proliferation and cardiac fibrosis, which was reversed with the PPAR-γ antagonist GW9662.[123] Statins reduced ROS production by augmenting the messenger ribonucleic acid (mRNA) expression of the PPAR-γ co-activator, which is an important regulator of mitochondrial biogenesis.[124] However, statins, especially the high intensity statins, increase the risk of diabetes.[125] Thus, the ability of the PPAR-γ agonists, thiazolidinediones, to lower blood sugar is in contrast to the effects of statins on PPAR-γ and demonstrates the complex nature of statin interactions with other pathways, including glucose metabolism.

CELLULAR EFFECTS OF STATINS

Statins and the Endothelium

Endothelial dysfunction is caused by hypercholesterolemia and is characterized by impaired bioavailability of endothelial-derived nitric oxide (NO). Endothelial NO is important for vasodilation, platelet aggregation, vascular smooth muscle proliferation, and endothelial-leukocyte interactions.[126] Statins increase endothelial NO production, in part, by upregulating endothelial NO synthase (eNOS), which may be a pleiotropic effect of statins (Table 4).[79,80] While the increase in eNOS is important, it should be noted that much of the animal studies examining eNOS and other cellular targets used significantly higher doses of statins than are used in clinical practice.

Statins upregulate eNOS through multiple mechanisms. One pathway involves Rho/ROCK signaling. *In vitro* studies show that Rho inhibition increases eNOS expression.[80] Increased ROCK activity downregulates eNOS, and ROCK inhibitors (Y-27632 and fasudil) increase eNOS expression.[127,128] The effects of statins on eNOS expression are not reversed by FPP or LDL-C, indicating that the effect is likely mediated through the geranylgeranylation of RhoA and ROCK signaling [80]

Statins also increase eNOS activity by post-translational activation of the phosphatidylinositol 3-kinase/protein kinase Akt (PI3k/Akt) pathway as eNOS is phosphorylated by Akt.[129] Inhibition of the Rho/ROCK pathway activates the PI3k/Akt pathway and cardioprotection.[130,131] ROCK is a negative regulator of the Akt pathway, possibly through activation of phosphatase and tensin homologue.[131]

Statins also act on caveolin 1 which is an integral membrane protein that binds to eNOS in caveolae, and directly inhibits NO production. [132] Statins decrease caveolin-1 expression in vitro and in mice, thereby promoting eNOS activity.[132]

Statins could also exert pleiotropic effects through the transcription factor kruppel-like factor -2 (KLF2). Statins induce KLF2 mRNA in ECs, which may be required for eNOS expression.[133] Statins reduce T proliferation through KLF2, which may explain some of their immunomodulatory effects.[134]

Statins may exert pleiotropic effects by enhancing the mobilization of EPCs. Impaired EPCs are associated with impaired endothelial function and decreased NO levels.[135] Atorvastatin increases EPCs in patients with CAD within one week. [136] This effect is apparently observed only at low statin concentrations, higher concentrations of statins tend to have angiostatic effects, which may explain why high-intensity statins are able to reduce intraplaque angiogenesis in patients with atherosclerosis.[137,138]

Statins and Vascular Smooth Muscle

The proliferation of vascular SMCs is important in vascular lesion pathogenesis.[139] Transplant arteriosclerosis is an immune response directed against donor ECs and vascular SMCs independent of hypercholesteremia that is still attenuated by statins.[140] Inhibition of isoprenoid synthesis by statins decreased platelet derived growth factor (PDGF)-induced

deoxyribonucleic acid (DNA) synthesis in vascular SMCs by increasing the cyclin-dependent kinase, $p27^{Kip1}$, which was possibly mediated by Rho GTPase.[141] Simvastatin decreases intimal thickening, reduces cellular proliferation, leukocyte accumulation, and PDGF receptor phosophorylation in LDL receptor deficient mice.[142] In vitro, atorvastatin reduces the effects of the pro-inflammatory cytokine IL-18, which inhibits SMC migration, nuclear factor-κB (NF-κB) activation, and matrix metalloproteinase (MMP)-9 expression.[143] In bovine pulmonary artery SMCs, atorvastatin inhibits migration of pulmonary artery SMC, which was reversed by GGPP and mevalonate, again implicating the potential for the Rho/ROCK pathway in SMC proliferation.[144]

Statins and the Myocardium

The GTP-binding proteins, Ras, Rho, and Rac play a critical role in cardiac hypertrophy.[145] Mice without Rac1 showed decreased NADPH oxidase activity and myocardial oxidative stress, confirming that Rac1 is essential for myocardial hypertrophy.[146] Rac1 also increases the activity of the mineralocorticoid receptor.[147] *In vitro,* statins increase small guanosine triphosphate binding protein guanosine diphosphate dissociation stimulator (SmgGDS) and decrease Rac1 in a lipid-independent fashion.[148] Statins decrease Rac1 levels, cardiomyocyte hypertrophy, and fibrosis in wild type mice, but not in mice that lack SmgGDS.[149] Rac1 contributes to doxorubicin-related cardiotoxicity through both a ROS-dependent and independent mechanism.[150] Finally, preoperative atorvastatin induced Rac1-mediated inhibition of NADPH in human atrial myocardium.[151]

The effect of statins on the myocardium is also mediated through RhoA and ROCK, because both lead to increased apoptosis and increased fibrosis, which could lead to the development of LVH and heart failure. Overexpression of RhoA in rat ventricular myocytes induces increased caspase-9 activation, DNA fragmentation, and apoptosis, which are blocked by ROCK inhibitors.[152] Mice with a genetic deletion of ROCK1 had less ischemic reperfusion-related fibrosis compared to wild type mice.[153] Mice without ROCK2 demonstrated less LVH, fibrosis, and apoptosis when exposed to Ang II or TAC compared to wild type mice.[154] In humans, leukocyte ROCK levels are 4.5 times higher in patients with LVH and hypertension compared to those with hypertension without LVH and ROCK activity also is increased with LVH in chronic kidney disease.[95,96] Statins increase NO bioavailability, which increases myocardial blood flow under hypoxic conditions and inhibits IL-6, IL-8, and vascular cell adhesion molecule-1 (VCAM-1).[155-157] *In vitro* studies show that statins reduce mitochondrial dysfunction and cardiomyocyte death.[158]

There is, however, conflicting data about whether statins improve outcomes in NICM. A cohort study demonstrated decreased mortality and a small randomized controlled trial showed improvement in ejection fraction, symptoms, and lower levels of pro-inflammatory cytokines.[159,160] However, both the large randomized GISSI-HF and CORONA trials did not show any benefit for either death or the composite endpoint.[161,162] While there are questions about the efficacy of statins for heart failure, it is possible that statin therapy is beneficial if started earlier in the disease course.

Statins and Platelets

Platelets are essential in the pathogenesis of ACS. Hypercholesteremia is associated with increased platelet reactivity and thrombin generation, which is decreased with pravastatin and is likely both cholesterol associated and LDL-C independent.[163] Mice treated with atorvastatin showed increased eNOS and down regulated platelet factor 4 and beta thromboglobulin in platelets, effects which are absent in eNOS knockout mice.[164] Fluvastatin acts through PPARα and PPAR-γ to reduce platelet aggregation in response to arachidonic acid and decreased platelet aggregation compared to colestimide.[165,166] Atorvastatin acutely inhibited platelet recruitment, decreased Nox2, Rac1, protein kinase C, platelet phospholipase A2 and thromboxane A2 while increasing NO levels.[167] Finally, the anti-thrombotic effect was also shown in the JUPITER trial because treatment with rosuvastatin was associated with decreased thromboembolism, an effect that is likely to be unrelated to cholesterol reduction since hypercholesterolemia is not a particularly strong risk factor for venous thromboembolism. [31]

DIRECT NON-LDL EFFECTS ON STATINS IN CARDIOVASCULAR DISEASE

Statins and Atherosclerosis

Atherosclerosis is a chronic inflammatory process of the vascular wall that is initiated by excessive LDL-C and is mediated by activated macrophages, T lymphocytes, B lymphocytes, and SMCs.[2] Statins are anti-inflammatory and reduce inflammatory cytokines and adhesion molecules, and acting on both the innate and adaptive immune responses.[168] Statins reduce Rac1-mediated ROS species production and reduce the oxidation sensitive inflammatory pathways.[169] Statins decrease inflammatory cytokines such as IL-6, IL-8, and MCP-1.[170] *In vitro* statins inhibited IL-6 induced monocyte chemotaxis and MCP-1 expression and inhibited Janus kinase and the signal transducers and activators of transmission pathway, an effect that was reversed by GGPP.[171] Statins reduce MMP-1, MMP-3 and MMP-9 from both SMCs and macrophages in a rabbit model, which was reduced by both GGPP and mevalonate.[172]

In the adaptive immune system, statins have effects on T-cell differentiation. Simvastatin reduced the differentiation of the proinflammatory IL-17 helper T cells (Th17) and enhanced the production of forkhead box P3 $(Foxp3)^+$ $Cd4^+$ regulatory T cells (Tregs) in a geranylgeranylation dependent manner. [173] Transforming growth factor beta (TGF-β) induces $Foxp3^+$ $Cd4^+$ Tregs and simvastatin acts through geranylgeranylation to inhibit the TGF-β inhibitors Smad6 and Smad7 and therefore increase TGF-β and $Foxp3^+$ $Cd4^+$ Treg expression.[174] $Cd4^+$ T lymphocytes from patients with ACS induced EC apoptosis through an upregulated TNF-related apoptosis inducing ligand (TRAIL) receptor DR5 on ECs and increased TRAIL expression on T lymphocytes, an effect that was blocked by statins and may provide a pathway for improved plaque stability by statins.[175]

Statins decrease the leukocyte and EC interaction that occurs in atherogenesis. ICAM-1 and VCAM-1 regulate the migration of leukocytes to ECs and platelet and endothelial cell adhesion molecute-1 (PECAM-1) is involved in leukocytes crossing ECs.[176,177] Statins inhibit VCAM-1 through PPARα and increased NO production.[157,178] RhoA inhibition

inhibits the clustering of VCAM-1 and ICAM-1 and decreases monocyte adhesion to ECs.[179] Lovastatin regulates PECAM-1 expression, which was reversed by GGPP and mevalonate, suggesting a role of Rho in regulating leukocyte migration.[180]

Statins and Stroke

While elevated cholesterol and LDL-C are risk factors for ischemic strokes in many epidemiological studies, it has not been established in every study and the link remains more controversial then the link for CAD.[181] Nonetheless statins reduce the risk of stroke by 25% in both the Heart Protection Study and the Treating to New Targets study and 48% in the JUPITER trial. [10,182,183] The SPARCL trial demonstrated that atorvastatin is effective for stroke secondary prevention.[184] While there has been debate if non-statin cholesterol medications effect stroke incidence the IMPROVE-IT trial showed the ezetimibe in addition to simvastatin had a 21% reduction in ischemic stroke.[56]

A potential pleiotropic target for statins in stroke is the effect of statins on eNOS, given that mice without eNOS demonstrate larger infarcts.[155] The effects of statins are likely mediated by Rho/ROCK because ROCK inhibitors upregulate eNOS and improve cerebral blood flow in mice.[128]

Conclusion

Given the cell culture and the animal studies as well as indirect evidence from clinical trials, it remains important to assess whether the non-LDL-C lowering effects of statins could be replicated by other cholesterol lowering therapies or by agents that act downstream of isoprenoid synthesis, e.g., squalene synthase inhibitors. Unfortunately, all of the current novel hyperlipidemia treatments are tested in patients receiving statins, which will only provide information regarding how much further to lower serum LDL-C, but does not exclude or include the potential pleiotropic effects of statins. The concept of statin pleiotropy has provided a window of opportunity to test and target other non-lipid-lowering signaling pathways that may affect cardiovascular disease. Agents that target inflammation alone, such as anti-IL-1β therapy (canakinumab) and methotrexate, are currently being tested in secondary prevention trials as adjunctive therapy to lipid lowering.[185,186] Furthermore, the ROCK inhibitors, fasudil and ripasudil, which are currently approved in Japan for the treatment of cerebral vasospasm after subarachnoid hemorrhage and to treat glaucoma, respectively, may be of interest as novel therapies for reducing cardiovascular diseases. Finally, the PCSK9i may help provide evidence for statin pleiotropy, especially when low-dose PCSK9i is compared with high-potency statins that are matched for equivalent LDL-C lowering. This design would provide the opportunity to definitively test the clinical relevance of statin pleiotropy on cardiovascular outcomes.

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Non-standard abbreviations and Acronyms

ApoB	Apolipoprotein B
LDL-C	low-density lipoprotein cholesterol
HMG-CoA	hydroxy-methylglutaryl coenzyme A
CHD	coronary heart disease
OATP	organic anion transporting polypeptide
LFA-1	leukocyte function-associated antigen-1
ICAM-1	intercellular adhesion molecule-1
AF	atrial fibrillation
CRP	C-reactive protein
ACS	acute coronary syndrome
CTT	Cholesterol Treatment Trialists
EPCs	endothelial progenitor cells
PCSK9i	proprotein convertase subtilisin kexin 9 inhibitors
IL	interleukins
TNFα	tumor necrosis factor alpha
EC	endothelial cells
FPP	farnesylpyrophosphate
ROCK	Rho kinase
Ang II	angiotensin II
TAC	transverse aortic constriction
MI	myocardial infarction

CAD	coronary artery disease
LVH	left ventricular hypertrophy
NADPH	nicotinamide adenine dinucleotide phosphate
ROS	reactive oxygen species
SMC	smooth muscle cells
NICM	non-ischemic cardiomyopathies
PPAR	peroxisome proliferator-activated receptor
LPSM	lipopolysaccharide
MCP-1	monocyte chemotactic protein-1
mRNA	messenger ribonucleic acid
NO	nitric oxide
eNOS	endothelial nitric oxide synthase
PI3K	phosphatidylinositol 3-kinase
KLF2	kruppel-like factor -2
PDGF	platelet derived growth factor
DNA	deoxyribonucleic acid
NFκB	nuclear factor κB
MMP	matrix metalloproteinase
SmgGDS	small GTP binding protein GDP dissociation stimulator
VCAM-1	vascular cell adhesion molecule-1
Tregs	regulatory T cells
CD	cluster of differentiation
Foxp3M	forkhead box P3
Th17	IL-17 helper T cells
TGF-β	Transforming growth factor beta
TRAIL	TNF related apoptosis inducing ligand
PECAM – 1	platelet and endothelial cell adhesion molecule-1

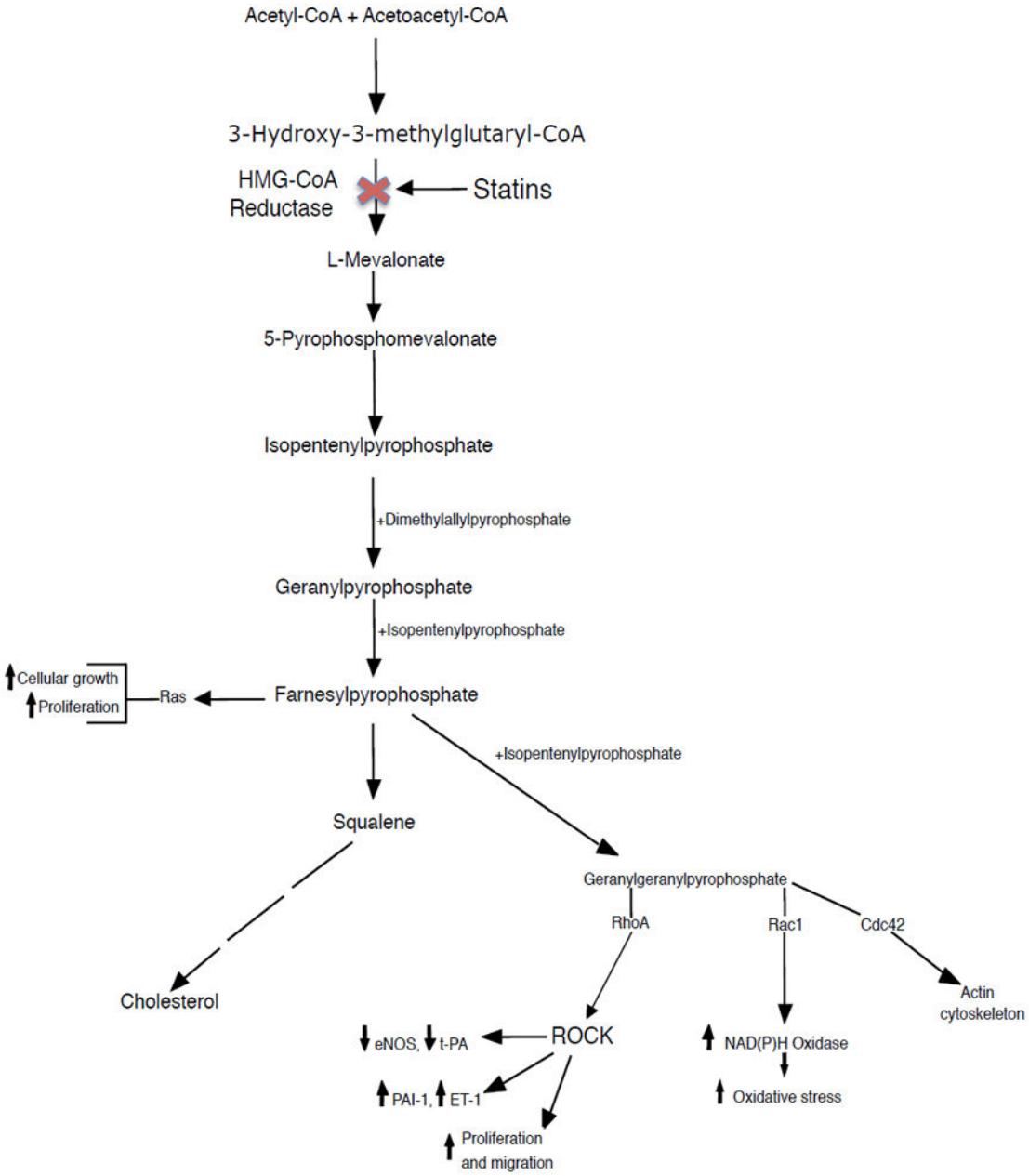

Figure 1.

Cholesterol and isoprenoid synthesis pathway which shows the inhibition of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase by statins. Decrease in isoprenylation of signaling molecules, such as Ras, Rho, and Rac, leads to the modulation of various signaling pathways. ROCK – rho associate protein kinase, NAD(P)H – nicotinamide adenine dinucleotide phosphate, eNOS – endothelial nitric oxide synthase, t-Pa – tissue-type plasminogen activator, ET-1 – endothelin 1, PAI-1 – plasminogen activator inhibitor 1.

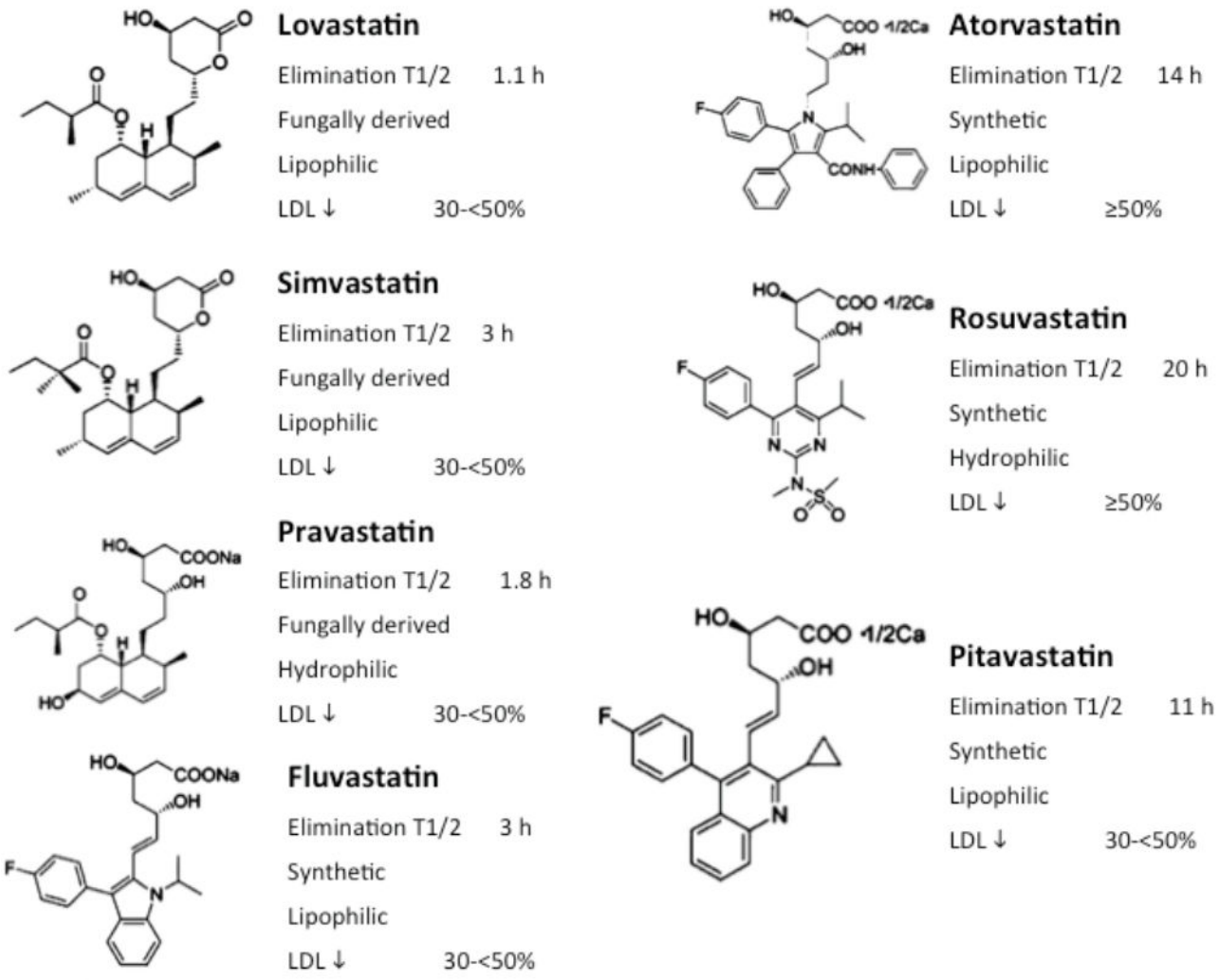

Figure 2.

The structure and pharmacokinetic properties of the commercially available statins.[32] LDL – low density lipoprotein, T1/2 – half life, h - hours

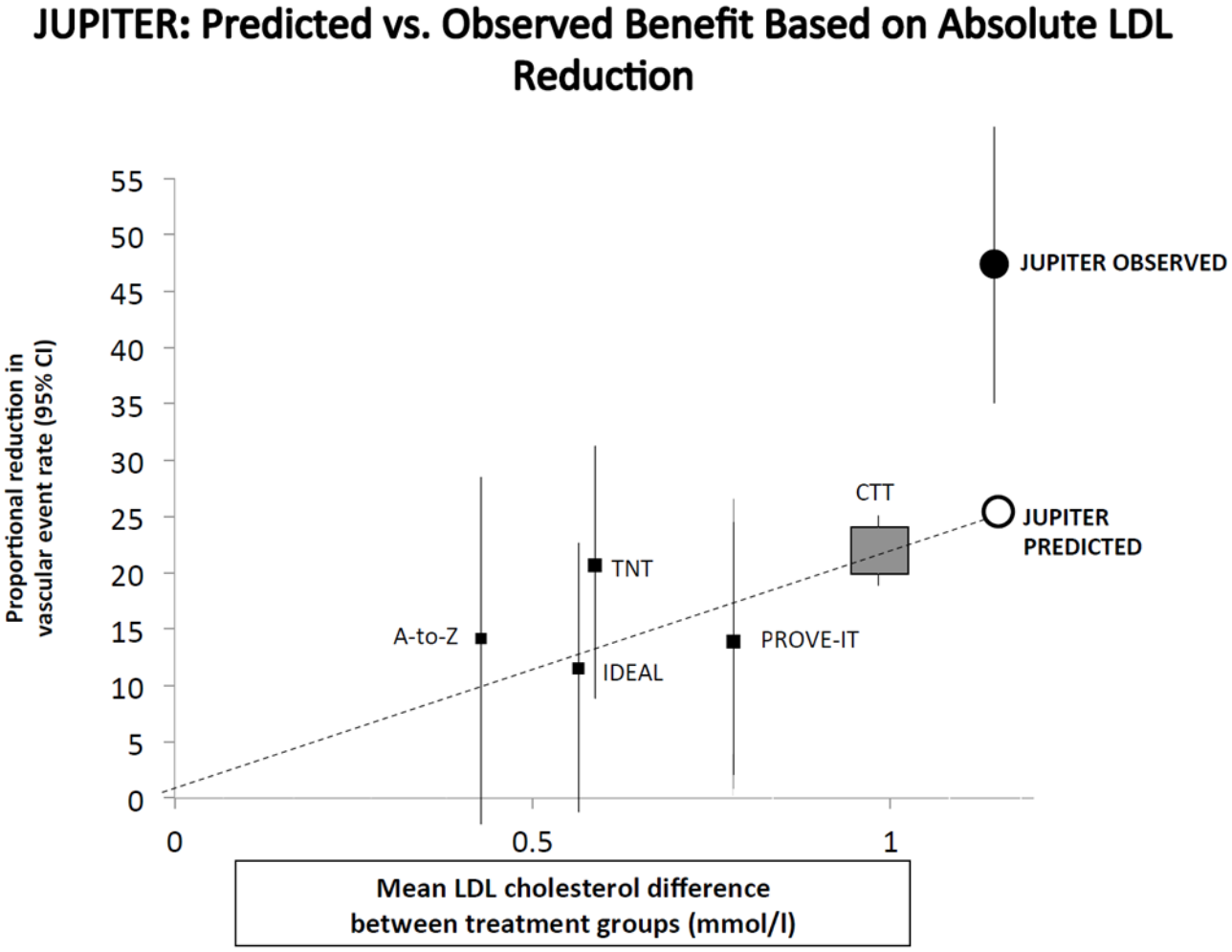

Figure 3.

The predicted reduction in vascular event rate from the JUPITER trial based on its low density lipoprotein (LDL) cholesterol lowering. The gray square represents the average effect of statins versus placebo based on the Cholesterol Treatment Trialists' collaboration regression line. The individual black squares represent individual trials, the open circle represents the predicted effect of atorvastatin in the Jupiter trial, the black circle represents the observed effect.[12]

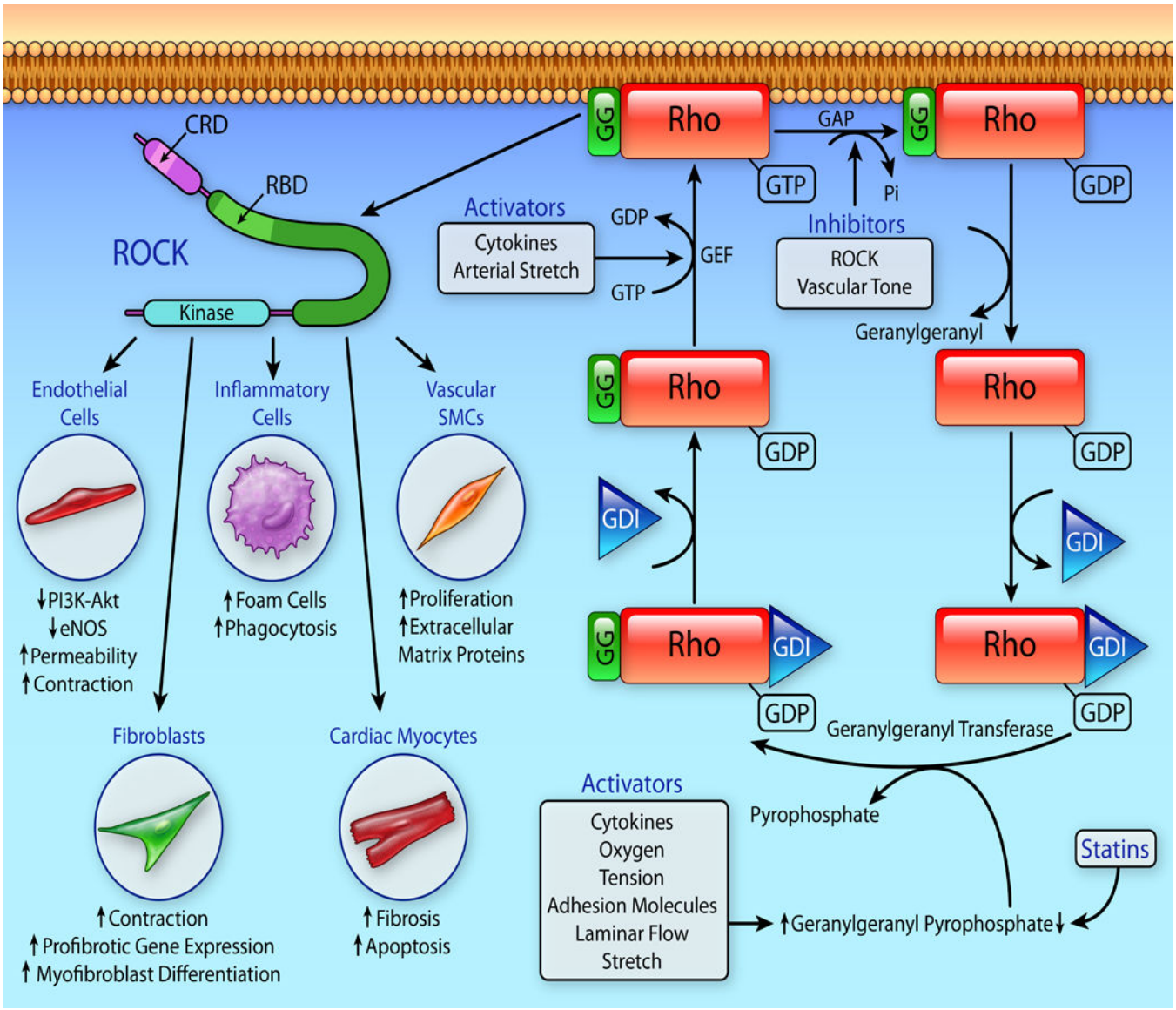

Figure 4.

Regulation of the Rho GTPase cycle. Rho cycles between an inactive, cytoplasmic, guanosine diphosphate (GDP) bound form and after geranylgeranylation is translocated to the plasma membrane and activated when it is bound to guanosine triphosphate (GTP). Inhibition of mevalonate synthesis by statins decreases geranylgeranyl pyrophosphate and prevents the geranylgeranylation of Rho and therefore its activation of Rho kinase (ROCK). ROCK mediates the downstream effects of Rho and has effect on endothelial cells, inflammatory cells, fibroblasts, cardiomyocytes, and vascular smooth muscle cells (SMC) that promote atherosclerosis and cardiac remodeling and may be responsible for the pleiotropic effects of statins. GG – geranylgeranyl, GDI – guanine nucleotide dissociation inhibitors, GEF – guanine nucleotide exchange factors, GAP – GTPase – activating proteins, PI3K - phosphatidylinositol 3-kinase, eNOS – endothelial nitric oxide synthase, CRD – Cysteine rich domain, RBD – Rho-binding domain

Table 1

The effect of statins on LDL-C independent diseases

Kidney disease	↓ Creatinine with normal and abnormal renal function[19,20]
Pneumonia	↓ Incidence[22]
	↓ Mortality[21]
Venous thromboembolism	↓ Incidence[31]
Multiple Sclerosis	↓ Whole brain atrophy[23]
	↓ Disability[23]
Bone strength	↓ Hip fracture in postmenopausal women[24]
Gastrointestinal	↓ Cholecystectomy for gallstones[25]
	↓ Pancreatitis with normal triglycerides[26]
Erectile dysfunction	↑ Function in sildenafil nonresponders[27]
Periodontal disease	↓ Periodontal inflammation[28]
Rheumatoid arthritis	↓ Mortality[29]
	↓ Inflammatory markers and improved disease activity score[30]

Table 2

Time to benefit for LDL-C lowering strategies

Non-statins	**Control**	**Trial**	**Time to benefit**
Cholestyramine	placebo	LRC-CPPT[57]	7.4 years
partial ileal bypass surgery	no surgery	POSCH[58]	9.7 years
Ezetimibe with simvastatin 40 mg	placebo with simvastatin 40 mg	IMPROVE-IT[56]	7.0 years
Statins			
Rosuvastatin	placebo	JUPITER[12]	1.9 years
Pravastatin	placebo	WOSCOPS[8]	5.0 years
	placebo	CARE[6]	5.0 years
	placebo	LIPID[17]	6.1 years
Atorvastatin	placebo	SPARCL[184]	4.9 years
	placebo	ASCOT-LLA[11]	3.3 years
Lovastatin	placebo	AFCAPS/Tex-CAPS[9]	5.2 years
Simvastatin	placebo	HPS[10]	2.0 years
	placebo	4S[5]	5.4 years
Fluvastatin	placebo	LIPS[16]	3.9 years

Table 3

Studies Showing Statin Inhibition of Rho Kinase

Author	Year	Sample	Effect
In vitro and animal studies			
Eto et al[84]	2002	Human endothelial cells	↓ tissue factor induction
McNeish et al[106]	2013	Rat middle cerebral arteries	↓ thromboxane receptor stimulation
Massaro et al[187]	2010	Human endothelial cells	↓ COX2 and MMP-9 expression
Li et al[144]	2007	Bovine pulmonary artery SMCs	↓ SMC mitogensis and migration
Ma et al[107]	2012	Rats with hypertension	↓ ROCK activity
Ohnaka et al[100]	2001	Human osteoblasts	↓ ROCK activity and BMP-2 expression
Tramontano et al[101]	2004	Human endothelial cells	↓ endothelial micorparticle levels
Gojo et al[102]	2007	Rats with diabetes	↓ urinary albumin and 8- hydroxydeoxyguanosine excretion
Yamanouchi et al[103]	2005	Rabbits with normal cholesterol and human endothelial cells	↓ ROCK activity and carotid intimal hyperplasia
Kozai et al[104]	2005	Human saphenous vein SMCs	↓ ROCK activity and carotid intimal hyperplasia
Trebicka et al[105]	2007	Rats with cirrhosis	↓ ROCK activity and ↑ eNOS
Human studies			
Rawlings et al[108]	2009	Humans with stable CAD	↓ ROCK activity
Liu et al[63]	2009	Humans with hyperlipidemia	↓ ROCK activity
Nohria et al[94]	2009	Humans with stable CAD	↓ ROCK activity

COX2 – cyclooxygenase 2;MMP - matrix metalloproteinase; SMC – smooth muscle cell; ROCK – rho kinase; BMP-2 – bone morphogenetic protein 2; eNOS – endothelial nitric oxide synthase; CAD – coronary artery disease

Table 4

The Pleiotropic Effects of Statins by Cell Type

Endothelial cells	↑ eNOS expression and activity[79,80]
	↓ Plasminogen activator inhibitor-1 expression, and ↑ Tissue-type plasminogen activator expression [188]
	↓ Endothelin-1 synthesis and expression[182]
	↓ ROS[113]
	↑ Peroxisome proliferator-activated receptor-α and γ expression[119-121]
	↓ Proinflammatory cytokines (IL-1β, IL- 6, cyclooxygenase-2) expression[157,187]
	↓ CD40 expression[189]
Vascular Smooth Muscle Cells	↓ Migration and proliferation[142,144]
	↓ ROS[113,115,116]
	↓ NADPH oxidase activity[111,115,116]
	↓ AT_1 receptor expression[113]
	↓ Platelet derived growth fator[141]
Myocardium	↓ NADPH oxidase activity[114,151]
	↓ ROS[124]
	↓ Left ventricular fibrosis and hypertrophy[114,149]
	↑ Nitric oxide[155,156]
	↓ Apoptosis[152]
Platelets	↓ Platelet reactivity[163,166]
	↓ Thromboxane A_2 biosynthesis[167]
Monocyte/Macrophages	↓ Macrophage growth[171]
	↓ MMP expression and secretion[143,172]
	↓ Tissue factor expression and activity[84]
	↓ Proinflammatory cytokines (IL-1β, IL- 6, IL-8, TNFα) expression[120,170]
	↓ Monocyte chemoattractant protein-1 secretion[170,171,189]
Vascular Inflammation	↓ CRP level[12,52]
	↓ Leukocyte-endothelial cell adhesion[41,42,168,176,177]
	↓ T cell activation[134,168,173,174]
	↓ Nuclear factor-κB activation[143,169]
Endothelial Progenitor Cells	↑ Mobilization of stem cells[136]

IL indicates interluekin; CD – cluster of differentiation; AT_1 – angiotensin type 1; TNF – tumor necrosis factor; NADPH – nicotamide adenine dinucleotide phosphate; ROS – reactive oxygen species; MMP - matrix metalloproteinase; eNOS – endothelial nitric oxide synthase