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Does it Matter Whether or Not a Lipid-Lowering Agent Inhibits Rho Kinase?

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Abstract

Lipid lowering agents, such as HMG CoA reductase inhibitors or statins, have been shown to reduce cardiovascular events. However, growing evidence from recent clinical trials suggest that some of the beneficial effects of statins may be unrelated to changes in LDL-C. In animal studies, many of the cholesterol-independent or “pleiotropic” effects of statins are mediated by inhibition of Rho kinase (ROCK). Indeed, ROCK has been implicated in the regulation of vascular tone, proliferation, inflammation, and oxidative stress. To what extent ROCK activity is inhibited in patients on lipid lowering therapy, in particular, statins, is not known, but may have important clinical and therapeutic implications. This review will attempt to make the case that, in addition to lipid lowering, inhibition of ROCK contributes to some of the benefits of statin therapy in patients with cardiovascular disease.

Introduction

Because serum cholesterol level is strongly associated with coronary heart disease, it has been generally assumed that cholesterol reduction by statins is the predominant, if not the only mechanism, underlying their beneficial effects. Data from a meta-analysis of lipid lowering trials suggest lipid modification alone accounts for the clinical benefits associated with statin therapy. Indeed, the slope of the relationship between cholesterol reduction and mortality risk reduction was the same for statins and non-statins, while the mortality risk reductions realized over statin treatment periods of 2 years and longer were found to be a consequence of cholesterol reduction alone. However, this type of meta-analysis does not take into account the differences in terms of the length of the individual trials with respect to cardiovascular benefits. Some of the non-statin lipid lowering trials such as the Lipid Research Clinic-Coronary Primary Prevention Trial (LRC-CPPT) using the bile acid resin, cholestyramine [1], or the Program on the Surgical Control of the Hyperlipidemias (POSCH) using partial ileal bypass surgery [2], reported benefits after 7.4 and 9.7 years, respectively; whereas most of the statin trials showed benefits at much earlier time points (e.g., within 5 years). Thus, if one compares the benefits after 5 years for all lipid-lowering trials, one finds that the non-statin trials no longer fall on the same slope of cholesterol:mortality risk reduction as do all of the statin trials. In fact, the benefits of cholesterol lowering after ileal bypass surgery in the POSCH study was not realized at 4.5 years, despite significant LDL-C reduction of 34% within the first 3 months after the surgical procedure. These results suggest that the beneficial effects of statins occur more rapidly and may not be entirely dependent on cholesterol reduction.

Rationale for Statin Pleiotropy in Clinical Trials

In the recent HPS and ASCOT trial, the relative risk reduction conferred by statin treatment was independent of the pre-treatment lipid levels [3,4]. These large prospective trials raise questions, whether individuals with CHD could benefit from statin drugs independently of

cholesterol levels. In agreement with these observations, subgroup analyses of previous clinical trials suggested that the beneficial effects of statins could extend to mechanisms beyond cholesterol reduction. For example, subgroup analysis of the West of Scotland Coronary Prevention (WOSCOP) and Cholesterol And Recurrent Events (CARE) studies indicate that despite comparable serum cholesterol levels among the statin-treated and placebo groups, statin-treated individuals have significantly lower risks for coronary heart disease compared to age-matched placebo-controlled individuals [5,6]. Indeed, when the statin treatment group was divided into quintiles of percentage LDL-C reduction it was found that there was no difference in the 4.4-year coronary event rate for quintiles 2 through to 5 (LDL-C reductions of 23–41%). Hence, there was no apparent association between coronary event rate and the level of LDL-C reduction. Furthermore, meta-analyses of cholesterol lowering 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 [7]. For example, application of the Framingham risk score to WOSCOPS produced a coincidence between predicted and observed risk in the placebo group, but underestimated the benefit of the pravastatin group by 31% [8].

Rationale for No Statin Pleiotropy in Clinical Trials

Despite the rapidity of benefits of statin therapy compared to other non-statin lipid-lowering therapies, it is still difficult to prove that pleiotropic effects of statins are real. First, patients receiving statin therapy invariably will have reduced lipid levels and it is often difficult to separate the lipid from the non-lipid lowering effects of statins in clinical trials. Second, many effects of statins such as improvement in endothelial function, decrease inflammation, increase plaque stability, and reduced thrombogenic response could all be accounted for, to some extent, by lipid lowering. Third, the concentrations used to demonstrate the biological effects of statins in cell culture and animal experiments, especially with regards to inhibition of Rho geranylgeranylation, but not PI3-kinase/Akt activation, appear to be much higher than what is prescribed clinically. Finally, both hydrophilic and lipophilic statins, which inhibit hepatic HMG-CoA reductase, appear to exert similar cholesterol-independent effects, despite the relative impermeability of hydrophilic statins in vascular tissues. Thus, it appears that lipid lowering contributes to many of the clinical benefits of statin therapy.

Finally, the lipophilic statins would be expected to penetrate cell membranes more effectively than the more hydrophilic statins, causing more side effects, but at the same time, eliciting more pleiotropic effects. 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 [9]. 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” probably does not reflect the hepatic versus non-hepatic effects of these agents.

Statins and Isoprenylated Proteins

By inhibiting L-mevalonic acid synthesis, statins also prevent the synthesis of other important isoprenoid intermediates of the cholesterol biosynthetic pathway, such as farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP) [10]. These intermediates serve as important lipid attachments for the post-translational modification of variety of proteins, including the β 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 [11]. Thus, protein isoprenylation permits the covalent attachment, subcellular localization, and intracellular trafficking of membrane-associated proteins. Members of the Ras and Rho GTPase family are major substrates for post-translational modification by prenylation [11,12]. By virtue of inhibiting isoprenoid synthesis, statins inhibit both Ras and Rho function in hepatic and peripheral tissues.

Because Rho is major target of geranylgeranylation, inhibition of Rho and its downstream target, Rho kinase (ROCK), is a likely mechanism mediating some of the pleiotropic effects of statins on the vascular wall [13,14]. Each member of the Rho family serves specific functions in terms of cell shape, motility, secretion, and proliferation, although overlapping functions between the members could be observed in overexpressed systems. The activation of Rho in Swiss 3T3 fibroblasts by extracellular ligands, such as platelet-derived lysophosphatidic acid, leads to myosin light chain phosphorylation and formation of focal adhesion complexes [11, 12,15]. Indeed, ROCK increases the sensitivity of vascular smooth muscle to calcium in hypertension [16] and coronary spasm [17]. In contrast, activation of Rac leads to the formation of lamellipodia, membrane ruffles, and oxidative stress, while activation of Cdc42 induces actin-rich surface protrusions called filopodia.

Role of ROCK in Cardiovascular Disease

There is growing evidence that abnormal ROCK function contributes to cardiovascular disease [18]. In the vascular wall, ROCK mediates vascular smooth muscle contraction, actin cytoskeleton organization, cell adhesion and motility [19]. Thus, abnormal ROCK activity may contribute to abnormal smooth muscle contraction observed in cerebral and coronary vasospasm [20,21], hypertension [22], and pulmonary hypertension [23]. In addition, ROCK could also regulate vascular tone and blood flow indirectly through negative effects on eNOS expression and activity [13,24] or via direct effects on the central nervous system [25,26]. Inhibition of ROCK leads to increase in cerebral blood flow and decrease in cerebral infarct size via upregulation of eNOS [27]. ROCK is also involved in vascular inflammation and remodeling [28], restenosis after balloon injury [29–31], ischemia-reperfusion injury [24,32, 33] and atherosclerosis [34,35]. Recent studies also suggest that long-term treatment with a ROCK inhibitor, fasudil, improved monocrotaline-induced fatal pulmonary hypertension in rats [23] and suppresses cardiac allograft vasculopathy in mice [36]. ROCK has also been implicated in the expression of a variety of genes, which are pertinent to vascular function, such as monocyte chemoattractant protein-1 (MCP-1) [37], plasminogen activator inhibitor-1 (PAI-1) [38], and osteopontin [39]. Indeed, ROCK is upregulated by inflammatory stimuli, such as angiotensin II and interleukin-1 β , in cultured cells [40], and by lipopolysaccharide (LPS) *in vivo* [41].

Recently, we measured leukocyte ROCK activity in Asian subjects with metabolic syndrome [42]. Compared to age- and sex-matched control subjects, ROCK activity was found to be higher in subjects with metabolic syndrome with a cutoff value for ROCK activity of 0.39 predicted the presence of metabolic syndrome with specificity and sensitivity rates of 70%. Each component of the metabolic syndrome such as body mass index, waist circumference, fasting glucose, and triglyceride levels, as well as the nonspecific inflammatory marker, high sensitivity-C-reactive protein (hs-CRP) were associated with elevated levels of ROCK activity. Indeed, the risk of elevated ROCK activity increased with the number of metabolic syndrome components, suggesting that leukocyte ROCK activity may be a novel serological marker of metabolic syndrome.

Rationale for the Development of ROCK Inhibitors

Because ROCK is involved in various aspects of vascular function and inflammatory conditions, the development of selective ROCK inhibitors has gained considerable interest in the pharmaceutical industry. Presently, Y-27632 and fasudil are non-isoform-selective ROCK inhibitors, which target their ATP-dependent kinase domains, and therefore, are equipotent in terms of inhibiting both ROCK1 and ROCK2. Neither fasudil nor Y27632 can distinguish between ROCK1 and ROCK2. Furthermore, at higher concentrations, these ROCK inhibitors could also inhibit other serine-threonine kinases such as PKA and PKC [27]. Nevertheless, compared to the other kinases, fasudil and its active metabolite, hydroxyfasudil, are relatively more selective for ROCKs, with hydroxyfasudil being slightly more selective than fasudil and Y27632 [27].

ROCK inhibitors such as fasudil have been shown to prevent cerebral vasospasm after subarachnoid hemorrhage [17,20]. Similarly, animal studies with Y-27632 showed that it could inhibit the development of atherosclerosis and arterial remodeling following vascular injury [29,34]. ROCK activity is involved in the expression of PAI-1 mediated by hyperglycemia, indicating that ROCK may function as a key regulator of cardiovascular injury in patients with diabetes mellitus [43]. Furthermore, ROCK has been reported to be involved in cerebral ischemia [44], erectile dysfunction [45], glomerulosclerosis [46], hypertension [16], myocardial hypertrophy [47], myocardial ischemia-reperfusion injury [24,33], neointima formation [29,48], pulmonary hypertension [23], and vascular remodeling [35].

Clinical Trials with ROCK Inhibitors

In a recent study using the ROCK inhibitor, fasudil, we showed that age and risk factor such as smoking, induces oxidative stress and aortic stiffness by increasing ROCK activity [49]. These findings suggest that ROCK activation may decrease the bioavailability of nitric oxide (NO) and promote inflammation, processes characteristic of atherosclerosis. Accordingly, we investigated whether inhibition of ROCK improves endothelial function and decreases markers of inflammation in human subjects with coronary artery disease (CAD) [50]. Endothelial function was assessed by flow-mediated, endothelium-dependent vasodilation using brachial artery ultrasonography. ROCK activity was determined in peripheral blood leukocytes. The change in endothelium-dependent vasodilation with fasudil was significantly greater than the change with placebo in CAD subjects, and was inversely proportional to ROCK inhibition. Fasudil, however, did not decrease markers of inflammation such as hs-CRP in either group. These findings suggest that ROCK may promote vascular dysfunction by decreasing NO bioavailability in humans with atherosclerosis.

Conclusion

There is growing evidence that ROCK plays an important pathophysiological role in cardiovascular diseases and that inhibition of ROCK may mediate many of the pleiotropic effects of statins. To date, a great number of cellular and physiological functions are mediated by ROCK, and ROCK activity is often elevated in disorders of the cardiovascular system. Thus, it may be important to know whether a lipid lowering therapy also inhibits ROCK activity. Indeed, we recently found that patients treated with equal lipid lowering doses of atorvastatin (40 mg) and rosuvastatin (10 mg), have decreased leukocyte ROCK activity and that the change in ROCK activity did not correlate with LDL lowering (unpublished data). This is the first study to demonstrate a reduction in ROCK activity with statins, suggesting that inhibition of ROCK activity may have important therapeutic implications for the benefits of statin therapy.

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Figure 1.

Figure 2.

Figure 3.