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Comparison of Effects of Rosuvastatin (10 mg) versus Atorvastatin (40 mg) on Rho Kinase (ROCK) Activity in Men with a Previous Atherosclerotic Event

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Abstract

In addition to inhibiting cholesterol biosynthesis, statins also inhibit the formation of isoprenoid intermediates, which are required for the activation of the Rho/Rho kinase (ROCK) pathway. Increased ROCK activity has been implicated in causing endothelial dysfunction and atherosclerosis. However, it is not known whether statins, at doses used to lower cholesterol levels, inhibit ROCK activity in humans with atherosclerosis. Furthermore, it is not known whether lipophilic and hydrophilic statins differ in their ability to inhibit ROCK activity. Accordingly, we enrolled 30 male subjects with stable atherosclerosis (low-density lipoprotein (LDL) ≥ 100 mg/dL) in a randomized, double-blind study comparing equivalent LDL-lowering doses of a hydrophilic statin (rosuvastatin 10 mg daily) to a lipophilic statin (atorvastatin 40 mg daily) for 28 days. We assessed the change in lipids, ROCK activity, and flow-mediated dilation of the brachial artery (FMD) before, and after statin therapy. Both treatment groups exhibited comparable 30–32% and 42–45% reductions in total and LDL cholesterol, respectively. Only atorvastatin reduced triglycerides and neither statin altered high-density lipoprotein cholesterol. While both statins inhibited ROCK activity ($p<0.0001$), the extent of inhibition was greater with rosuvastatin (18±2% vs. 8±2%, $p=0.0006$). Statins also improved FMD from 7.4±0.6 to 9.3±0.4 ($p=0.003$) with rosuvastatin being slightly better than atorvastatin. The inhibition of ROCK activity by statins did not correlate with reductions in LDL ($p=0.57$), but was associated with improvement in FMD. These findings provide direct clinical evidence that statins, at clinically relevant doses, could differentially inhibit ROCK activity and improve endothelial function by cholesterol-independent mechanism.

Keywords

atherosclerosis; hypercholesterolemia; endothelial function; statins; Rho kinase

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DISCLOSURES

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Rho is a member of a large family of small GTPases, and together with its downstream target, ROCK, regulate organization of cytoskeletal proteins and other vital cellular functions.[1] Abnormal activation of ROCK has been observed in animal models of vascular injury.[2] In addition to inhibiting cholesterol synthesis, statins prevent the formation of isoprenoid intermediates such as geranylgeranyl pyrophosphate.[3] These isoprenoid intermediates are lipid attachments that are required for the membrane translocation and GTP-binding activity of Rho. Indeed, the inhibition of Rho/ROCK pathway has been implicated as a potential mechanism for some of the beneficial effects of statin therapy. Direct inhibition of Rho/ROCK pathway in cell culture or *in vivo* increases endothelial nitric oxide synthesis,[4] decreases vascular smooth muscle cell contraction and proliferation,[2,5,6] decreases cytokine formation and inflammatory cell trafficking and proliferation,[7–9] and reduces thrombogenicity of the vessel wall.[10–12] Statins have been shown to recapitulate these vascular benefits *in vitro* via inhibition of Rho.[4,9,13–16] However, it is not yet known whether statins, at doses given for lipid lowering, mediate these vascular benefits in humans via inhibition of the Rho/ROCK pathway. The primary aim of this study was to test the hypothesis that statins inhibit ROCK activity in humans and this reduction coincides with improvements in vascular dysfunction that are characteristic of atherosclerosis. The secondary aim of this study was to test whether there are any differences between these statins in their ability to inhibit ROCK activity despite differential cellular uptake.[17]

METHODS

The Human Research Committee at Brigham and Women's Hospital approved this study. The study included 30 male subjects with stable atherosclerosis and fasting LDL cholesterol levels ≥ 100 mg/dL off statin therapy. Subjects were recruited from the cardiology clinics at Brigham and Women's and Faulkner Hospitals. The presence of atherosclerosis was determined by ≥ 50% stenosis in at least one coronary artery at cardiac catheterization, by history of prior myocardial infarction, prior angioplasty, prior coronary artery bypass graft surgery, prior ischemic stroke, or documented peripheral arterial disease. Exclusion criteria included unstable angina or revascularization within 3 months of study enrollment, malignancy, chronic inflammatory disease, acute infection, history of myositis/myopathy, liver transaminases > 2X upper limit of normal, creatine phosphokinase > upper limit of normal, and reluctance to discontinue statin therapy.

This study had a randomized, double-blinded, parallel design. After an initial screening visit, subjects who signed informed consent and met the inclusion/exclusion criteria were asked to discontinue statin therapy for a minimum of two weeks. This period is sufficient to restore endothelial function and lipid profiles to pre-statin treatment levels.[18] Subjects were then randomized to receive one month of atorvastatin (40 mg) or rosuvastatin (10 mg) daily. Atorvastatin (lipophilic) was chosen based on the results of the CURVES trial, which showed that atorvastatin at 8 weeks lowered LDL cholesterol significantly more than simvastatin, pravastatin, lovastatin and fluvastatin ($p<0.01$), with similar tolerability and safety profile.[19] Rosuvastatin (hydrophilic) was chosen because it has proven to be the most effective of the hydrophilic statins in cholesterol lowering.[20] The 10mg dose of rosuvastatin was chosen because it has been shown to be equivalent to the 40mg dose of atorvastatin in LDL lowering ability.[20] Blood for fasting lipid profiles, ROCK activity, and safety assessment was collected before and one month after statin treatment. Brachial artery ultrasonography was also performed to evaluate endothelial function before and one month after statin treatment.

Fasting serum lipids (total and high density lipoprotein (HDL) cholesterol and triglycerides) were measured with an Olympus AU 400 autoanalyzer using enzymatic methods. LDL cholesterol was calculated according to the methods of Friedewald et al.[21] ROCK activity was assayed in peripheral blood leukocytes as the proportion of phospho-Thr^{853} in the myosin

binding subunit (MBS) of myosin light chain phosphatase.[22] Blood was collected at room temperature in heparinized tubes (20U/ml) with 10mM fasudil (Asahi Chemical Industry Co. Ltd., Japan). Leukocytes were isolated from peripheral blood using a variation of previously published methods.[23] The leukocyte pellet was suspended in Media 199 solution (M199) (Sigma Chemical, IL) and then diluted to achieve 5×10^6 cells/mL. Fixative solution (50% trichloroacetic acid (Sigma Chemical, IL), 50 mmol/L dichlorodiphenyltrichloroethane (Sigma Chemical, IL), and protease inhibitors (Calbiochem, EMD Biosciences, Inc, Germany) were then added to the solution and the resulting precipitate was stored at −80°C for Western blot analysis.

Western blot analysis was performed as previously described[23] using rabbit anti-phospho-specific Thr^{853}-MBS polyclonal antibody and rabbit anti-MBS polyclonal antibody (Covance Laboratories, IN). NIH 3T3 cell lysates were used as a positive control and to standardize the results of Western blot analyses from several membranes. ROCK activity was expressed as the percent of phosphoThr^{853}-MBS in each sample/phosphoThr^{853}-MBS in each positive control divided by MBS in each sample/MBS in each positive control.

Endothelium-dependent dilation of the brachial artery was measured before and one month after statin treatment.[24] Brachial artery diameter was measured under basal conditions and during reactive hyperemia following 5 minutes of an ischemic stimulus. A blood pressure cuff placed on the upper arm was inflated to suprasystolic pressures for 5 minutes to induce forearm ischemia. Acquisition and analysis of the digitized images were performed using software from Information Integrity, Inc. (Boston, MA). Subjects were asked to withhold all vasoactive medications on study days until after the study procedures. All measurements were performed after an overnight fast in the supine position using the same arm and site.

Atorvastatin (40 mg) and rosuvastatin (10 mg) are both FDA approved drugs with an indication to treat elevated LDL cholesterol. Atorvastatin and rosuvastatin tablets were obtained from AstraZeneca Pharmaceuticals, LLP (Westborough, MA) and Pfizer, Inc. (Cambridge, MA), respectively. The tablets were encapsulated into matching capsules, placed in subject-specific bottles, randomized, and dispensed by the Investigational Drug Pharmacy at Brigham and Women's Hospital.

The primary endpoint of this study was the change in leukocyte ROCK activity after one month of statin therapy compared to baseline. The secondary endpoints included change in lipid profiles and change in FMD with statin therapy compared to baseline. We also correlated the change in ROCK activity with change in LDL and change in FMD. Additionally, we evaluated whether both atorvastatin (lipophilic) and rosuvastatin (hydrophilic) inhibited ROCK activity.

All statistical analyses were conducted using SAS 9.1 (SAS Institute Inc., NC). Baseline characteristics and experimental measures are expressed as mean ± SE. The endpoints of interest (ROCK activity, lipids, and FMD) before and one month after statin treatment were compared using a paired Student's t-test for normally distributed variables and a Sign rank test for non-parametric variables. All correlations were made using Pearson's or Spearman's correlation coefficient, for parametric and non-parameteric variables, respectively. Baseline characteristics of the two statin groups were compared using unpaired Student's t-test or Wilcoxon rank sum test for continuous variables and chi-square analysis for discrete variables. Comparisons between the two statin groups were made using an independent *t*-test or Wilcoxon rank sum test. A two-sided p value of < 0.05 was considered statistically significant.

RESULTS

All enrolled subjects had a history of coronary atherosclerosis, except one subject who had suffered a cerebrovascular event without any evidence of coronary disease. The baseline

characteristics of the entire study cohort are shown in Table 1. Baseline characteristics were also compared between subjects randomized to the two statin drugs (atorvastatin vs. rosuvastatin). The distribution of traditional cardiac risk factors and cardiac medications was similar between the two statin arms (Table 1). Baseline values of fasting lipids, leuckocyte ROCK activity, and FMD were also similar between the two treatment arms.

For the entire patient cohort, ROCK activity decreased by 21% from 62 ± 7% at baseline to 48 ± 5% ($p<0.0001$) after one month of statin therapy (Table 2 and Figure 1). Subjects randomized to 4 weeks of rosuvastatin 10 mg daily (n=15) experienced a decline in ROCK activity from 64 ± 10% at baseline to 46 ± 8% ($p<0.0001$) after treatment. For subjects randomized to atorvastatin 40 mg daily (n=15), ROCK activity also decreased from 55 ± 7% at baseline to 47 ± 6% ($p<0.0001$) after treatment. Although both statins effectively reduced ROCK activity, the extent of ROCK inhibition was significantly greater with rosuvastatin compared to atorvastatin, even after controlling for baseline differences in ROCK activity ($p=0.0003$) (Figure 1).

Statins improved FMD by 40 ± 12% from a baseline of 7.4 ± 0.6% to 9.3 ± 0.4% ($p=0.003$) after one month of therapy (Figure 2). Rosuvastatin improved FMD in the brachial artery by 34 ± 14% from 7.3 ± 1.2 at baseline to 8.8 ± 4.7 after one month of therapy ($p=0.03$) and atorvastatin improved FMD by 45 ± 18% from 7.4 ± 1.5 to 9.5 ± 5.1 ($p=0.06$). The improvement in endothelial function relative to baseline was similar with both statin drugs ($p=1.00$) although improvement in FMD was significant with rosuvastatin, but not atorvastatin. Statin therapy reduced total cholesterol levels by 31% from 225 ± 8 mg/dL to 155 ± 8 mg/dL ($p<0.0001$) and LDL levels by 43% from 141 ± 6 mg/dL to 79 ± 5 mg/dL ($p<0.0001$). Both rosuvastatin (10 mg) and atorvastatin (40 mg) resulted in equivalent reductions in total cholesterol ($p=0.57$) and LDL cholesterol ($p=0.28$) after one month of therapy (Table 2). Atorvastatin, but not rosuvastatin, also decreased triglyceride levels significantly ($p=0.04$). Neither drug affected HDL levels during this study.

The hsCRP levels vary widely in our small cohort of patients. Baseline plasma hsCRP levels (CRP1) were 1.3 and 2.5 mg/L in the rosuvastatin and atorvastatin groups, respectively (p=NS) (Table 2). The hsCRP levels after treatment with rosuvastatin and atorvastatin (CRP2) were 1.1 and 1.3 mg/L, respectively, which were not different from baseline ($p=0.35$ and $p=0.57$).

There was no correlation between the changes in ROCK inhibition and the changes in lipid levels ($p=0.57$). ROCK inhibition was associated with improvement in endothelial function. However, because of the lack of a placebo group and limited number of patients, a definitive correlation between ROCK inhibition and improvement in endothelial function could not be statistically shown in this study ($p=0.71$).

Discussion

Our results demonstrate that statin therapy, at clinically relevant doses used for lipid lowering, inhibits ROCK activity in humans. This reduction in ROCK activity is accompanied by an improvement in flow-mediated vasodilation, an indicator of endothelial function. Furthermore, our results demonstrate that statins appear to inhibit ROCK activity and improve endothelial function, despite their differential cellular uptake. These findings suggest that lipophilicity of a particular statin may not be the only determinant of their effects on ROCK. Perhaps other factors such as potency for HMG-CoA reductase inhibition and in vivo half-life of a particular statin may also play a role in leukocyte ROCK inhibition.

In addition to decreasing cholesterol formation, statins inhibit isoprenoid formation.[3] Isoprenoids are intermediates in the mevalonate pathway but diverge from cholesterol synthesis. The isoprenoid geranylgeranylpyrophosphate is required for the post-translational

modification and activation of a group of small GTPases including Rho, Rac and cdc42. Thus, by inhibiting isoprenoid formation, statins decrease the membrane translocation and activation of these small GTPases and their down-stream effectors, such as ROCK.[3] Mounting experimental evidence suggests that isoprenoid inhibition by statins might explain the clinical observations that statins improve cardiovascular outcomes even in subjects with atherosclerosis and normal cholesterol levels.[25] However, these basic observations have not been previously translated in humans. This study, therefore, provides a mechanistic possibility for the putative lipid-independent effects of statin therapy.

Experimental data suggests that ROCK inhibition increases nitric oxide bioavailability via post-transcriptional stabilization of eNOS mRNA[4] and increased phosphorylation of eNOS by activation of the phosphatidylinositol-3-kinase/protein kinase Akt pathway.[26] Based on these findings, we would anticipate that ROCK inhibition would improve nitric oxide bioavailability and hence endothelial function in humans with atherosclerosis. Indeed, treatment with the direct ROCK inhibitor, fasudil, improves flow-mediated vasodilation in the brachial artery of humans with hyperlipidemia and atherosclerosis, without influencing LDL cholesterol levels.[24] Consistent with these previous findings, we found that statins reduce ROCK activity and improve endothelial function in men with atherosclerosis. Statins have been shown to inhibit hs-CRP.[27] However, in our study, probably due to the small sample size, there were no observable effects of rosuvastatin or atorvastatin on hs-CPR levels.

Rosuvastatin contains a polar methyl sulfonamide moiety that makes it relatively hydrophilic compared with atorvastatin.[17] The importance of this characteristic is that in contrast to more lipophilic statins such as atorvastatin, rosuvastatin has lower rates of passive diffusion into non-hepatic cells. However, our results demonstrate that both hydrophilic (rosuvastatin) and lipophilic (atorvastatin) statins could inhibit ROCK activity in non-hepatic tissues in humans with atherosclerosis. This is an important observation in the clinical setting since it suggests that in addition to lipid lowering, the ROCK inhibitory effects of statin therapy may apply to both lipophilic and hydrophilic statins. Further studies with more lipophilic and hydrophilic statins are needed in order to determine whether there are any differences between lipophilicity of a statin and inhibition of ROCK activity.

The limitations of this study are that it was small, not long enough, and compared only 2 statins with no clinical endpoints. Given the ethical limitations of withdrawing statin therapy in men with atherosclerosis, this study did not include a placebo arm. Although, our results showed a consistent and significant decline in ROCK activity before and after statin therapy, inclusion of a placebo arm would make this result more robust. Indeed, our prior study examining the effects of the direct ROCK inhibitor, fasudil, has shown that ROCK inhibition is correlated with flow-mediated vasodilation in the absence of lipid modification [24] Thus, it is likely that with more patients and a placebo arm, inhibition of ROCK would have been correlated with improvement in FMD. Finally, comparison of statins to lipid-lowering agents that lower cholesterol without inhibiting HMG-CoA reductase, i.e, ezetimibe, may shed more light on the lipid-independent effects of ROCK inhibition by statin therapy.

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References

1. Loirand G, Guerin P, Pacaud P. Rho kinases in cardiovascular physiology and pathophysiology. Circ Res 2006;98:322–334. [PubMed: 16484628]
2. Sawada N, Itoh H, Ueyama K, Yamashita J, Doi K, Chun TH, Inoue M, Masatsugu K, Saito T, Fukunaga Y, Sakaguchi S, Arai H, Ohno N, Komeda M, Nakao K. Inhibition of rho-associated kinase results in suppression of neointimal formation of balloon-injured arteries. Circulation 2000;101:2030–2033. [PubMed: 10790342]
3. Liao JK. Rho-kinase: a potential link between hypercholesterolemia and abnormal vascular smooth muscle contraction. Circ Res 2006;99:238–239. [PubMed: 16888249]
4. Laufs U, Liao JK. Post-transcriptional regulation of endothelial nitric oxide synthase mRNA stability by Rho GTPase. J Biol Chem 1998;273:24266–24271. [PubMed: 9727051]
5. Seasholtz TM, Majumdar M, Kaplan DD, Brown JH. Rho and Rho kinase mediate thrombin-stimulated vascular smooth muscle cell DNA synthesis and migration. Circ Res 1999;84:1186–1193. [PubMed: 10347093]
6. Bolz SS, Vogel L, Sollinger D, Derwand R, de Wit C, Loirand G, Pohl U. Nitric oxide-induced decrease in calcium sensitivity of resistance arteries is attributable to activation of the myosin light chain phosphatase and antagonized by the RhoA/Rho kinase pathway. Circulation 2003;107:3081–3087. [PubMed: 12796138]
7. Wojciak-Stothard B, Williams L, Ridley AJ. Monocyte adhesion and spreading on human endothelial cells is dependent on Rho-regulated receptor clustering. J Cell Biol 1999;145:1293–1307. [PubMed: 10366600]
8. Miyata K, Shimokawa H, Kandabashi T, Higo T, Morishige K, Eto Y, Egashira K, Kaibuchi K, Takeshita A. Rho-kinase is involved in macrophage-mediated formation of coronary vascular lesions in pigs in vivo. Arterioscler Thromb Vasc Biol 2000;20:2351–2358. [PubMed: 11073837]
9. Ito T, Ikeda U, Shimpo M, Ohki R, Takahashi M, Yamamoto K, Shimada K. HMG-CoA reductase inhibitors reduce interleukin-6 synthesis in human vascular smooth muscle cells. Cardiovasc Drugs Ther 2002;16:121–126. [PubMed: 12090904]
10. Ishibashi T, Nagata K, Ohkawara H, Sakamoto T, Yokoyama K, Shindo J, Sugimoto K, Sakurada S, Takuwa Y, Teramoto T, Maruyama Y. Inhibition of Rho/Rho-kinase signaling downregulates plasminogen activator inhibitor-1 synthesis in cultured human monocytes. Biochim Biophys Acta 2002;1590:123–130. [PubMed: 12063175]
11. Nakakuki T, Ito M, Iwasaki H, Kureishi Y, Okamoto R, Moriki N, Kongo M, Kato S, Yamada N, Isaka N, Nakano T. Rho/Rho-kinase pathway contributes to C-reactive protein-induced plasminogen activator inhibitor-1 expression in endothelial cells. Arterioscler Thromb Vasc Biol 2005;25:2088–2093. [PubMed: 16123329]
12. Rikitake Y, Liao JK. Rho-kinase mediates hyperglycemia-induced plasminogen activator inhibitor-1 expression in vascular endothelial cells. Circulation 2005;111:3261–3268. [PubMed: 15956119]
13. Essig M, Nguyen G, Prie D, Escoubet B, Sraer JD, Friedlander G. 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors increase fibrinolytic activity in rat aortic endothelial cells. Role of geranylgeranylation and Rho proteins. Circ Res 1998;83:683–690. [PubMed: 9758637]
14. Laufs U, Marra D, Node K, Liao JK. 3-Hydroxy-3-methylglutaryl-CoA reductase inhibitors attenuate vascular smooth muscle proliferation by preventing rho GTPase-induced down-regulation of p27 (Kip1). J Biol Chem 1999;274:21926–21931. [PubMed: 10419514]
15. Bourcier T, Libby P. HMG CoA reductase inhibitors reduce plasminogen activator inhibitor-1 expression by human vascular smooth muscle and endothelial cells. Arterioscler Thromb Vasc Biol 2000;20:556–562. [PubMed: 10669656]
16. Eto M, Kozai T, Cosentino F, Joch H, Luscher TF. Statin prevents tissue factor expression in human endothelial cells: role of Rho/Rho-kinase and Akt pathways. Circulation 2002;105:1756–1759. [PubMed: 11956113]
17. McTaggart F. Comparative pharmacology of rosuvastatin. Atheroscler Suppl 2003;4:9–14. [PubMed: 12714032]

18. Stroes ES, Koomans HA, de Bruin TW, Rabelink TJ. Vascular function in the forearm of hypercholesterolaemic patients off and on lipid-lowering medication. Lancet 1995;346:467–471. [PubMed: 7637480]

19. Jones P, Kafonek S, Laurora I, Hunninghake D. Comparative dose efficacy study of atorvastatin versus simvastatin, pravastatin, lovastatin, and fluvastatin in patients with hypercholesterolemia (the CURVES study). Am J Cardiol 1998;81:582–587. [PubMed: 9514454]

20. Jones PH, Davidson MH, Stein EA, Bays HE, McKenney JM, Miller E, Cain VA, Blasetto JW. Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses (STELLAR* Trial). Am J Cardiol 2003;92:152–160. [PubMed: 12860216]

21. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502. [PubMed: 4337382]

22. Amano M, Mukai H, Ono Y, Chihara K, Matsui T, Hamajima Y, Okawa K, Iwamatsu A, Kaibuchi K. Identification of a putative target for Rho as the serine-threonine kinase protein kinase N. Science 1996;271:648–650. [PubMed: 8571127]

23. Liu PY, Chen JH, Lin LJ, Liao JK. Increased Rho kinase activity in a Taiwanese population with metabolic syndrome. J Am Coll Cardiol 2007;49:1619–1624. [PubMed: 17433952]

24. Nohria A, Grunert ME, Rikitake Y, Noma K, Prsic A, Ganz P, Liao JK, Creager MA. Rho Kinase Inhibition Improves Endothelial Function in Human Subjects With Coronary Artery Disease. Circ Res. 2006

25. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet 2002;360:7–22. [PubMed: 12114036]

26. Wolfrum S, Dendorfer A, Rikitake Y, Stalker TJ, Gong Y, Scalia R, Dominiak P, Liao JK. Inhibition of Rho-kinase leads to rapid activation of phosphatidylinositol 3-kinase/protein kinase Akt and cardiovascular protection. Arterioscler Thromb Vasc Biol 2004;24:1842–1847. [PubMed: 15319269]

27. Jialal D, Stein D, Balis D, Grundy SM, Adams-Huet B, Devaraj S. Effect of hydroxymethyl glutaryl coenzyme A reductase inhibitor therapy on high sensitive C-reactive protein levels. Circulation 2001;103:1933–1935. [PubMed: 11306519]

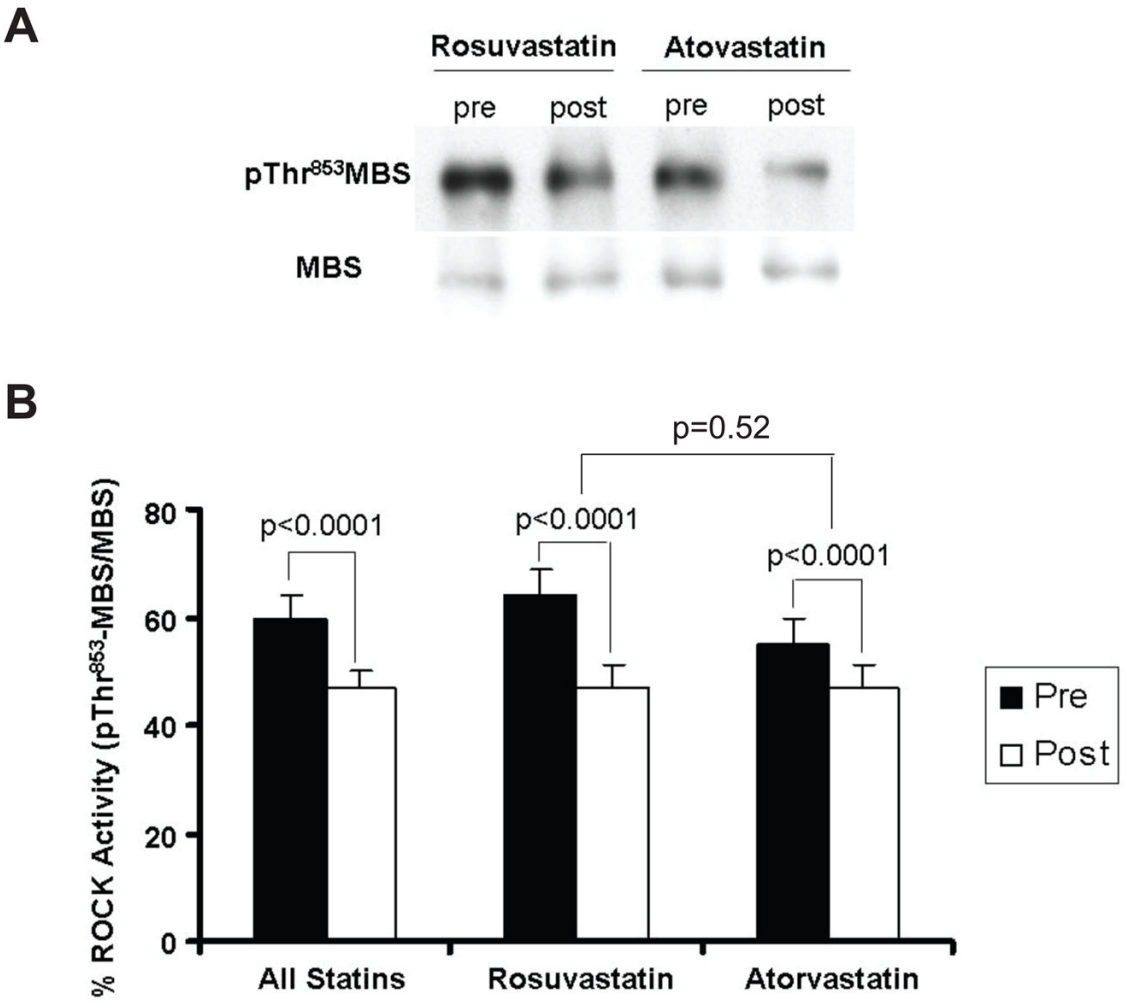

Figure 1.

Reduction in ROCK (ROCK) activity with statin therapy. ROCK activity in leukocytes was assessed by Western blot analysis before and after one month of statin therapy. ROCK activity was calculated as the percent phosphorylated myosin binding subunit ($pThr^{853}MBS$) of myosin light chain phosphatase (ROCK target) relative to total myosin binding subunit (MBS). Panel A demonstrates a representative Western blot analysis showing a decline in the relative staining for $pThr^{853}MBS$ with both rosuvastatin and atorvastatin therapy. Panel B demonstrates that ROCK activity declined significantly with all statin therapy and with both hydrophilic (rosuvastatin) and lipophilic (atorvastatin) statins.

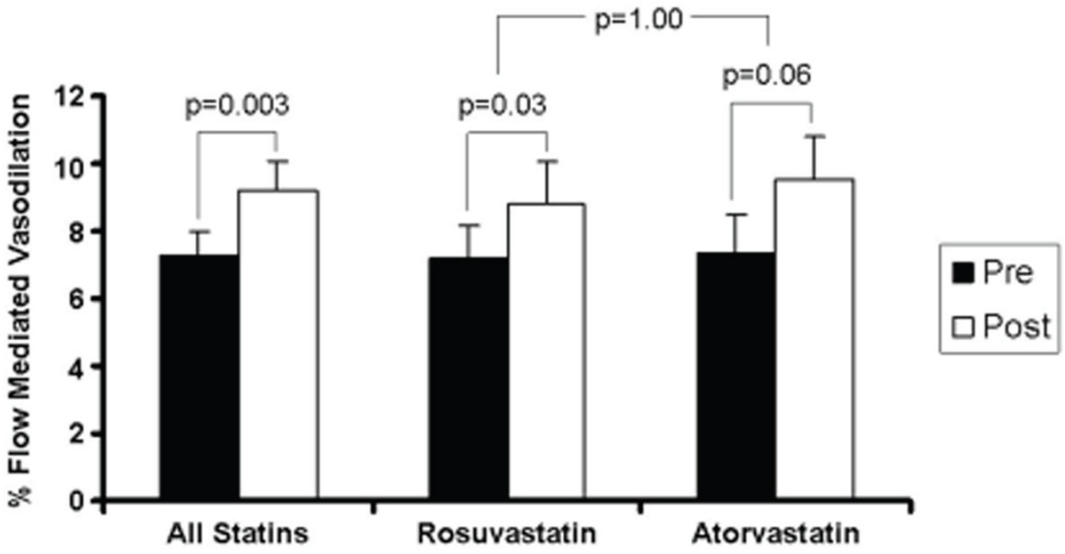

Figure 2.

Effect of statin therapy on brachial artery flow-mediated dilation (FMD). Brachial artery FMD was assessed before and after one month of statin therapy. Statin therapy improved flow-mediated vasodilation in the study cohort as a whole. Both rosuvastatin (hydrophilic) and atorvastatin (lipophilic) improved FMD to a similar extent.

Table 1

Baseline characteristics between treatment groups

Variable	Rosuvastatin (n=15)	Atorvastatin (n=15)	P Value
Age (years)	65.2 ± 1.6	65.0 ± 2.0	0.67
Systemic hypertension	12 (80%)	13 (87%)	0.64
Diabetes mellitus	5 (33%)	4 (26%)	0.50
Past history			
Smoker	7 (47%)	9 (53%)	0.69
Myocardial infarction	10 (67%)	11 (73%)	0.58
Percutaneous coronary intervention	10 (67%)	10 (67%)	0.83
Coronary artery bypass surgery	5 (33%)	6 (40%)	0.39
Cerebrovascular disease	2 (13%)	0	0.13
Peripheral artery disease	1 (7%)	1 (7%)	0.96
Medications			
Aspirin	15 (100%)	15 (100%)	0.68
β-blocker	13 (87%)	12 (80%)	0.72
Calcium channel blocker	3 (20%)	5 (33%)	0.18
Angiotensin converting enzyme inhibitor	12 (80%)	11 (73%)	0.62
Nitrate	1 (7%)	2 (13%)	0.50
Diuretic	5 (33%)	5 (33%)	0.84

Data are expressed as mean ± SE for continuous variables or number (%) for dichotomous variables.

Table 2

Comparison of changes in lipid parameters and high sensitivity-C reactive protein in all subjects and between Rosuvastatin and Atorvastatin treatment groups

	All subjects			Rosuvastatin 10 mg daily			Atorvastatin 40 mg daily			
Parameters	**Baseline**	**Post-treatment**	**P value**	**Baseline**[****]	**Post-treatment**	**Δ%**	**Baseline**[****]	**Post-treatment**	**Δ%**	**P value**[*]
Total cholesterol (mg/dL)	225 ± 8	155 ± 8	<0.0001	232 ± 10	165 ± 9[**]	−28.3	217 ± 7	144 ± 8[**]	−31.7	0.57
Triglycerides (mg/dL)	192 ± 24	147 ± 25	0.0005	184 ± 28	174 ± 32	−6.0	193 ± 33	116 ± 9[***]	−40.2	0.06
High density lipoprotein (mg/dL)	46 ± 5	47 ± 4	0.26	48 ± 4	49 ± 4	3.1	44 ± 5	45 ± 4	1.6	0.85
Low density lipoprotein (mg/dL)	141 ± 6	79 ± 5	<0.0001	146 ± 9	80 ± 7[***]	−45.2	135 ± 8	77 ± 6[***]	−42.5	0.28
High sensitivity C-reactive protein (mg/L)	1.8 [0.9, 3.1]	1.2 [0.8, 3.2]	0.61	1.3 [0.8–2.4]	1.1 [0.8–1.9]	−0.2	2.5 [1.1–3.8]	1.3 [0.6–5.7]	−1.2	0.84

Data are expressed as mean ± SE or ranged with lower (25% percentile) and higher (75% percentile) for asymmetrically distributed continuous variables, or % for dichotomous variables. Δ% = (baseline − after treatment)/baseline × 100%. Other abbreviations please see Table 2.

*: compared to Δ% between 2 treatment groups;

**: p<0.05 compared with baseline level,

***: p<0.01 compared with baseline level.

****No difference between 2 groups for all parameters on their baseline level.