Published in final edited form as:

Atherosclerosis. 2012 April ; 221(2): 521–526. doi:10.1016/j.atherosclerosis.2012.01.001.

Increased Leukocyte Rho-associated Coiled-Coil Containing Protein Kinase Activity Predicts the Presence and Severity of Coronary Vasospastic Angina

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Abstract

Objective—Although inhibition of Rho-associated coiled-coil containing protein kinase (ROCK) has been shown to prevent coronary vasospastic angina (CVA), direct evidence linking ROCK activity and CVA is lacking. Accordingly, we investigated whether ROCK activity is an independent marker for CVA and is altered after treatment with antispastic medications.

Methods and Results—We prospectively studied 31 Taiwanese patients who were diagnosed with CVA and 33 control subjects. Subject demographics were recorded, and blood samples were obtained at baseline in all participants and in CVA patients after 3 months of antispastic treatment. Compared with control subjects, leukocyte ROCK activity was greater in CVA patients (136% versus 91%, $P < 0.001$). A cutoff value for leukocyte ROCK activity of 104% predicted the presence of CVA with specificity and sensitivity rates of 88% and 84%, respectively. ROCK activity increased with the severity of CVA (P for trend < 0.001). Following 3-month treatment of antispastic agents, leukocyte ROCK activity, high-sensitivity C-reactive protein, and interleukin-6 levels were reduced by 43%, 42% and 27%, respectively ($P < 0.05$ for all).

Conclusions—Increased levels of leukocyte ROCK activity independently predicted the presence of CVA and correlated with CVA severity. Treatment with antispastic agents substantially reduced the level of leukocyte ROCK activity.

Keywords

angina; calcium antagonist; coronary vasospasm; rho-kinase

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Disclosures: None.

Introduction

Coronary vasospastic angina (CVA) is a cause of ischemic heart disease, including acute coronary syndromes [1,2]. However, the precise mechanisms responsible for the occurrence of CVA are not known. Previous studies involving histological evaluation of coronary plaques or arteries in patients with coronary spasm-related variant angina have reported evidence of intimal injury, such as neointimal hyperplasia with infiltration of inflammatory cells [3]. Recently, we noted that the serum levels of inflammatory markers are elevated in CVA patients without obstructive coronary artery disease (CAD) [4]. These findings suggest that despite angiographically normal coronary arteries, patients with CVA exhibit increased inflammatory response.

Rho-associated coiled-coil containing protein kinase (ROCK) is a serine/threonine protein kinase that mediates the downstream signaling of the small guanosine triphosphate (GTP)-binding protein, Rho, on the actin cytoskeleton. Human studies have shown that ROCK inhibition can improve endothelial function in patients with CAD and CVA [5,6]. In a porcine model of variant angina, it was shown that the activation of ROCK is involved in the development coronary vasospasm [7]. Although inhibition of ROCK attenuates CVA, it does not necessarily mean that ROCK is the cause of CVA. Furthermore, it is not known whether increased ROCK activity is associated with CVA and whether ROCK activity correlates with CVA severity in humans. Thus, clinical studies linking ROCK with CVA are lacking.

In this prospective case-control study, we sought to determine whether leukocyte ROCK activity is an independent marker of CVA, whether it correlates with other inflammatory and risk markers in CVA, and whether it is decreased after treatment with antispastic medications.

Methods

Patients Selection

Consecutive patients who underwent cardiac catheterization for suspected ischemic heart disease between August 2007 and December 2007 were included in the present study. Criteria for enrollment into the CVA group included: 1) an attack occurring at rest and ST-segment deviation or T-wave inversion on the electrocardiogram; 2) an attack relieved by sublingual administration of nitroglycerin; 3) no significant obstructive CAD (<50% luminal narrowing of major coronary arteries) after intra-coronary nitroglycerin; and 4) a positive response to intra-coronary methylergonovine infusion (see Supplementary Methods). The Institutional Review Board of Chang Gung Memorial Hospital (96-0817B and 97-0407B) approved this study. Written informed consent was obtained from all patients.

Clinical Data

Medical records were reviewed for cardiac risk factors, including cigarette smoking habits, diabetes mellitus status, and details regarding hypercholesterolemia and hypertension (see Supplementary Methods).

Cardiac Catheterization

All anti-spastic drugs (nitrates and calcium channel blockers) were withdrawn ≥ 24 hours before cardiac catheterization, except for sublingual nitroglycerin, which was withdrawn ≥ 2 hours before cardiac catheterization. Quantitative coronary angiography was performed and analyzed as described (see Supplementary Methods). Only patients exhibiting non-

obstructive CAD (<50% reduction in the lumen diameter) underwent a subsequent intra-coronary methylergonovine provocation test.

Intra-coronary Methylergonovine Provocation Test

Intra-coronary methylergonovine (Methergin®, Novartis, Basel, Switzerland) provocation was performed as described (see Supplementary Methods). CVA was defined as a reduction of over 70% in the diameter of an arterial lumen with concurrent chest pain or ischemic ST-T changes during the provocation testing [8]. Real-time quantitative coronary angiography was used to measure changes in arterial lumen diameter.

Biochemical Analysis

Blood specimens were collected in citrate-treated tubes at 2 time points: 1) immediately before coronary angiography after an overnight fast for control and CVA groups and 2) after a period of 3 months of treatment with calcium antagonist(s) and/or nitrate in the CVA group. Plasma nitric oxide measurement was performed in duplicate using L-citrulline detection kit (BioSupply UK, Bradford, UK), which detects nitric oxide by indirectly measuring L-citrulline; the product stoichiometry of L-citrulline and nitric oxide is 1:1. This kit is based on the colorimetric change at absorbance of 540 nm, which occurs when the reaction of L-citrulline with 2,3-Butanedione monoxime. Plasma hsCRP was measured in duplicate by enzyme linked immunosorbent assays (IMMULITE hsCRP, Diagnostic Products Corporation, Los Angeles, California). Plasma concentrations of interleukin-6, tissue necrosis factor-alpha (TNF- α), and adiponectin were also measured by enzyme-linked immunosorbent assay kits (R&D Systems, Inc., Minneapolis, Minnesota).

Leukocyte ROCK Assay

Leukocytes from peripheral blood (10 mL) were isolated, and ROCK expression and activity were performed as described (see Supplementary Methods). ROCK protein expression was expressed as the ratio of ROCK to glyceraldehydes-3-phosphate dehydrogenase protein expression. ROCK activity was expressed as the ratio of phosphorylation level of myosin binding subunit (pMBS) to total MBS in each positive control.

Statistical Analyses

The frequencies between the CVA group and the controls were compared using the Chi-Square test. Continuous variables with a skewed distribution, i.e., p value <0.05 by Kolmogorov-Smirnov testing, are presented as medians (25th, 75th percentiles), and those with normal distribution are expressed as means $\pm$ standard deviation. Independent-sample t test and Mann-Whitney U test were used to compare the differences in continuous variables between patients with coronary spasm and the controls. The multivariate logistical model's discriminative ability was assessed by Harrell's C statistic. To evaluate the effect of CVA severity on ROCK activity, we considered multi-vessel (≥ 2) coronary vasospasm and recent angina (i.e. angina duration of ≤ 1 week and angina frequency of ≥ 2 /week) as components of increased CVA severity. Comparison of biochemical data and ROCK activity before and after treatment with anti-spastic agents was performed using the Mann-Whitney U test. A p value of <0.05 was considered statistically significant. All statistical analyses were conducted with the SPSS statistical package for Windows version 15.0 (SPSS Inc., Chicago, Illinois).

Results

Baseline Characteristics

A total of 228 patients underwent diagnostic coronary angiography to evaluate coronary artery disease without coronary intervention. Of these patients, 92 of them underwent intracoronary methylergonovine provocation testing. Twelve patients refused to participate in the study. Because CVA patients were more sensitive to vasospasm, the mean methylergonovine dose that was used to elicit vasospasms was lower than that used for control subjects. The mean age of the remaining 80 patients was 57 ± 11 years, with men constituting 61% of the sample population. Initially, there were 41 patients in the CVA group and 39 patients in the control group. In the CVA group, 10 patients were on statin treatment and in the control group, 6 patients were being treated with statins. These patients and controls were excluded because statin treatment could affect ROCK activity [9,10]. The final study population included 31 patients in the CVA group and 33 subjects in the control group. The average age, gender, body mass index, prevalence of diabetes mellitus, hypertension, and hypercholesterolemia, and types of medications before angiography were similar between both groups (Table 1). The percentage of current smokers was greater among CVA patients. Compared with the control group, CVA patients had higher peripheral leukocyte, lymphocyte, and monocyte counts. In the CVA group, 8 patients presented with ST-segment elevation and the rest presented with ST-segment depression and/or T-wave inversion. Single-vessel coronary vasospasm was the most common finding in CVA patients, with spasm provoked mostly in the right coronary artery.

L-citrulline, Inflammatory Markers, Adipokine, and ROCK Activity

No significant difference of L-citrulline level was found between control and CVA groups. Among the inflammatory markers and adipokine measured, plasma hsCRP and interleukin-6 levels were greater in CVA patients than in control subjects, but not TNF- α or adiponectin (Table 1). ROCK1 protein levels and ROCK activity were higher in CVA patients than in controls (136 % [CI: 114-155] versus 91 % [CI: 75-101]) (Fig. 1A). However, ROCK2 protein levels were lower in CVA patients than in control subjects. Our final multivariate model controlled for smoking, left ventricular ejection fraction, hsCRP, interleukin-6, ROCK1, and ROCK2. After controlling these covariates, multivariate analysis for the association between ROCK activity and CVA showed that baseline ROCK activity was an independent predictor of CVA (Table 2). Harrell's C statistic of the multivariate model showed a high discriminative ability (0.933, CI: 0.875-0.990). The cut-off value for ROCK activity that predicted the presence of CVA was $>104\%$ by receiver-operator characteristic analysis (area under the curve = 0.92 ± 0.03 , [95% CI: 0.86 to 0.99], $P < 0.001$) (see Supplementary Data). The sensitivity, specificity, positive predictive, negative predictive and positive likelihood ratio values for this ratio in detecting CVA in our cohort were 84%, 88%, 87%, 88%, and 7.20, respectively.

To determine whether ROCK activity is a novel risk marker for CVA and whether it correlates with severity of CVA, we performed an association analysis to determine the correlation coefficient of ROCK activity with clinical and inflammatory markers in patients with CVA. After adjusting for smoking status, peripheral leukocyte count ($r=0.275$, $P=0.029$), lymphocyte count ($r=0.252$, $P=0.046$), monocyte count ($r=0.371$, $P=0.003$), interleukin-6 ($r=0.293$, $P=0.020$) and ROCK1 protein expression ($r=0.290$, $P=0.021$) were positively associated with increased levels of ROCK activity. In contrast, ROCK2 protein expression ($r=-0.322$, $P=0.010$) was negatively associated with ROCK activity. The L-citrulline and hsCRP levels were not associated with ROCK activity. There was no association between peripheral leukocyte count, lymphocyte count, monocyte count, L-citrulline level and ROCK protein expression. Additionally, there was no association

between L-citrulline level and inflammatory markers. After adjusting for age and smoking, patients with greater CVA severity (≥ 2) had higher odds ratios for elevated ROCK activity when compared with those with < 2 components (Table 3). These findings suggest that elevated ROCK activity correlates with inflammation (interleukin-6, a primary stimulant for hepatic production of acute-phase proteins) and CVA severity.

Effects of Antispastic Agents

As seen in Table 1, antispastic agents (i.e. calcium antagonists and/or nitrates) were the most frequently prescribed medications at discharge in all 31 patients who had CVA. After excluding patients who did not agree to have follow-up ROCK assays and who received statin therapy, a total of 11 patients underwent follow-up biochemical tests. After 3 months of antispastic therapy, the ROCK activity decreased significantly from 128% (CI: 105-164) to 85% (CI: 74-99) (Fig. 1B). In addition, the hsCRP and interleukin-6 levels had also decreased significantly (Table 4). No recurrent angina was noted in CVA patients. These findings suggest that antispastic agents attenuate ROCK activity and inflammation in CVA patients.

Discussion

We have shown that increased leukocyte ROCK activity independently predicted the diagnosis of CVA in patients with non-obstructive CAD. Furthermore, increased ROCK activity correlated with CVA severity and treatment with antispastic agents was associated with decreased ROCK activity and ischemic events in CVA patients. These findings indicate that increased ROCK activity is a novel risk marker for CVA and suggest that ROCK may be involved in the pathogenesis of CVA.

ROCK activation has been shown to be involved in coronary vasospasm in a porcine model of coronary vasospasm [7]. In addition to preventing actin-myosin interaction in spastic coronary segments, direct inhibition of Rho or its downstream effector, ROCK, augments endothelial nitric oxide synthesis [11], decreases vascular smooth muscle cell contraction and proliferation [12], and decreases cytokine formation and leukocyte recruitment [13]. Thus, activation of ROCK may be a critical mediator that leads to the pathogenesis of CVA such as endothelial dysfunction, inflammation, and smooth muscle hypercontraction [1,2].

Although the precise mechanism by which ROCK activity is increased among CVA patients is not known, several possible mechanisms could explain the observed findings. For example, the activity and the expression of ROCK are enhanced in arteriosclerotic lesions, possibly leading to increased myosin light chain phosphorylation and coronary vasospasm [14]. Other possible mechanism by which increased ROCK activity could contribute to coronary vasospasm may involve decreased endothelium-derived nitric oxide (NO) bioavailability. Indeed, endothelial dysfunction and oxidative stress has been identified as a possible mechanism for coronary vasospasm and decreased NO bioavailability increases RhoA/ROCK activity [15,16]. These results strongly suggest that increased leukocyte ROCK activity is a cause rather a consequence of CVA.

We found that CVA patients had increased leukocyte ROCK activity as well as increased leukocyte ROCK1 protein expression. Interestingly, ROCK1 in leukocytes appears to play a greater role in the development of neointima formation and vascular inflammation after flow cessation-induced vascular injury [17]. Indeed, we found that ROCK activity correlated with ROCK1 protein expression and independently predicted the diagnosis of CVA after multivariate analysis. Surprisingly, our study also showed decreased leukocyte ROCK2 protein in CVA patients. It is not clear why leukocyte ROCK2 expression is lower in CVA patients compared to controls, despite overall increase in leukocyte ROCK activity in CVA

patients. Nevertheless, ROCK1 appears to be more important in leukocytes than ROCK2 [17], whereas ROCK2 appears to be more important in vascular smooth muscle cells rather than in circulating leukocyte cells [18]. These findings suggest that high ROCK activity in circulating leukocytes could affect neointima proliferation and vascular smooth muscle contraction.

Multi-vessel coronary vasospasm has been shown to be one of the independent predictors of poor prognosis in patients with variant angina [19], suggesting that disease severity is an important factor in determining the angina attack. In addition to considering multi-vessel coronary vasospasm, we also considered the clinical presentation of recent angina as a sign of increased disease activity, i.e. angina duration of ≤ 1 week and angina frequency of ≥ 2 /week. Our findings suggest that elevated ROCK activity requires the presence of several CVA severity assessments that are associated with unstable presentation of CVA. In our study, calcium antagonists reduced ROCK activity in CVA patients to the same level observed in the control group. Calcium antagonists have been found to enhance NO production via inhibition of NO dissolution by the inhibition of superoxide production (an antioxidative effect) and via the induction of endothelial NO synthase expression (an anti-inflammatory effect) [20]. In addition, NO-mediated vasodilation is through the inhibition of ROCK constrictor activity in the intact rat aorta [21]. Thus, it is likely that antispastic agents decrease CVA through inhibition of ROCK activity and enhancement of endothelium-derived NO production [22].

Interestingly, increased ROCK activity was observed in 2 CVA patients, who did not experience angina. Therefore, we were unable to demonstrate a correlation between decreased ROCK activity and reduced symptoms. The occurrence of angina attacks in patients with CVA is difficult to assess because their frequency tends to fluctuate spontaneously and the attacks are not necessarily accompanied by symptoms [23]. Previous studies have confirmed the favorable outcomes of CVA patients in the absence of significant obstructive CAD [19]. Although complications exist, they cannot be predicted in these patients. Thus, based on the observation of recurrent coronary vasospasm with or without symptoms, it is suggested that antispastic treatment should be continued in patients with CVA, even when the patients are asymptomatic [1,19].

In summary, we have shown that increased levels of ROCK activity independently correlated with the diagnosis of CVA and CVA severity. Treatment with antispastic agents significantly decreased the level of ROCK activity in these patients. These findings strongly implicate ROCK in the pathogenesis of CVA and establish leukocyte ROCK activity as a novel marker of CVA. Indeed, increased ROCK activity in peripheral leukocytes has been observed in patients with coronary artery disease and hypertension [10,24]. Thus, measuring peripheral leukocyte ROCK activity on a regular basis is suggested because it provides additional information other than history in assessing patients with chest pain, especially in those without obstructive CAD. However, it remains to be determined whether leukocyte ROCK activity is a useful predictor of cardiovascular outcome in patients with acute coronary syndrome.

Limitations

Some limitations of this study should be noted. First, we did not treat control patients with antispastic medications to see what are their effects on ROCK activity and inflammatory markers in subjects without CVA. Second, angiography may not be the best predictor of coronary atherosclerosis burden, and therefore, may underestimate the propensity for CVA. Further studies with coronary intravascular ultrasound may provide a better quantification of the relationship between atherosclerosis burden and the risk of CVA. Third, CVA can be detected in myocarditis as the myocardial inflammation results in coronary endothelial

dysfunction and smooth muscle hyperreactivity [25]. In our study, we tried to exclude patients who had inflammatory manifestations of infections and autoimmune disorders and who had fever of unknown origin; however, we could not definitely exclude patients who had coexisting CVA and myocarditis. Fourth, we did not measure L-citrulline level from the coronary sinus as the difference in serum nitric oxide level between the coronary sinus and the aorta has been demonstrated after intracoronary provocation test in CVA patients with a -786T>C polymorphism in the endothelial nitric oxide synthase gene [26]. In that study, there were no significant differences of baseline aortic nitric oxide levels between control and CVA groups. Fifth, we did not have information on coronary microcirculation. Further studies on coronary microcirculation may provide more information of CVA. Finally, we did not examine whether increased ROCK activity could distinguish CAD patients (>50% stenosis) with CVA versus CAD patients without CVA.

Conclusions

Increased levels of ROCK activity independently correlated with the diagnosis of CVA and CVA severity. Treatment with antispastic agents significantly decreased the level of ROCK activity in these patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Sources of Funding This study was supported by Grant CMRPG 260501 from Chang Gung Memorial Hospital, Keelung, Taiwan, and the National Institutes of Health (HL052233).

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Highlights

- ROCK activity increased with the severity of CVA
- Increased ROCK activity predicted the presence of CVA
- Treatment with antispastic agents reduced the level of ROCK activity

Figure 1. ROCK Activity in CVA Patients

Rho-associated coiled-coil containing protein kinase (ROCK) activity (30 μ g protein per lane) is greater among coronary vasospastic angina (CVA) patients (A) and is reduced after antispastic treatment for 3 months (B). Data are median values (interquartile range).

Table 1

Baseline characteristics of coronary vasospasm patients and control subjects

	Control n = 33	Vasospasm n = 31	P Value
Age (years)	55 ± 9	57 ± 11	0.391
Male	17 (52)	22 (71)	0.111
Body mass index (kg/m ²)	25 ± 3	26 ± 3	0.429
Smoking	8 (24)	15 (48)	0.044
Diabetes mellitus	5 (15)	7 (23)	0.447
Hypertension	13 (39)	8 (26)	0.247
Hypercholesterolemia	12 (36)	17 (55)	0.138
Total cholesterol (mg/dL)	196 ± 44	210 ± 40	0.185
Low-density cholesterol (mg/dL)	139 ± 40	150 ± 38	0.236
High-density cholesterol (mg/dL)	37 ± 13	36 ± 12	0.764
Peripheral leukocyte (/mm ³)	6084 ± 1016	7281 ± 2598	0.017
Segment (/mm ³)	3610 ± 932	4262 ± 2196	0.123
Lymphocyte (/mm ³)	1931 (1712, 2313)	2322 (1960, 2822)	0.014
Monocyte (/mm ³)	256 (210, 325)	338 (275, 393)	0.005
Eosinophil (/mm ³)	152 ± 84	162 ± 129	0.709
Basophil (/mm ³)	28 ± 13	36 ± 32	0.200
Left ventricular ejection fraction (%)	70 ± 8	66 ± 9	0.036
Spasm-provoked coronary artery			
Right		23 (58)	
Left anterior descending		12 (30)	
Left circumflex		5 (12)	
Number of spastic arteries			
1		23 (74)	
2		7 (23)	
3		1 (3)	
Dose of methylergonovine (μg)	92	83 ± 15	0.001

Medications	A	D	A	D	A	D
Calcium antagonists	6 (18)	21 (64)	5 (16)	29 (94)	0.828	0.004
ACEI/ARB	2 (6)	3 (9)	2 (6)	8 (26)	0.949	0.137
β-blockers	6 (18)	2 (6)	7 (23)	3 (10)	0.662	0.727
Diuretics	1 (3)	3 (9)	2 (6)	4 (13)	0.518	0.795
Aspirin	21 (64)	10 (30)	18 (58)	14 (45)	0.648	0.461
Nitrates	6 (18)	4 (12)	9 (29)	11 (35)	0.306	0.062
Statins	0	2 (6)	0	4 (13)		0.465
L-citrulline (μmol/L)	419 ± 476		577 ± 582		0.237	
Inflammatory markers						
hsCRP (mg/L)	1.05 (0.51, 2.01)		2.20 (0.97, 5.19)		0.006	

Medications	A	D	A	D	A	D
Interleukin-6 (pg/mL)	1.72 (1.16, 2.60)		2.47 (1.62, 3.16)		0.042	
TNF- α (pg/mL)	1.28 (1.02, 1.88)		1.41 (1.13, 2.06)		0.310	
Adiponectin (μ g/mL)	58.0 $\pm$ 42.5		59.1 $\pm$ 50.5		0.923	
ROCK 1 (%)	0.55 (0.14, 1.08)		0.90 (0.35, 1.83)		0.013	
ROCK 2 (%)	7.21 (4.38, 14.15)		1.96 (0.46, 6.73)		<0.001	

Categorical data are expressed as numbers of patients (percentages). Continuous data with and without skewness are presented as: medians (25th, 75th percentiles) and means $\pm$ SDs, respectively. A, before angiography; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensinogen receptor blocker; D, at discharge; hsCRP, high-sensitivity C-reactive protein; ROCK, Rho-associated coiled-coil containing protein kinase; TNF- α , tissue necrosis factor- α .

Table 2

Relationships between CVA and clinical risk factors, plasma inflammatory markers, and leukocyte ROCK expression and activity.

	Univariate			Multivariate		
	Odds ratio	95% CI	P	Odds ratio	95% CI	P
Smoking	2.930	1.012-8.482	0.047	1.292	0.219-7.625	0.777
Left ventricular ejection fraction, per 10%	1.518	0.892-2.583	0.124	1.791	0.715-4.486	0.213
hsCRP, per 1 mg/L	1.529	1.062-2.202	0.023	1.709	1.043-2.801	0.034
Interleukin-6, per 1 pg/mL	1.151	0.956-1.384	0.138	1.023	0.731-1.432	0.895
ROCK1, per 1%	1.509	0.951-2.392	0.080	1.170	0.800-1.710	0.419
ROCK2, per 1%	0.883	0.803-0.971	0.010	0.952	0.834-1.086	0.465
ROCK activity, per 1%	1.106	1.049-1.167	<0.001	1.102	1.034-1.174	0.003
ROCK activity >104%	29.120	7.552-112.288	<0.001	19.447	3.728-101.433	<0.001

Abbreviations as in Table 1.

Table 3

Adjusted Odds ratio of Increased ROCK Activity (p-MBS/MBS >104%) with Duration and Frequency of CVA and the Number of Spastic Coronary Arteries.

Clinical Characteristics	Odds Ratio (95% CI)	P
A. Angina Duration		
0	1.0	
≤ 1 week *	30.4 (4.92-188)	<0.001
> 1 week	24.1 (4.77-122)	<0.001
Trend	6.20 (2.55-15.1)	<0.001
B. Angina Frequency		
0	1.0	
≥ 2/week *	33.4 (5.53-201)	<0.001
≤ 1/week	21.8 (4.18-114)	<0.001
Trend	6.35 (2.54-15.8)	<0.001
C. Spastic Artery		
0	1.0	
1	25.6 (5.78-114)	<0.001
≥2 *	30.4 (2.88-321)	0.005
Trend	13.2 (3.82-45.6)	<0.001
D. Severity of CVA (number of *)		
0	1.0	
1	12.3 (2.77-54.3)	0.001
≥2	22.0 (2.32-209)	0.007
Trend	7.26 (2.42-21.8)	<0.001

* more severe of CVA in this category; CI, confidence interval; MBS, myosin binding subunit; p-MBS, phosphorylation level of myosin binding subunit; other abbreviations as in Table 1.

Table 4

Baseline and 3-month follow-up plasma hs-CRP and interleukin-6 levels and ROCK protein expression in subset CVA patients with/without statin therapy after cardiac catheterization

	Include statin use (n=14)		Exclude statin use (n=11)		P Value
	Baseline	Follow-up	Baseline	Follow-up	
L-citrulline (μmol/L)	655 ± 630	420 ± 369	573 ± 607	439 ± 418	0.397
hs-CRP (mg/L)	2.62 (0.95, 6.52)	1.28 (0.95, 2.68)	2.20 (0.89, 5.19)	1.27 (0.88, 2.66)	0.028
Interleukin-6 (pg/mL)	2.54 (1.87, 2.88)	1.97 (1.12, 2.31)	2.47 (1.73, 2.79)	1.80 (1.10, 2.22)	0.021
TNF-α (pg/mL)	1.41 (1.16, 2.09)	1.51 (1.30, 2.92)	1.41 (1.17, 2.06)	1.41 (1.29, 4.64)	0.155
Adiponectin (μg/mL)	39 ± 28	43 ± 39	42 ± 30	46 ± 41	0.608
ROCK 1 (%)	0.579 (0.307, 3.126)	0.467 (0.210, 0.923)	0.418 (0.266, 1.384)	0.466 (0.141, 0.576)	0.424
ROCK 2 (%)	1.009 (0.366, 3.504)	5.023 (0.403, 10.945)	1.078 (0.401, 2.162)	4.106 (0.174, 10.653)	0.374

Data with and without skewness are presented as: medians (25th, 75th percentiles) and means ± SDs, respectively. Abbreviations as in Table 1