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RAPID COMMUNICATION

Enhanced Rho-Kinase Activity in Patients With Vasospastic Angina After the Great East Japan Earthquake

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Background: It remains unclear whether disease activity of vasospastic angina (VSA) is altered during a disaster.

Methods and Results: Before and after the Great East Japan Earthquake, we examined Rho-kinase activity in circulating neutrophils of 11 VSA patients and their mental stress with the post-traumatic stress disorder (PTSD) questionnaire. Rho-kinase activity was significantly increased at 6 months after the Earthquake, and was returned to baseline level at 12 months. Importantly, percent change in Rho-kinase activity was significantly correlated with the PTSD score.

Conclusions: These results indicate that the Rho-kinase activity of VSA patients was transiently enhanced associated with disaster-related mental stress. (*Circ J* 2012; **76:** 2892–2894)

Key Words: Angina; Stress; Vasospasm

On March 11, 2011, we experienced the Great East Japan Earthquake in our Tohoku area. The Earthquake-related physical and psychological stress persisted for several months. Coronary vasomotor reactivity is known to be enhanced by stress[1] and we have previously demonstrated that Rho-kinase plays a key role in the molecular mechanisms of vasospastic angina (VSA).[2] We have also recently demonstrated that Rho-kinase activity in circulating neutrophils is a useful biomarker for the diagnosis and disease activity assessment of VSA.[3] We thus examined whether Rho-kinase activity was increased in VSA patients after the Earthquake.

Methods

The protocol of the present study was approved by the Ethical Committees of Tohoku University and all patients provided written informed consent. In 11 patients with VSA, all of whom had been previously diagnosed based on spasm provocation test with acetylcholine in accordance with the Guidelines for Diagnosis and Treatment of Patients with Vasospastic Angina of the Japanese Circulation Society,[4] we were able to measure Rho-kinase activity in circulating neutrophils at 3 time points: before and 6 and 12 months after the Earthquake. Rho-kinase activity was defined by the ratio of the phosphorylated form/total form of myosin-binding subunit, a substrate of Rho-kinase.[3] We also quantified the extent of mental stress at 6 and 12 months after the Earthquake with the post-traumatic stress disorder (PTSD) questionnaire that is widely used for screening of PTSD.[5] The PTSD questionnaire consists of 22 questions for major PTSD symptoms in order to categorize symptom severity into 5 different levels (0–4), where the cut-off value for PTSD is 25 points.[5] All results are expressed as mean±standard deviation (SD) and P values <0.05 were considered to be statistically significant.

Results

The mean age of the 11 patients (8 males, 3 females) was 62±11 years. The prevalence of hypertension, diabetes mellitus and dyslipidemia was 55%, 27% and 91%, respectively. Their left ventricular ejection fraction was well-preserved (71±9%) and 2 of them continued to smoke after the Earthquake. All patients continued their medical treatment with calcium-channel blockers (CCBs) and although there was no change in the levels of serological markers after the Earthquake, including high-sensitivity C reactive protein and lipid profiles (**Table**), Rho-kinase activity in circulating neutrophils was significantly increased at 6 months after the Earthquake and was returned

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Table. Changes in Serological Markers After the Great East Japan Earthquake

	Baseline (n=11)	6 months (n=11)	12 months (n=11)	P value
WBC (/μl)	4,900 (4,600, 6,100)	5,200 (4,600, 5,900)	4,500 (4,200, 7,100)	0.78
Hb (g/dl)	13.4±1.1	13.5±1.2	13.7±1.4	0.87
Ht (%)	39.2±3.2	39.5±3.5	39.4±4.1	0.98
hs-CRP (mg/dl)	0.084 (0.031/0.173)	0.046 (0.022/0.254)	0.052 (0.036/0.166)	0.86
LDL (mg/dl)	108±23	106±32	113±26	0.59
HDL (mg/dl)	46±13	50±16	48±10	0.45
TG (mg/dl)	169±81	171±92	123±54	0.23
Fasting serum glucose (mg/dl)	113±18	117±35	110±37	0.54
HbA_{1c} (%)	5.7±0.6	5.8±0.6	5.8±0.8	0.83

Values are mean±SD or median (25^{th} percentile/75^{th} percentile).
WBC, white blood cells; Hb, hemoglobin; Ht, hematocrit; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides.

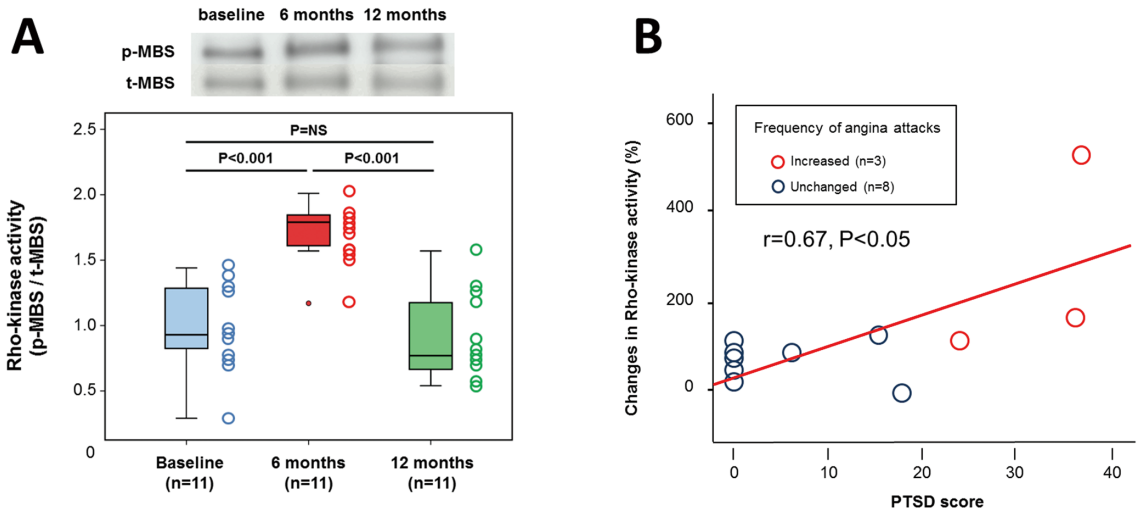

Figure. Changes in Rho-kinase activity in circulating neutrophils of patients with vasospastic angina (VSA). (**A**) Representative Western blotting of phosphorylated myosin-binding subunit (MBS) and total MBS in circulating neutrophils. Comparisons of Rho-kinase activity at 3 time points were performed with one-way ANOVA followed by Tukey's test. (**B**) Positive correlation between the PTSD score and the percent change in Rho-kinase activity from the baseline at 6 months after the Great East Japan Earthquake. Red circles represent VSA patients with increased symptoms after the Earthquake, and blue circles those with unchanged symptoms after the Earthquake, respectively. The relationship between the 2 values was examined with linear regression analysis. PTSD, post-traumatic stress disorder.

to the baseline level at 12 months (0.99±0.34 at baseline, 1.71±0.23 at 6 months, 0.90±0.34 at 12 months) (Figure A). Among the 11 patients, 3 were directly hit by the tsunami and complained of increased frequency of angina attacks after the Earthquake. In these 3 patients, as compared with the remaining 8 patients without direct tsunami-hit or worsening symptoms, both PTSD score (32±7 vs. 5±8, P=0.01) and the percent change in Rho-kinase activity from the baseline (268±232% vs. 55±40%, P<0.05) were significantly higher at 6 months after the Earthquake. Importantly, there was a significant positive correlation between the PTSD score and the percent change in Rho-kinase activity from the baseline at 6 months after the Earthquake (r=0.67, P<0.05) (Figure B). We also confirmed that elevated levels of PTSD score at 6 months after the Earthquake declined at 12 months, as in the case of Rho-kinase, although there was no significant correlation between the changes in the PTSD score and those in Rho-kinase activity from 6 to 12 months after the Earthquake (Table S1).

Discussion

The novel findings of the present study were that Rho-kinase activity in circulating neutrophils of VSA patients was changed dynamically in one year after the Earthquake and that the percent change in Rho-kinase activity from the baseline was positively correlated with the PTSD score in those patients. We have recently demonstrated that a ratio of 1.18 is the best cut-off level of Rho-kinase activity for the diagnosis of VSA and that medical treatment with CCBs ameliorated the level to below the cut-off value in VSA patients.[3] In the present study, although all the patients continued to take CCBs and no significant changes were noted for coronary risk factors or sys-

temic inflammation, Rho-kinase activity was significantly increased to 1.71 at 6 months after the Earthquake, a much higher level than the cut-off level of 1.18 for the diagnosis of VSA, and was returned to the baseline level at 12 months. Indeed, only 3 patients hit by the tsunami had exacerbated symptoms of VSA. However, since approximately two-thirds of the attacks of VSA patients were free of symptoms,[6] it is highly possible that the remaining 8 patients without worsening angina also had asymptomatic ischemic attacks when their Rho-kinase activity was increased after the Earthquake. VSA is known to be worsened by mental stress.[1] In the present disaster with a series of earthquakes and tsunami, mental stress was enhanced in many people in the Tohoku area. Thus, we quantified mental stress with the PTSD score that were established in the Hanshin-Awaji Earthquake in 1995 in Japan[5] and examined the relationship between the severity of PTSD and VSA disease activity. Importantly, we found a positive correlation between these 2 values at 6 months after the Earthquake. Particularly, the VSA patients who were directly hit by the tsunami and complained of increased angina attacks showed higher PTSD score and increased Rho-kinase activity. These findings indicate that coronary vasospastic activity, as reflected by the Rho-kinase activity, was significantly enhanced by the disaster-related mental stress. Coronary spasm plays an important role in the pathogenesis of ischemic heart disease and other systemic disorders.[7,8] Furthermore, it is conceivable that the transient enhanced coronary vasospastic activity may have increased cardiovascular events, such as sudden cardiac death, fatal arrhythmia and heart failure hospitalization, after the Earthquake, as we have recently reported.[9,10] Disaster stress could cause activation of the sympathetic nervous system and exacerbation of risk factors including hypertension, blood viscosity and platelet aggregating activity.[11] These disaster-related risk factors could also exacerbate VSA through Rho-kinase activation, although detailed mechanisms remain to be clarified in future studies.

The limitations of this study include the relatively small number of subjects and the lack of information on Rho-kinase activity in non-VSA subjects.

In conclusions, we were able to demonstrate the transient but significant increase in Rho-kinase activity in VSA patients after the Great East Japan Earthquake Disaster, in proportion to the extent of PTSD score, suggesting the importance of careful management of VSA patients after a disaster.

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Disclosures

Conflicts of Interest: None.

References

1. Yoshida K, Utsunomiya T, Morooka T, Yazawa M, Kido K, Ogawa T, et al. Mental stress test is an effective inducer of vasospastic angina pectoris: Comparison with cold pressor, hyperventilation and master two-step exercise test. *Int J Cardiol* 1999; **70:** 155–163.
2. Shimokawa H, Takeshita A. Rho-kinase is an important therapeutic target in cardiovascular medicine. *Arterioscler Thromb Vasc Biol* 2005; **25:** 1767–1775.
3. Kikuchi Y, Yasuda S, Aizawa K, Tsuburaya R, Ito Y, Takeda M, et al. Enhanced Rho-kinase activity in circulating neutrophils of patients with vasospastic angina: A possible biomarker for diagnosis and disease activity assessment. *J Am Coll Cardiol* 2011; **58:** 1231–1237.
4. JCS Joint Working Group. Guidelines for diagnosis and treatment of patients with vasospastic angina (coronary spastic angina) (JCS2008): Digest version. *Circ J* 2010; **74:** 1745–1762.
5. Asukai N, Kato H, Kawamura N, Kim Y, Yamamoto K, Kishimoto J, et al. Reliability and validity of the Japanese-language version of the impact of event scale-revised (IES-R-J): Four studies of different traumatic events. *J Nerv Ment Dis* 2002; **190:** 175–182.
6. Yasue H, Kugiyama K. Coronary spasm: Clinical features and pathogenesis. *Intern Med* 1997; **36:** 760–765.
7. Maseri A, Beltrame JF, Shimokawa H. Role of coronary vasoconstriction in ischemic heart disease and search for novel therapeutic targets. *Circ J* 2009; **73:** 394–403.
8. Koga S, Ikeda S, Nakata T, Yasunaga T, Takeno M, Koide Y, et al. Low glomerular filtration rate is associated with high prevalence of vasospastic angina. *Circ J* 2011; **75:** 1691–1695.
9. Nakano M, Kondo M, Wakayama Y, Kawana A, Hasebe Y, Shafee MA, et al. Increased incidence of tachyarrhythmias and heart failure hospitalization in patients with implanted cardiac devices after the Great East Japan Earthquake Disaster. *Circ J* 2012; **76:** 1283–1285.
10. Aoki T, Fukumoto Y, Yasuda S, Sakata Y, Ito K, Takahashi J, et al. The Great East Japan Earthquake Disaster and cardiovascular diseases. *Eur Heart J* 2012 August 28 [Epub ahead of print].
11. Kario K. Disaster hypertension: Its characteristics, mechanism, and management. *Circ J* 2012; **76:** 553–562.

Supplementary Files

Supplementary File 1

Table S1. Rho-Kinase Activity and PTSD Score at 6 and 12 Months After the Great East Japan Earthquake

Please find supplementary file(s);
http://dx.doi.org/10.1253/circj.CJ-12-1238