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Original article

Increased levels of the oxidative stress marker, nitrotyrosine in patients with provocation test-induced coronary vasospasm

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ABSTRACT

Background: Endothelial dysfunction of the coronary arteries caused by oxidative stress plays an important role in the pathogenesis of coronary vasospasm. However, it is not clear whether circulating biomarkers for oxidative stress are altered after coronary vasospasm. We investigated temporal changes in the levels of oxidative stress biomarkers after coronary vasospasm induced by intracoronary acetylcholine provocation testing, resulting in transient myocardial ischemia.

Methods and results: Thirty consecutive patients with suspected vasospastic angina pectoris (VSAP) were enrolled in the study. Patients were categorized into the VSAP-positive group ($n = 14$) and the VSAP-negative group ($n = 16$) on the basis of test results. Serum samples were examined for the levels of the oxidative stress markers 4-hydroxynonenal (HNE) and nitrotyrosine (NT) before, and 15 min, 3 h, and 12 h after the provocation test. The serum HNE levels did not change in either group after the test. The serum NT levels in the VSAP-positive group significantly increased at 3 h and 12 h after the test (11.3 ± 3.3 μg/ml at 3 h, $p = 0.015$, and 12.1 ± 5.7 μg/ml at 12 h, $p = 0.03$), as compared with baseline (8.1 ± 3.2 μg/ml). In the VSAP-negative group, the serum NT levels significantly decreased from baseline at each of the 3 time points.

Conclusions: Serum NT significantly increased after coronary vasospasm induced by acetylcholine provocation, suggesting that serum NT could be a biomarker of transient myocardial ischemia and could contribute to the development of VSAP.

Introduction

Coronary vasospasm is involved in the pathophysiology of variant angina, acute myocardial infarction, and sudden cardiac death [1–5]. Endothelial dysfunction of the coronary arteries caused by the overproduction of oxidative stress plays an important role in the pathogenesis of coronary spasm [6–10]. Episodes of vasospastic angina pectoris (VSAP) are characterized by transient myocardial ischemia caused by total or near-total occlusion of a coronary artery. Oxidative stress produced by ischemia/reperfusion injury is known to oxidize lipids and proteins, thereby inducing endothelial dysfunction, atherosclerotic lesions, and myocardial stunning [11–13]. 4-Hydroxynonenal (HNE) is a well-known lipid peroxide produced during cardiac ischemia [14]. Nitrotyrosine (NT) is produced by the modification of protein tyrosine residues by peroxynitrite generated from the reaction of nitric oxide (NO) and superoxide [15]. HNE resides in atherosclerotic lesions and particularly in foam cells, whereas HNE enhances metalloproteinase-2 production in vascular smooth muscle cells and may underlie plaque rupture [16,17]. Peroxynitrite production in the endothelium decreases NO bioavailability and prostacyclin production, decreases vasorelaxation, and changes the vascular tone [18–21], and endothelial dysfunction and peroxynitrite formation are early events in angiotensin-induced cardiovascular disorders [22]. However, no clinical evidence has been provided for HNE and NT in the pathogenesis of VSAP, despite the potential importance of oxidative stress. This study aimed to investigate the effect of oxidative stress on the course of VSAP. To this end, we investigated

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temporal changes in HNE and NT levels after coronary vasospasm induced by intracoronary acetylcholine provocation testing in patients with VSAP and in controls.

Methods

Subjects

Thirty-two consecutive Japanese patients suspected of having VSAP, who had atypical chest pain at rest, were admitted to our hospital and diagnostic coronary angiography was performed. Two patients whose coronary angiography results showed ≥50% organic stenosis were excluded from this study. Therefore, we performed the intracoronary acetylcholine provocation test on the remaining 30 patients who did not have any significant organic stenosis.

The study protocol conformed to the Declaration of Helsinki and was approved by the Human Ethics Committee of Kanazawa Medical University, and all patients provided written informed consent.

Catheterization procedure, angiographic analysis, and blood sample collection

At least 24 h before the provocation test, nitrates, calcium channel blockers, and other anti-anginal drugs, except sublingual nitroglycerin, were withdrawn from all patients after admission. All coronary angiography procedures were performed through a femoral approach by using the standard Judkins technique after the administration of 2000 U of heparin in the morning. In the provocation test, acetylcholine was injected through a catheter into the right coronary artery at a dose of 20 or 50 μg and into the left coronary artery at 50 or 100 μg, each within 1 min. At 3 min from the initiation of each injection, angiography was performed. A standard 12-lead electrocardiogram was recorded continuously to assess ST-segment shift. The patients informed the examiner if they experienced chest pain during the provocation test. When angina developed with chest pain and/or ST-segment shift, angiography was immediately performed, and subsequently 0.25 mg of nitroglycerin was injected into the responsible coronary artery to relieve the symptoms. The heart rate and blood pressure of each patient were monitored continuously during the procedure. Hemodynamic variables were measured before and at the end of acetylcholine infusion. The double product was calculated by multiplying the systolic blood pressure and the heart rate at the same time point. Coronary vasospasm was defined as the transient total or near total (>90%) occlusion of the provoked coronary artery, which was reversible with nitroglycerin. Patients were categorized as test-positive if they developed angina with ST-segment shift and chest pain and showed coronary vasospasm on coronary angiography. Among the 30 patients subjected to the intracoronary acetylcholine provocation test, 14 were positive (VSAP-positive group), and 16 were negative (VSAP-negative group). After the provocation, 0.25 mg of nitroglycerin was injected into both coronary arteries of VSAP-negative patients or the nonresponsible coronary artery of VSAP-positive patients to obtain maximal coronary dilation, and final angiography was performed. Blood samples were collected from the femoral sheath before and 15 min after the provocation test. Follow-up blood samples were obtained from an antecubital vein 3 and 12 h after completion of the provocation test. Serum samples were prepared from each blood sample through centrifugation and were stored at −80 °C until assay.

Measurement of HNE and NT in the serum of patients

Time-resolved fluoroimmunoassay, which is 100 times more sensitive than conventional enzyme-linked immunosorbent assay, was performed to measure HNE and NT, as previously reported [23–25]. The fluorescence intensity was measured at 340 nm excitation and 615 nm emissions, using an Arvo SX multilabel counter (Perkin-Elmer Life Sciences, Boston, MA, USA).

Statistical analyses

Data are expressed as mean ± SD. The Student's *t* test was used to compare the values between the 2 groups. ANOVA for repeated measures was used to assess the differences in the values measured at baseline and at each of the 3 time points. Categorical variables in the patient's clinical background were compared using the χ^2 test or a nonparametric test. Data analysis was performed using StatView software, version 5.0 (SAS Institute, Cary, NC, USA) and R software, version 2.5.1 (http://www.r-project.org/). Statistical significance was defined as $p < 0.05$.

Results

Clinical characteristics of the study group

Table 1 shows the clinical characteristics of patients in the VSAP-positive and VSAP-negative groups. No significant differences were found between the 2 groups with respect to mean age, sex, and the prevalence of coronary risk factors such as hypertension, hyperlipidemia, diabetes mellitus, or smoking status. In the VSAP-positive group, 9 (64%) patients experienced chest pain during the provocation test. Two (14%) and 12 (86%) patients showed total and near-total occlusion, respectively, on the coronary angiogram. All patients with total occlusion showed transient ST-segment elevation, whereas all patients with near-total occlusion showed transient ST-segment depression. No patients in the VSAP-negative group had significant narrowing of the involved coronary artery, electrocardiographic changes, or chest pain during the procedure. None of the studied patients had major complications such as ventricular rhythm disturbance, refractory spasm, or myocardial infarction. The frequency of prior use of nitrates and/or calcium channel blockers before the test was lower in the VSAP-negative

Table 1
Clinical characteristics of the study patients.

	VSAP-positive group	VSAP-negative group	*p*-Value
Total population, *n*	14	16	
Age, years (means ± SD)	66 ± 8.2	63 ± 11	0.62
Men, *n* (%)	5 (36)	9 (56)	0.34
Coronary risk factors, *n* (%)			
Hypertension	7 (50)	7 (44)	0.73
Hyperlipidemia	7 (50)	6 (38)	0.49
Diabetes mellitus	1 (7)	1 (6)	0.92
Smoking	4 (29)	2 (13)	0.27
Chest pain, *n* (%)	9 (64)	0 (0)	
Provoked form, *n* (%)			
100%	2 (14)		
99%	12 (86)		
Provoked coronary, *n* (%)			
LAD	9 (64)		
LCX	1 (7)		
RCA	7 (50)		
Medication at admission, *n* (%)			
Nitrate	7 (50)	3 (19)	0.07
CCB	9 (64)	5 (31)	0.07
Nitrate and/or CCB	12 (86)	6 (38)	0.007
Statins	3 (21)	2 (13)	0.51
Aspirin	5 (36)	4 (25)	0.64
ACEI/ARB	1 (7)	5 (31)	0.08

ACE, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blockers; CCB, calcium channel blockers; LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery; RCA, right coronary artery.