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Relationship between c-reactive protein and the asymmetric dimethylarginine-induced endothelial dysfunction pathway in vasospastic angina

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Vasospastic angina (VSA) is a special form of atherosclerotic disease. There is some evidence suggesting a relationship between inflammation and VSA. We sought to demonstrate the relationship between high-sensitivity c-reactive protein (hs-CRP), a sensitive marker of inflammation, and the asymmetric dimethylarginine (ADMA)-induced endothelial dysfunction pathway in patients with VSA. We studied 68 patients who were diagnosed with VSA with typical symptoms and had a positive hyperventilation test. We determined plasma levels of hs-CRP, ADMA, brachial flow-mediated dilation (FMD), and other biochemical parameters in these 68 VSA patients and 68 age-matched non-VSA subjects. Multivariate logistic regression indicated that hs-CRP (OR, 3.81 $P < 0.001$) and a history of smoking (OR, 3.06 $P = 0.008$) were independently associated with the incidence of VSA. Moreover, we found that CRP was directly related to ADMA ($r = 0.69$, $P < 0.001$) but inversely related to brachial FMD ($r = -0.66$, $P < 0.001$) in patients with VSA. Additionally, ADMA was inversely related to brachial FMD ($r = -0.75$, $P < 0.001$) in patients with VSA. These results indicate that there is a relationship between CRP and the ADMA-induced endothelial dysfunction pathway in patients with VSA. Anti-inflammatory agents may be a potential strategy for the treatment of endothelial dysfunction in VSA.

1. Introduction

Vasospastic angina (VSA) is characterized by attacks that occur at rest and are associated with transient ST segment elevation in an electrocardiogram (ECG). VSA is a special form of atherosclerotic disease that can result clinically in cardiovascular events involving chest pain, myocardial infarction, arrhythmias, and even sudden death (Schulze et al. 2006; Shin et al. 2012). As a type of angina, VSA is caused by coronary spasm (Group JCSJW 2010; Tanaka et al. 2011).

The link between inflammation and vasospastic angina was first reported by Lewis et al. in 1978. Recent evidence has suggested a relationship between inflammation and coronary vasospasm (Li et al. 2007). Hung et al. prospectively investigated the association between high-sensitivity c-reactive protein (hs-CRP), a sensitive marker of inflammation, and coronary vasospasm in a sample of 428 patients. These patients underwent coronary angiography, and no significant coronary artery stenosis was found. The results showed that high-sensitivity CRP levels were independently associated with the diagnosis of vasospastic angina (Hung et al. 2005). However, the pathogenesis underlying the involvement of inflammation in vasospastic angina is not yet clear.

Endothelial dysfunction of the coronary arteries plays a role in the pathogenesis of coronary spasm (Miwa et al. 2009). Asymmetric dimethylarginine (ADMA), which is recognized as an endogenous competitive inhibitor of NO synthase, accounts for this endothelial dysfunction (Davis et al. 2011). It has been shown in some studies that the plasma ADMA concentration in the coronary sinus is higher in VSA patients than in controls (0.42 ± 0.06 μmol/L *vs.* 0.37 ± 0.06 μmol/L, $P < 0.05$) (Hori et al. 2003), and the endothelial function of the coronary arteries is hindered in patients with VSA (Miwa et al. 2009). Therefore, the ADMA-induced endothelial dysfunction pathway plays a role in the pathogenesis of VSA.

The aim of the present study was to investigate the relationship between CRP and the ADMA-induced endothelial dysfunction pathway in patients with VSA.

2. Investigations and results

A total of 68 patients with VSA and 68 subjects without VSA were included in this study. The clinical characteristics and biochemical parameters of the subjects are shown in Table 1. The patients with VSA displayed higher levels of hs-CRP, ADMA, and low-density lipoprotein (LDL), an increased history of cigarette smoking, and a lower level of brachial FMD compared to the non-VSA group. The serum hs-CRP level of the VSA group was significantly higher than that of the non-VSA group (2.31 ± 1.24 mg/L vs. 1.24 ± 0.57 mg/L, $P < 0.001$). The plasma concentration of ADMA was significantly higher in the VSA group than the non-VSA group (0.62 ± 0.14 μmol/L vs. 0.46 ± 0.14 μmol/L, $P < 0.001$). The prevalence of cigarette smoking was also significantly greater in the VSA group than in the non-VSA group (68% vs. 34%, $P < 0.001$). Furthermore, the low-density lipoprotein (LDL) levels of the VSA

Table 1: Clinical characteristics and biochemical parameters of the study groups

	Non-VSA Group (n = 68)	VSA Group (n = 68)	P value
Age (years)	57.3 ± 10.5	60.54 ± 10.2	0.067
Male gender (%)	37 (55)	39 (58)	0.608
Body mass index (kg/m^2)	23.99 ± 2.52	24.75 ± 2.99	0.113
Smoker (%)	23 (34%)	47 (68%)	< 0.001
Hypertension (%)	29 (42)	27 (39)	0.391
Diabetes mellitus (%)	13 (19)	12 (18)	0.826
Hypercholesterolemia (%)	4 (6)	8 (12)	0.231
Total cholesterol (mmol/L)	5.20 ± 0.87	5.40 ± 0.79	0.161
LDL cholesterol (mmol/L)	3.45 ± 0.63	3.69 ± 0.73	0.043
HDL cholesterol (mmol/L)	1.40 ± 0.30	1.30 ± 0.31	0.055
Fasting glucose (mg/dL)	81.75 ± 6.68	80.63 ± 5.74	0.293
hs-CRP (mg/L)	1.24 ± 0.57	2.31 ± 1.24	< 0.001
Plasma ADMA (μmol/L)	0.46 ± 0.14	0.62 ± 0.14	< 0.001
Systolic blood pressure (mmHg)	135.4 ± 18.3	139.7 ± 17.8	0.235
FMD, Brachial flow-mediated dilation responses (%)	4.70 ± 0.67	3.31 ± 0.68	< 0.001

The presented values are the mean ± SD or total numbers (%). Probabilities determined via Student's unpaired t test or X^2 tests. ADMA, asymmetric dimethylarginine; HDL, high-density lipoprotein; FMD, Brachial flow-mediated dilation responses; LDL, low-density lipoprotein; NS, not significant.

group were higher than in the non-VSA group (3.69 ± 0.73 vs. 3.45 ± 0.63, P = 0.043). In contrast, the brachial FMD of the VSA group was significantly lower than that of the non-VSA group (3.31 ± 0.68% vs. 4.70 ± 0.67%, $P < 0.001$). There were no significant differences in terms of age, gender, body mass index, or rates of other disease states between the two groups. Baseline of blood glucose levels and lipid profiles were also comparable between these two groups.

To assess factors predicting the incidence of VSA, univariate and multivariate logistic regression analyses were performed (Table 2). In the univariate analysis, hs-CRP (OR, 4.38 $P < 0.001$), a history of cigarette smoking (OR, 4.09 $P < 0.001$), LDL-C levels (OR, 1.67 P = 0.046), age (OR, 1.03 P = 0.070), and body mass index (OR, 1.11 P = 0.116) were found to be associated with the incidence of VSA. Multivariate analysis showed that hs-CRP levels (OR, 3.81 $P < 0.001$) and a history of cigarette smoking (OR, 3.06 P = 0.008) were independent predictors of VSA.

As shown in the Fig. 1, we discussed the relationships between pairs of the factors hs-CRP, ADMA, and FMD in the VSA group. From this figure, it can be observed that hs-CRP was positively related to ADMA (r = 0.69, $P < 0.001$) (Fig. a) and inversely associated with brachial FMD (r = −0.66, $P < 0.001$) (Fig. b) in the VSA group. Moreover, ADMA was inversely associated with brachial FMD (r = −0.75, $P < 0.001$) in the VSA group (Fig. c).

3. Discussion

The results of this study suggest that CRP is associated with the ADMA-induced endothelial function pathway in patients with VSA.

As a type of angina, vasospastic angina is caused by coronary spasm (Group JCSJW 2010). Smoking, endothelial dysfunction, endothelial nitric oxide synthetase (eNOS) polymorphysms, and markers of oxidative stress are regarded as the most important risk factors for coronary spasm.

The present study found that plasma levels of hs-CRP, a highly sensitive marker of inflammation, are higher in patients with coronary spasm than in those without coronary spasm (Pearson et al. 2003; Pepys and Hirschfield 2003). A multivariate analysis revealed that only a history of smoking and elevated CRP levels, with a cutoff point of 2 mg/L, were independently and significantly associated with coronary spasm (Danesh et al. 2004; Ridker et al. 2005).

Endothelial dysfunction is known to be involved in the pathogenesis of coronary spasm (Kugiyama et al. 1996; Yasue et al. 2008). Motoyama et al. (1998) demonstrated that endothelial function of the brachial artery is impaired in patients with VSA. These authors found that flow-dependent vasodilation was significantly decreased in patients with VSA compared to controls (3.1 ± 1.8 *vs.* 7.1 ± 2.5%, $p < 0.001$). Interestingly, a multivariate logistic regression analysis conducted in the present study indicated that hs-CRP was independently associated with the incidence of VSA. Moreover serum hs-CRP was inversely associated with brachial FMD (r = −0.66, $P < 0.001$) in patients with VSA. Therefore, inflammation may impair endothelial function and be involved in the pathogenesis of coronary spasm.

Nitric oxide (NO) is an elusive mediator that causes vascular dilatation (Palmer et al. 1987). Endothelial dysfunction has been ascribed to either reduced NO release from endothelial cells or reduced bioavailability of NO (Quyyumi et al. 1995). ADMA, as a principal endogenous inhibitor of nitric oxide synthesis (NOS), reduces the formation of NO and causes endothelial dysfunction (Cardounel et al. 2007; Liu et al. 2012).

ADMA is derived from the methylation of arginine residues within proteins *via* the activity of redox-sensitive S-adenosylmethionine–dependent protein arginine N-methyltransferases (Boger et al. 2000). Proinflammatory stimuli have been shown to inhibit dimethylarginine dimethylaminohydrolase activity and the enzymes that degrade ADMA in cell culture studies (Tran et al. 2003). These findings support the observation that ADMA plasma levels are elevated in subclinical inflammatory states (Patel et al. 2008). Thus, it is likely that ADMA may be a key molecule mediating the association between inflammation and endothelial dysfunction (Antoniades et al. 2011). In the present study, serum hs-CRP was found to be directly related to serum ADMA but inversely related to brachial FMD in patients with VSA. Furthermore, serum ADMA was inversely associated with brachial FMD in patients with VSA.

These findings suggest that CRP is associated with the ADMA-induced endothelial function pathway in patients with VSA. Thus, anti-inflammatory agents may be a potential strategy for the treatment of endothelial dysfunction in VSA.

Table 2: Logistic regression analysis for VSA

Factor	Univariate analysis			Multivariate analysis		
	OR	95%CI	P value	OR	95%CI	P value
Age (years)	1.03	0.99−1.07	0.070	1.02	0.98−1.06	0.454
Male gender (%)	1.19	0.61−2.35	0.605			
Body mass index (kg/m^2)	1.11	0.98−1.25	0.116	1.09	0.94−1.26	0.273
Smoker (%)	4.09	2.00−8.36	< 0.001	3.06	1.34−7.02	0.008
Hypertension (%)	1.35	0.68−2.67	0.387			
Diabetes mellitus (%)	0.91	0.47−2.55	0.825			
Hypercholesterolemia (%)	2.13	0.61−7.45	0.235			
LDL cholesterol (mmol/L)	1.67	1.01−2.78	0.046	1.47	0.80−2.69	0.214
Fasting glucose (mg/dL)	1.01	0.99−1.02	0.251			
hs-CRP (mg/L)	4.38	2.41−7.97	< 0.001	3.81	2.04-7.09	< 0.001
Systolic blood pressure (mmHg)	1.01	0.99−1.03	0.234			

LDL, low-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; OR, odds ratio; CI, confidence interval.

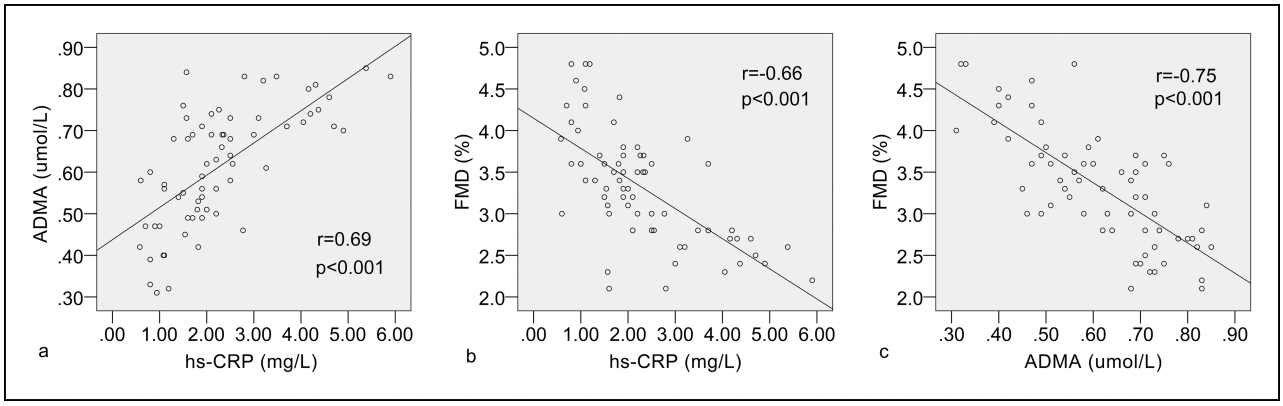

Fig. 1: Relationship between hs-CRP and ADMA (a); hs-CRP and FMD (b); ADMA and FMD (c) in the VSA group high-sensitivity C-reactive protein–hs-CRP; Asymmetric dimethylarginine–ADMA; Flow-mediated dilation–FMD; Vasospastic angina–VSA.

4. Experimental

4.1. Study subjects

Non-vasospastic angina group (Non-VSA group): Sixty-eight age- and gender-matched healthy subjects were included in this study. These subjects were recruited from a physical examination test center of the First Affiliated Hospital of Harbin Medical University. They showed no clinical evidence of vasospastic angina according to Japanese Circulation Society (JCS) Guidelines (Group JCSJW 2010). They also presented no spontaneous chest pain, no ECG findings (a transient ST elevation of 0.1 mV or more recorded in at least two contiguous leads in 12-lead ECG), and no significant coronary artery stenosis (< 50% luminal diameter of major coronary arteries) upon coronary CT angiography (CTA), but they did display a negative hyperventilation test.

vasospastic angina group (VSA group): The study group included 68 patients with VSA referred to our hospital for assessment from October 2009 to January 2012. According to Japanese Circulation Society (JCS) Guidelines (Group JCSJW 2010), the entry criteria were as follows: angina occurred at rest and was associated with a transient ST segment elevation of $\geq$ 1 mV observed via standard ECG or 24-hour ambulatory ECG Holter monitoring; the attack was relieved by sublingual administration of nitroglycerin; a positive hyperventilation test was defined as an ST segment elevation of $\geq$ 1 mV observed on the monitor or 12-lead ECG after an explanation of hyperventilation; and there was no evidence of acute myocardial infarction after the pain and no significant coronary artery stenosis (< 50% luminal diameter of major coronary arteries) upon coronary angiography.

The protocol conformed to the ethical guidelines of our institutions, and informed consent was obtained from each participant.

4.2. Hyperventilation test

A hyperventilation test was conducted at rest in the early morning after an interval of at least 48 h following the administration of vasoactive drugs. Patients promoted vigorously hyperventilation (target respiratory rate of 25 times/min or higher) for 6 min to the extent that was possible. These patients were monitored with a 12-lead ECG during hyperventilation and for 10 min after its completion. Hyperventilation was discontinued immediately as soon as an anginal attack or a significant ST-T change was observed in the ECG. When an anginal attack occurred, the patient was immediately administered a fast-acting nitrate (Group JCSJW 2010).

4.3. Measurement of brachial FMD

Endothelial function was assessed by measuring the flow-mediated dilation (FMD) of the right brachial artery following a method described previously (McDonald 1990; Nerla et al. 2012). Briefly, after a 10 min rest period, images of the right brachial artery were obtained approximately 5 cm proximal to the antecubital crease using a 7.0-MHz linear array transducer and an iE33 xMATRIX echocardiography system (Philips Ultrasound, Philips Medical Systems, Bothell, WA). Measurements were performed twice, at baseline and then 1 to 15 min after the release of an upper arm arterial occlusion that had been maintained at 220 mm Hg for 5 min. The FMD was calculated as the maximum percent change in the brachial artery diameter during hyperemia compared to baseline measurements. Independent observers calculated the mean value of the two measurements.

4.4. Coronary angiography

Left and right coronary angiography was performed for all patients with VSA using standard techniques, and the results were analyzed by at least two experienced physicians.

4.5. Coronary CT angiography (CTA)

All 68 non-VSA subjects underwent coronary CT angiography (TOSHIBA).

4.6. Laboratory analysis

Blood samples were taken from the subjects after an overnight fast. Total cholesterol, high-density lipoprotein cholesterol, triglycerides and glucose were measured using standard methods. Low-density lipoprotein cholesterol was calculated using the Friedewald formula. hs-CRP levels were measured via immunological turbidity (Beckman Coulter IMMAGE800, United States

of America). Plasma ADMA concentrations were determined through high-performance liquid chromatography (Teerlink et al. 2002).

4.7. Statistical analysis

The results are presented as the mean ± SD for continuous variables and as a percentage of the total number of patients for categorical variables. Student's unpaired *t*-test and chi-square tests were used for comparing continuous and categorical variables, respectively. If the data were not normally distributed, the Mann-Whitney *U*-test was employed.

Logistic regression analysis was performed to evaluate the relationship between the incidence of VSA and the following parameters: age, male gender, body mass index, smoking, hypertension, diabetes, LDL cholesterol, hypercholesterolemia, fasting glucose, and hs-CRP. Only the variables showing significant associations upon univariate analysis ($P < 0.20$) were included in the multivariate analysis. ADMA and brachial FMD were not entered into the univariate or multivariate logistic regression analysis because they were associated with hs-CRP.

Relationships between paired parameters were analyzed using the Pearson product moment correlation coefficient. We tested the independent relationships between hs-CRP and ADMA; hs-CRP and brachial FMD; and serum ADMA and brachial FMD in patients with VSA. All P-values were 2-sided, and a $P < 0.05$ was considered significant. All calculations were performed with a standard statistical package (SPSS for Windows version 13.0).

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