Endothelial dysfunction is an independent factor responsible for vasospastic angina

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A B S T R A C T

In order to evaluate peripheral endothelial function in patients with vasospastic angina (VSA), we measured flow-mediated dilation (FMD) of the brachial artery in patients with VSA and compared it with FMD in patients without VSA. Endothelial dysfunction is considered one of the mechanisms underlying VSA. However, its exact role remains to be clarified. The study included 30 patients with positive spasm-provocational test results without evidence of significant coronary stenosis (VSA group) and 30 patients with negative spasm-provocational test results without evidence of significant coronary stenosis (control group). In each patient, brachial artery diameter responses to hyperemic flow and glyceryl trinitrate spray were measured using high-resolution ultrasound. The carotid intima-media thickness was also measured as a marker of systemic atherosclerosis. FMD was lower in the VSA group (4.8±0.5%) compared with the control group (9.4±0.7%, P<0.0001). In the VSA group, FMD was not affected by coronary risk factors or the presence of atherosclerotic changes on coronary angiography. Glyceryl trinitrate-induced dilation did not differ between the two groups. The intima-media thickness was comparable between the VSA (0.85±0.04 mm) and control groups (0.81±0.05 mm). These findings indicated that peripheral endothelial function is impaired only in the VSA group, whereas the atherosclerotic changes were similar in the two groups. We conclude that endothelial dysfunction may be an independent factor responsible for the development of VSA.

INTRODUCTION

Coronary spasm, which results from increased vaso-motor tone in the epicardial coronary arteries, can cause myocardial ischaemia [1]. The precise mechanisms underlying coronary spasm remain to be elucidated. However, several factors, including changes in autonomic tone [2], enhanced α-adrenergic receptor activity [3], hyper-reactivity of the coronary smooth muscle [4] and magnesium deficiency [5], have been implicated in the genesis of coronary spasm.

Although several studies have suggested that endothelial dysfunction may contribute to the genesis of coronary spasm [6–8], other studies have shown that endothelial function is preserved in patients with vasospastic angina (VSA) [4,9–11]. Therefore, to evaluate whether peripheral endothelial dysfunction is present in patients with VSA, we measured flow-mediated dilation (FMD) of the brachial artery, as an index of endothelial function, and the intima-media thickness (IMT) of the carotid artery, as a marker of systemic atherosclerosis, in patients with VSA. Furthermore, we compared these...
parameters with those from patients without VSA, as well as patients with significant coronary stenosis.

METHODS

Subjects
We studied 30 Japanese patients with VSA (VSA group: mean age, 64 years; 19 men, 11 women) who fulfilled the following inclusion criteria: (1) presence of spontaneous chest pain associated with ST segment elevation or depression on a resting 12-lead ECG or ambulatory ECG, (2) a positive result on spasm-provocation testing, and (3) the absence of significant organic coronary artery stenosis (> 50%) based on coronary angiography. We also studied 30 patients with a negative result on spasm-provocation testing and no evidence of significant coronary stenoses (control group). The two groups were well matched with respect to age, gender and body mass index. In addition, we studied 30 patients with coronary artery disease (CAD group), who fulfilled the following criteria: (1) the presence of exertional chest pain, (2) a positive result on exercise testing and/or stress myocardial perfusion imaging, and (3) angiographic evidence of significant coronary stenosis. Patients with previous myocardial infarction, heart failure or other serious diseases were excluded from the study. Written informed consent was obtained from all of the patients before entry into the study. The protocol was approved by the ethics committee of our institution.

Ultrasound measurements
All vasodilatory therapies were withheld for at least 24 h before the study, except for the unrestricted use of sublingual glyceryl trinitrate (GTN), which was withheld 1 h before the study. All studies were performed early in the morning (6:00–8:00 hours) before coronary angiography, using a 10 MHz linear array ultrasound probe attached to an ultrasound system (EUB-8000; Hitachi Medical, Tokyo, Japan).

Brachial artery study
High-resolution ultrasound was used to measure changes in the diameter of the brachial artery in response to reactive hyperaemia (FMD), as an index of endothelium-dependent dilation, and to GTN, as an index of endothelium-independent dilation, as described previously [12–14]. Each patient rested in the supine position for 10 min before the first scan was performed and remained supine throughout the study. The right brachial artery was scanned in a longitudinal view above the elbow to find the clearest images of the anterior and posterior wall layers. All measurements were performed with the same position of the transducer and the arm. Blood flow velocity in the brachial artery was measured with a pulse-Doppler signal oriented at a 70° angle to the vessel placed in the centre of the artery. Blood flow was calculated by multiplying the velocity–time integral of the Doppler-flow signal by the heart rate and vessel cross-sectional area. After the baseline brachial artery diameter and blood flow velocity had been measured, a blood pressure cuff was placed around the forearm and inflated to a pressure of 250 mmHg. After 4.5 min, the cuff was deflated. Blood flow velocity was measured 15 s after cuff deflation, and the arterial diameter was measured 45–60 s after cuff deflation. After 10 min, baseline measurements were repeated. GTN spray (0.3 mg) was then administered, and 4 min later the final measurements were obtained.

All images were recorded on super-VHS videotape and later analysed by the same investigator who was blinded to the patients’ characteristics. The diameter was always measured at end-diastole (peak of the R-wave on the ECG) using electronic calipers. Measurements were the mean of five cardiac cycles. FMD is expressed as the percentage change in diameter after reactive hyperaemia compared with the baseline value. The increase in flow is expressed as the percentage increase in blood flow during reactive hyperaemia relative to the blood flow at baseline. Likewise, GTN-induced dilation is expressed as the percentage change in diameter after GTN administration compared with the baseline value. In our studies, the intra- and inter-observer variability for the repeated measurements of resting arterial diameter were 0.021 ± 0.003 mm and 0.054 ± 0.004 mm respectively. Furthermore, when this study was performed at the same time on two separate days in 18 patients, the between-occasion within-patient difference for the measurement of the percentage increase in the arterial diameter during reactive hyperaemia was 1.3 ± 0.3%.

IMT measurement
The IMT of the carotid artery was determined as described previously [15,16]. This measurement was determined for the far wall of the right carotid artery. The common carotid artery was studied in the longitudinal plane from the level of the common carotid artery to the bifurcation of the common carotid artery. The images were recorded from the approach showing the greatest distance between the lumen–intima interface and the media–adventitia interface. All scans were recorded on super-VHS videotape for subsequent analysis. The IMT was measured from the B-mode screen to within 10 mm proximal to the bifurcation, synchronized with the R-wave peak by one investigator who was blinded to the patient characteristics. The mean IMT was calculated as the mean of ten measurements using electronic calipers. In our studies presented here, the intra- and inter-observer variabilities for the repeated measurement of
Table 1  Demographic and clinical characteristics of the study patients

Results are expressed as the means ± S.E.M. Abbreviation: NS, not significant.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>VSA (n = 30)</th>
<th>Control (n = 30)</th>
<th>CAD (n = 30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64 ± 1</td>
<td>63 ± 2</td>
<td>64 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>Men/women</td>
<td>19/11</td>
<td>19/11</td>
<td>20/10</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.1 ± 0.5</td>
<td>23.8 ± 0.6</td>
<td>23.7 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Coronary risk factor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>5 (17)</td>
<td>7 (23)</td>
<td>11 (37)</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>16 (53)</td>
<td>18 (60)</td>
<td>21 (70)</td>
<td>NS</td>
</tr>
<tr>
<td>Hypercholesterolemia (%)†</td>
<td>9 (30)</td>
<td>7 (23)</td>
<td>14 (47)</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>4 (13)</td>
<td>5 (17)</td>
<td>11 (37)</td>
<td>NS</td>
</tr>
<tr>
<td>Having any coronary risk factors</td>
<td>24 (80)</td>
<td>24 (80)</td>
<td>29 (97)</td>
<td>NS</td>
</tr>
<tr>
<td>Left ventricular ejection fraction</td>
<td>68 ± 1</td>
<td>70 ± 2</td>
<td>70 ± 1</td>
<td>NS</td>
</tr>
<tr>
<td>Coronary angiogram</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum stenosis (%)</td>
<td>25 ± 3</td>
<td>23 ± 3</td>
<td>78 ± 3</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Number of diseased vessels</td>
<td>0</td>
<td>0</td>
<td>1.6 ± 0.1</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Atherosclerotic change (%)</td>
<td>21 (70)</td>
<td>20 (67)</td>
<td>30 (100)</td>
<td>0.0023</td>
</tr>
</tbody>
</table>

* On therapy and/or blood pressure ≥ 140/90 mmHg.
† On therapy and/or total cholesterol ≥ 240 mg/dl.

IMT were 0.08 ± 0.01 mm and 0.13 ± 0.02 mm respectively.

Coronary angiography

All anti-anginal therapy was discontinued for at least 24 h before catheterization, except for the unrestricted use of sublingual GTN. Diagnostic coronary angiography was performed using the percutaneous brachial approach. A 5 F temporary pacing electrode catheter (Bard, Tewksbury, MA, U.S.A.) was placed in the right ventricular apex via the right internal jugular vein, and connected to a temporary pacemaker, which was set at a rate of 50 beats/min. After baseline coronary angiograms were obtained, incremental doses of acetylcholine were infused into the left coronary artery (3 µg/min, 30 µg/min and 100 µg/min) for 2 min with 5-min intervals between consecutive doses until coronary spasm was induced. Once coronary spasm was induced, the infusion of acetylcholine was stopped. If coronary spasm was not induced by acetylcholine, then incremental doses of methylergometrine maleate were infused into the left coronary artery (10 µg/min, 20 µg/min and 30 µg/min) for 1 min with 1-min intervals between consecutive doses. If coronary spasm was not induced by the infusion of methylergometrine maleate into the left coronary artery, incremental doses were infused into the right coronary artery (15 µg/min and 25 µg/min) using the same method as described for the left coronary artery. When coronary spasm was induced, GTN (200 µg) was given as an intra-coronary injection. The spasm-provocational test was not performed in patients with CAD.

When coronary spasm was induced, GTN (200 µg) was given as an intra-coronary injection. The spasm-provocational test was not performed in patients with CAD.

The arterial diameters were measured without knowledge of the clinical characteristics of the patients. The end diastolic frame was selected, and images were analysed using a computer-assisted coronary angiographic analysis system (CAAS II/QUANTCOR; Siemens AG, Berlin and Munich, Germany). The average value of three measurements of luminal diameter and percentage stenosis were used for analysis. A positive spasm-provocational test was defined as a ≥ 50% reduction in the diameter of the artery during coronary angiography associated with ST segment changes and/or typical chest pain after intracoronary injection of the drugs. Significant coronary stenosis was defined as a ≥ 50% stenosis of the major coronary arteries. Atherosclerotic changes were defined as < 50% stenosis.

Statistics

All results are expressed as the means ± S.E.M. Differences between the three groups were analysed by one-way analysis of variance and best-fit tests. Scheffe’s test for multiple comparisons was used when the analysis of variance revealed significant differences between the groups. Differences between the two subgroups in the VSA group were analysed by Mann–Whitney U test. \( P < 0.05 \) was considered statistically significant.

RESULTS

Clinical characteristics

The clinical characteristics of the patients are summarized in Table 1. The age, gender, body mass index and left ventricular ejection fraction were similar in the three
Table 2  Brachial artery diameter and blood flow
Results are expressed as the means ± S.E.M. Abbreviation: NS, not significant.

<table>
<thead>
<tr>
<th>Variable</th>
<th>VSA</th>
<th>Control</th>
<th>CAD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline mean blood pressure (mmHg)</td>
<td>109 ± 4</td>
<td>105 ± 4</td>
<td>105 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline heart rate (beats/min)</td>
<td>59 ± 2</td>
<td>62 ± 2</td>
<td>61 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>Brachial diameter (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.79 ± 0.13</td>
<td>3.70 ± 0.12</td>
<td>3.67 ± 0.10</td>
<td>NS</td>
</tr>
<tr>
<td>After cuff deflation</td>
<td>3.96 ± 0.11</td>
<td>4.04 ± 0.12</td>
<td>3.77 ± 0.10</td>
<td>NS</td>
</tr>
<tr>
<td>After GTN spray administration</td>
<td>4.46 ± 0.13</td>
<td>4.34 ± 0.12</td>
<td>4.27 ± 0.10</td>
<td>NS</td>
</tr>
<tr>
<td>Brachial blood flow</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (ml/min)</td>
<td>190 ± 20</td>
<td>182 ± 23</td>
<td>199 ± 27</td>
<td>NS</td>
</tr>
<tr>
<td>Increase in flow after hyperaemia (%)</td>
<td>384 ± 43</td>
<td>402 ± 44</td>
<td>374 ± 43</td>
<td>NS</td>
</tr>
</tbody>
</table>

Brachial artery diameter and blood flow
The brachial artery diameter and blood flow parameters are summarized in Table 2. The mean blood pressure and heart rate at baseline were similar in the three groups. The brachial diameter at baseline, after hyperaemia and after GTN administration did not differ between the three groups. Brachial blood flow at baseline and percentage increase in blood flow after hyperaemia were also comparable among the three groups.

FMD
FMD was 4.8 ± 0.5% in the VSA group, 9.4 ± 0.7% in the control group and 2.6 ± 0.3% in the CAD group (Figure 1). FMD in the VSA group was lower compared with the control group (P < 0.0001), but it was not as depressed as in the CAD group (P = 0.0149 VSA group compared with the CAD group). In addition, FMD was lower in the VSA group than in the control group, irrespective of the presence of atherosclerotic changes in the coronary arteries (presence of atherosclerotic changes: 5.0 ± 0.6% in the VSA group, 9.4 ± 0.9% in the control group, P < 0.0001) (absence of atherosclerotic changes: 4.3 ± 1.0% in the VSA group, 9.4 ± 0.8% in the control group, P =

Figure 1  FMD (A) and GTN-induced dilation (B)
FMD in the VSA group (4.8 ± 0.5%) was lower than in the control group (9.4 ± 0.7%), but it was not as severe as in the CAD group (2.6 ± 0.3%). GTN-induced dilation did not differ between the three groups (18.5 ± 1.5%, 17.5 ± 1.0% and 16.6 ± 1.2% in the VSA, control and CAD groups respectively). ●, individual values; ○, means ± S.E.M.
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Figure 2  FMD based on the presence (A) or absence (B) of atherosclerotic changes of the coronary arteries in the VSA and control groups

FMD was lower in the VSA group compared with the control group, both in the subgroups with atherosclerotic changes (5.0 ± 0.6% in the VSA group, 9.4 ± 0.9% in the control group) and the subgroups without atherosclerotic changes (4.3 ± 1.0% in the VSA group, 9.4 ± 0.8% in the control group).

GTN-induced dilation

GTN-induced dilation was 18.5 ± 1.5% in the VSA group, 17.5 ± 1.0% in the control group, and 16.6 ± 1.2% in the CAD group (Figure 1). GTN-induced dilation did not differ between the three groups.

IMT

The IMT was similar between the VSA (0.85 ± 0.04 mm) and control groups (0.81 ± 0.04 mm), but was smaller compared with the CAD group (1.03 ± 0.06 mm).

DISCUSSION

In the present study, we evaluated endothelium-dependent and -independent dilation of the brachial artery, as well as the IMT of the common carotid artery in patients with VSA, and compared these parameters with those in patients without VSA. Our results demonstrate that endothelial function is impaired in patients with VSA, whereas endothelium-independent dilation and IMT are comparable in patients with and patients without VSA. These findings suggest that endothelial dysfunction may contribute to the genesis of coronary spasm.

With respect to endothelial function of coronary arteries in patients with VSA, there are two contradictory theories. One theory suggests that coronary endothelial function is impaired [6,8], whereas the other suggests that endothelial function is preserved [4,9]. The same controversy exists about endothelial function of the brachial artery. Motoyama and co-workers [7,17,18] demonstrated that endothelial function of the brachial artery is impaired in patients with VSA. In contrast, Botker et al. [10] and Ito et al. [11] showed that endothelial function of...
the brachial artery in patients with VSA is the same as in normal individuals. In the present study, FMD was lower in patients with VSA compared with the control group, indicating that endothelial dysfunction is present in patients with VSA, which is in keeping with the results reported by Motoyama and co-workers [7,17,18]. Several factors may account for these conflicting results. Firstly, the examination time of the brachial artery study may affect FMD. We performed the brachial artery ultrasound study early in the morning (6:00–8:00 hours). Coronary spasm often occurs early in the morning [19], and it is possible that in patients with VSA FMD may be minimal early in the morning. However, there are no results concerning circadian changes in FMD in patients with VSA. Secondly, racial differences may affect FMD. It has been reported that the response of the coronary artery to acetylcholine differs between Japanese and Caucasians [20]. The same may be true for the brachial artery. Moreover, it has been reported that measurements of FMD are affected by the placement of the blood pressure cuff [21] and the basal diameter of the brachial artery [22]. These differences may account for the conflicting FMD results in patients with VSA.

FMD did not differ between the VSA subgroups. As a matter of fact, the present study may be underpowered because only a small number of patients with VSA were included. However, a large percentage of the study patients had coronary risk factors. Therefore, the finding may contribute to the small influence of coronary risk factors on FMD in the present study. In addition, we have reported previously [23] that significant stenosis (> 50%) can affect FMD, whereas atherosclerotic changes cannot affect FMD. Therefore, we conclude that endothelial dysfunction may be present in the absence of associated coronary risk factors or atherosclerotic changes, and that endothelial dysfunction may be one of the mechanisms responsible for VSA.

There has been only one study demonstrating that the IMT is greater in patients with VSA than in control patients [24]. In the report by Shinozaki et al. [24], a high percentage of patients with insulin resistance and low concentrations of low-density lipoprotein cholesterol were included in their group of patients with VSA. In our study population, the incidence of coronary risk factors were similar between the VSA and control groups, although insulin sensitivity was not assessed in our study. Therefore, differences in the study populations may have led to the conflicting results. We found that the IMT, a marker of systemic atherosclerosis, was similar in patients with VSA compared with the control group, indicating that the atherosclerotic burden was similar in the two groups. The finding that patients with VSA had impaired FMD and preserved IMT supports our hypothesis that in patients with VSA, endothelial dysfunction is an independent factor contributing to the genesis of coronary spasm.

In patients with VSA, it has been reported that an increased concentration of reactive oxygen species inactivates endothelial NO and leads to endothelial dysfunction [18,25]. Smoking, which increases the concentration of reactive oxygen species [26], occurred at the same frequency in the VSA and control groups in the present study. However, in patients with VSA, other factors, including insulin resistance [18,24,27] and vitamin C deficiency [18], may cause an increase in the concentration of reactive oxygen species. It has also been reported that mutations in the endothelial NO synthase gene occur more frequently in patients with VSA [28]. These factors may cause the endothelial dysfunction in patients with VSA.

There are several limitations to the present study. Only a small number of patients with VSA were included in our study. With this small number of patients, statistically significant differences in the VSA subgroups could not be identified. We analysed major coronary risk factors, such as smoking, presence of hypertension, hypercholesterolaemia and diabetes mellitus, but we did not examine metabolic factors such as insulin resistance, which might affect endothelial function. Our spasm-provocation testing protocol may be aggressive. We have adopted this protocol because of the following reasons: (1) we can observe coronary endothelial function and induce coronary vasospasm with intracoronary infusion of acetylcholine, and (2) the ability to induce coronary spasm seems to be greater with intracoronary infusion of ergonovine than acetylcholine [29,30]. Therefore, we infused acetylcholine at first and then ergonovine when the acetylcholine-provocation test was negative. However, this protocol increases the percentage of patients with the false-positive tests (i.e., patients who have coronary severe vasocostriction, but no symptoms of VSA). Therefore, we used the three inclusion criteria to identify 30 patients with typical VSA.

In conclusion, endothelial function of the brachial artery is impaired in patients with VSA compared with endothelial function in patients without VSA. Endothelial dysfunction in patients with VSA did not differ based on the presence of coronary risk factors or atherosclerotic changes of the coronary arteries. Furthermore, the IMT of the common carotid artery, a marker of systemic atherosclerosis, is preserved in patients with VSA. These findings indicate that endothelial dysfunction may be an independent factor contributing to the genesis of coronary spasm.

ACKNOWLEDGMENTS

We are grateful to Koichiro Nakashima (Department of Internal Medicine, Shobara Red Cross Hospital), and Yukihito Higashi, Hidekazu Hirao, Shota Sasaki, Yukihiro Fukuda, Keiji Matsuda and Keigo Nakagawa.
(First Department of Internal Medicine, Hiroshima University School of Medicine) for their technical assistance and helpful comments. We also thank Miss Yuko Omura for her secretarial assistance.

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Received 23 April 2001/18 July 2001; accepted 5 September 2001