Impaired Endothelium-Dependent Vasodilation in the Brachial Artery in Variant Angina Pectoris and the Effect of Intravenous Administration of Vitamin C

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Endothelial dysfunction in the coronary artery contributes to the pathogenesis of variant angina, and endothelial dysfunction in variant angina may be associated with increased oxidant stress in the systemic arteries. We investigated whether endothelial dysfunction exists in the peripheral artery in patients with variant angina, and also examined the effect of vitamin C, an antioxidant, on endothelium-dependent vasodilation. Using high-resolution ultrasound, both the flow-mediated vasodilation (FMD, endothelium-dependent vasodilation) and sublingual nitroglycerin-induced vasodilation (NTG-D, endothelium-independent vasodilation) in the brachial artery were measured in 28 patients with variant angina and 24 control subjects who had normal coronary arteries. FMD was significantly impaired in patients with variant angina compared with control subjects (1.8 ± 2.2% vs 6.4 ± 4.9%, p <0.001). FMD and NTG-D before and after intravenous administration of either vitamin C or placebo were measured in 17 patients with variant angina. FMD significantly improved after the administration of vitamin C (from 2.2 ± 2.4% to 4.5 ± 1.6%, p <0.01), but not after adminstration of the placebo (from 2.0 ± 2.6% to 1.7 ± 1.9%). The improved FMD due to vitamin C in patients with variant angina, however, was not significantly different from that in the control subjects. NTG-D was not significantly different between patients with variant angina and control subjects (14.0 ± 7.8% vs 13.6 ± 5.0%) and it was also not affected by vitamin C. In conclusion: (1) FMD in the brachial artery is impaired in patients with variant angina, and (2) the acute administration of the antioxidant, vitamin C, was observed to reverse this endothelial dysfunction. These findings support the theory that the systemic inactivation of nitric oxide due to oxidative stress might exist in patients with variant angina. ©2001 by Excerpta Medica, Inc.

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Coronary artery spasm is involved in the pathogenesis of not only variant angina but also of fixed coronary artery disease, acute myocardial infarction, and sudden cardiac death. Several factors, including endothelial injury and/or vascular smooth muscle hypersensitivity to vasoconstrictor stimuli, have been considered to play a role in the mechanism of coronary artery spasm.[1] Previous studies have shown that impaired endothelial function plays an important role in this abnormal coronary vasoreactivity.[1,2] Recent studies have demonstrated decreased endothelial nitric oxide activity in the coronary artery to be associated with the pathogenesis of variant angina.[3,4] Decreased nitric oxide activity in the coronary artery induces impairment of endothelial-dependent vasodilation (flow-mediated vasodilation [FMD]). Vitamin C is a typical water-soluble antioxidant and effectively scavenges free radical species. Increased oxidative stress has been shown to inactivate endothelium-derived nitric oxide. The link of increased oxidative stress and decreased levels of endothelium-dependent vasodilation has been shown in patients with atherosclerotic risk factors; of these factors, smoking has been shown to be a major risk for variant angina and cigarettes contain large amount of free radicals.[5] A recent study demonstrated that administration of vitamin C attenuated abnormal vasomotor reactivity in the coronary artery in variant angina, thus suggesting that increased oxidant stress causes endothelial dysfunction in variant angina.[6] However, the effect of vitamin C on systemic endothelial function in variant angina has not yet been elucidated. The purpose of the present study is: (1) to confirm whether or not systemic endothelial dysfunction exists in variant angina, and (2) to investigate whether the acute administration of vitamin C can restore such systemic endothelial dysfunction if patients with variant angina demonstrate endothelial dysfunction in the brachial artery.

METHODS

Study populations: This study comprised 2 study groups. The first study consisted of 28 patients with variant angina (20 men and 8 women, mean age 59 ± 7 years) and 24 control subjects (17 men and 7 women, mean age 56 ± 12 years) who underwent coronary angiography and had normal coronary arteries. The second group was comprised of 17 patients

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with variant angina (11 men and 6 women, age 58 ± 6 years). All patients with variant angina had spontaneous chest pain at rest and underwent diagnostic coronary angiography. The diagnosis of variant angina was made if the following criteria were satisfied: (1) no significant luminal diameter stenosis of a major branch of coronary artery (<50%) on angiogram, (2) a documented ST-segment elevation during angina immediately relieved by sublingual nitroglycerin, or the presence of coronary spasm induced by intracoronary injection of acetylcholine that was associated with chest pain and ischemic ST-T changes. The control subjects underwent diagnostic coronary angiography for an evaluation of atypical chest pain or electrocardiographic abnormality; all control subjects had normal coronary arteries. All vasoactive medications were withheld for ≥12 hours before starting the study. Patients with unstable angina, previous myocardial infarction, heart failure, or any other serious cardiovascular diseases were excluded. Any patients in whom the administration of vitamin C was inappropriate, such as those with renal dysfunction or bronchial asthma, were also excluded. Informed consent was obtained from all subjects.

Brachial artery vasodilator response study: The brachial artery vasodilator responses were measured by ultrasound techniques that have all been previously validated.[7–12] A 7.5-MHz linear array transducer and ultrasound machine (SONOS 2000, Hewlett Packard Company, Andover, Massachusetts) were used to image the brachial artery. All imaging was performed with the subjects resting in a supine position in a temperature-controlled room. Patients refrained from ingesting any beverages containing caffeine or alcohol and any meals for ≥12 hours before performing the brachial vasodilator response study. The brachial study was performed around 8:00 A.M. at all phases of the present study. The subject's right arm (the predominant arm) was comfortably immobilized in an extended position to image the brachial artery. For each subject, optimal brachial artery images in a longitudinal fashion were obtained between 2 and 10 cm above the antecubital fossa. This location was then marked, and all subsequent imaging was obtained at the same location. All images were recorded on super VHS videotapes for later analysis. First, baseline 2-dimensional images were obtained to measure the baseline arterial diameter. The pulse Doppler blood flow velocity at baseline was then measured with the signal at a 60° angle to the artery and the range gate was adjusted to 1.0 mm and positioned in the center of the vessel. To induce hyperemia, the blood pressure cuff was inflated to occlusive pressure (≥50 mm Hg over the systolic blood pressure) at the most proximal position of the forearm. The artery was occluded for 5 minutes and then the cuff was rapidly deflated. Pulse Doppler signals were recorded to determine the maximal hyperemic flow velocity for the first 15 seconds after cuff deflation. Two-dimensional images of the artery during reactive hyperemia were obtained for 60 to 90 seconds after cuff deflation to determine the flow-mediated vasodilator response.

Nitroglycerin-induced vasodilation (NTG-D), endothelial-independent response, was assessed as follows: ≥10 minutes after measuring the brachial vasodilator response (to allow the vessel diameter to return to prehyperemia size), 2-dimensional images at baseline, and 3 to 5 minutes after the sublingual administration of nitroglycerin (0.3 mg) were obtained.

To measure brachial artery diameter, 2-dimensional images at the same points of the cardiac cycle that were at peak systole (close to end of the T wave on the electrocardiogram) were selected and a 10- to 20-mm segment of the artery was identified for analysis using the anatomic landmark in each patient by playing back the videocassette recorder. A quantitative coronary artery angiography analysis device (Cardio 500, Kontron Elektronik Corp., Munich, Germany), which contained a digitizing board, was used for digitizing the artery diameter. Three separate images were digitized and the average segment diameter was thus determined. FMD during hyperemia and NTG-D were determined as the percent change in the artery diameter. Brachial artery blood flow at baseline and during reactive hyperemia was obtained by a previously described method.[8,11] Blood flow volume was calculated by multiplying the vessel cross-sectional area (πr^2) and the velocity-time integral of the Doppler flow signal using a commercial software package (SONOS 2000, Hewlett Packard Company). The relative increase in the blood flow at reactive hyperemia was calculated as the maximum flow recorded in the first 15 seconds after cuff deflation divided by the baseline flow. This technique has been shown to be reproducible and reliable. A previous study showed that the intraobserver variability (coefficient of variation) for repeated measures of vessel diameter to be <3%.[12] All these measurements were performed by the same investigator to avoid any interobserver variability; this investigator was blinded to any measurement phases or treatments.

Study protocol: The study patients were allocated into 2 study protocols. In the first study, the brachial artery vasodilator responses were evaluated in 28 patients with variant angina and in 24 control subjects. In the patients in the first study, the endothelial dependent- and independent-vasodilator responses were assessed to elucidate whether or not systemic endothelial dysfunction exists in variant angina.

To investigate the effect of vitamin C, 17 patients with variant angina were assigned to the second study. The second study consisted of a single, blind, placebo-controlled crossover design. Brachial artery vasodilator responses to vitamin C and placebo (saline) administration were evaluated in 17 patients on separate days within 1 week. Vitamin C (ascorbic acid; Daiichiseiyaku, Tokyo) was infused via the antecubital vein (50 mg/min over 20 minutes, total dosage of 1,000 mg). Both the vitamin C and the placebo was randomly allocated. As a result, 9 patients received vitamin C, whereas 8 patients received the placebo. The flow-mediated and nitroglycerin-induced vasodilator responses were assessed before and after intravenous vitamin C and placebo administration. In ad-

TABLE 1 Characteristics of Study Patients

	Patients With Variant Angina		Control Subjects (n = 24)
	First Study (n = 28)	Second Study (n = 17)	
Age (yrs)	59 ± 7	58 ± 6	56 ± 12
Men	20 (71%)	11 (65%)	17 (71%)
Body mass index (kg/m^2)	22.8 ± 3.1	23.3 ± 3.4	23.6 ± 4.1
Current smoker	16 (57%)	10 (59%)	9 (38%)
Systemic hypertension	6 (21%)	2 (12%)	7 (29%)
Diabetes mellitus	3 (11%)	3 (18%)	1 (4%)
Hyperlipidemia	12 (43%)	6 (35%)	13 (54%)
Total cholesterol (mg/dl)	207 ± 29	207 ± 35	217 ± 40
Triglyceride (mg/dl)	148 ± 63	154 ± 69	163 ± 120
HDL cholesterol (mg/dl)	56 ± 17	54 ± 15	57 ± 14
Fasting blood sugar (mg/dl)	111 ± 46	120 ± 58	100 ± 16

Data are expressed as the mean ± SD.

Hypertension was defined as blood pressure ≥160/95 mm Hg; diabetes mellitus was defined as fasting blood sugar ≥126 mg/dl; and hyperlipidemia was defined as plasma total cholesterol of ≥230 mg/dl.

HDL = high-density lipoprotein.

dition, 10-ml blood samples were withdrawn from the antecubital vein before and after vitamin C administration. The plasma concentration of vitamin C before and after administration was determined by high-performance liquid chromatography. FMD, NTG-D, and the pulse Doppler signal were measured for each condition (before and during, or shortly after hyperemia at baseline; before and during, or shortly after hyperemia under vitamin C or placebo administration; before and after sublingual nitroglycerin administration).

Statistical analysis: All data are expressed as the mean ± SD. Student's *t* test was applied for the comparison of data in patients with variant angina and in control subjects. Paired Student's *t* test was utilized for comparisons of the data in the subjects taking vitamin C and the placebo. Frequency distributions were tested by either chi-square test or Fisher's exact probability test. Differences were considered significant if the p value was <0.05.

RESULTS

Selected clinical characteristics are shown in Table 1. Generally, there were no significant differences in the clinical characteristics between patients with variant angina and control subjects in the 2 study groups. Age, gender, body mass index, and serum total cholesterol, triglyceride, and high-density lipoprotein cholesterol levels were almost identical in the patients with variant angina and the control subjects. The incidences of current smokers and diabetics were slightly higher in patients with variant angina than in the control subjects. However, these differences did not reach statistical significance.

The brachial artery vasodilator response in patients with variant angina was significantly different from that in the control subjects. The FMD was significantly decreased in patients with variant angina compared with the control subjects (1.8 ± 2.2% vs 6.4 ± 4.9%, $p<0.001$; Figure 1). However, the NTG-D was not significantly different between the patients with variant angina and the control subjects (14.0 ± 7.8% vs 13.6 ± 5.0%). Baseline heart rate, blood pressure, vessel diameter, and the calculated brachial blood flow were not substantially different between the patients with variant angina and the control subjects. Reactive hyperemic blood flow and the relative increase in the blood flow at reactive hyperemia compared with baseline were also identical (Table 2).

The effect of vitamin C administration on the flow-mediated vasodilator response in the brachial artery in patients with variant angina is shown in Figure 2. Intravenous vitamin C administration significantly increased the flow-mediated, endothelium-dependent, vasodilator response in 17 patients with variant angina (2.2 ± 2.4% to 4.5 ± 1.6%, $p<0.01$), whereas, no significant increase was seen in the FMD after placebo administration (2.0 ± 2.6% to 1.7 ± 1.9%). In contrast, there was no significant difference in the sublingual nitroglycerin-induced, endothelium-independent, vasodilator response between vitamin C and placebo administration (17.2 ± 7.1% vs 16.7 ± 6.9%). The improved value of the flow-mediated vasodilator response by vitamin C in the second study was not significantly different from that in the control subjects in the first study. The plasma level of vitamin C increased significantly after vitamin C administration (from 27 ± 22 to 228 ± 126 μmol/L, $p<0.001$). Changes in heart rate, blood pressure, baseline vessel diameter, baseline blood flow, and the relative increase in blood flow at reactive hyperemia, were not influenced by either vitamin C or placebo administration (Table 3). In this study, the flow-mediated vasodilator response measurements were repeated twice a week (before vitamin C and placebo administration) and the reproducibility obtained from these measurements were found to be acceptable (Figure 3).

DISCUSSION

The present study demonstrated that systemic endothelial dysfunction exists in variant angina and that the acute administration of vitamin C can restore systemic endothelial dysfunction. We examined the FMD of the brachial artery to test whether a systemic vasomotor dysfunction exists in patients with variant angina and found the flow-mediated, endothelium-dependent, vasodilation in the brachial artery to be significantly decreased in patients with variant angina compared with control subjects who were matched for age, gender, vessel diameter, and cardiovascular risk factors. Because such factors as vessel diameter and cardiovascular risk factors significantly influence FMD in the brachial artery,[13] factor-matched comparisons between patients with variant angina and control

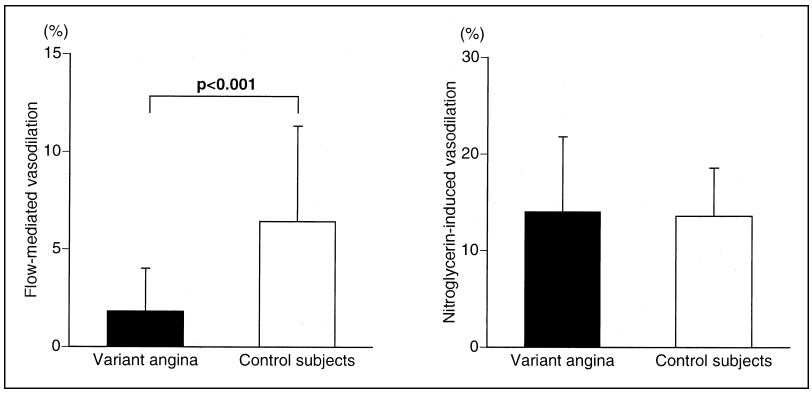

FIGURE 1. FMD *(left)* and NTG-D *(right)* in patients with variant angina and control subjects. FMD was significantly impaired in patients with variant angina (p <0.001). All data are expressed as mean ± SD.

TABLE 2 Hemodynamics and Brachial Artery Parameters

	Patients With Variant Angina (n = 28)	Control Subjects (n = 24)
Baseline heart rate (beats/min)	62 ± 8	69 ± 16
Baseline systolic blood pressure (mm Hg)	132 ± 18	124 ± 17
Baseline diastolic blood pressure (mm Hg)	80 ± 10	76 ± 11
Baseline vessel diameter (mm)	4.31 ± 0.84	3.92 ± 0.72
Baseline blood flow (ml/min)	136 ± 68	161 ± 98
Reactive hyperemic blood flow (ml/min)	542 ± 223	554 ± 206
Relative increase in blood flow at hyperemia	4.3 ± 1.9	4.1 ± 1.9

Data are expressed as the mean ± SD.
Relative increase in blood flow at hyperemia means the ratio of the reactive hyperemic blood flow to the baseline blood flow.

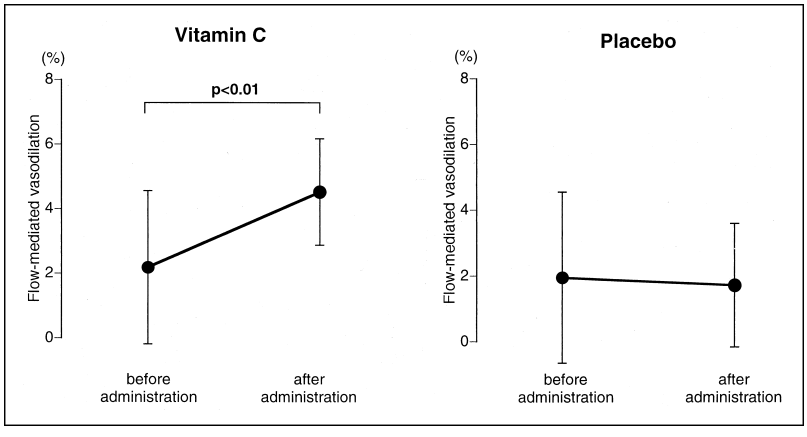

FIGURE 2. FMD in patients with angina before and after administration of vitamin C *(left)* and a placebo *(right)*. FMD was significantly improved by vitamin C administration (p <0.01). All data are expressed as mean ± SD.

subjects are important, and the present observation of a decreased endothelial function in the brachial artery in variant angina is thus substantiated.

Because atherosclerosis is a systemic disorder, endothelial dysfunction can also develop in not only the coronary artery but also in the systemic artery. Previous studies have shown that the extent of coronary artery endothelium-dependent vasoreactivity to acetylcholine or increased shear stress was closely correlated to that of the brachial artery flow-mediated vasodilation in patients with suspected coronary artery disease.[7,8] However, conflicting results have been reported regarding the impairment of systemic artery endothelial function in patients with variant angina, although endothelial dysfunction in the coronary artery has been confirmed.[3,4] Recently, Ito et al[14] reported that the systemic endothelial function was preserved in patients with variant angina. However, their study was limited because the study population only included men. In addition, Botker et al[15] showed that there were no significant differences in the endothelial function in the brachial artery between patients with variant angina and normal subjects. This study was also limited because the study population only included whites. In contrast, Motoyama et al[16,17] agreed with our findings, which indicated an impaired endothelial function in the brachial artery in patients with variant angina. Their study populations consisted of male and female patients, who were all Japanese. Some mutations of endothelial nitric oxide synthase gene have been reported to be associated with coronary spasm.[18,19] These conflicting results therefore may be due to differences in sex, and race and/or genetic abnormalities in study populations, although we did not find any significant differences in the endothelial function due to any gender differences in the patients with variant angina in the present study (data not shown).

This study also showed that acute intravenous administration of vitamin C improved FMD of the brachial artery in patients with variant angina. Vitamin C had no effect on the baseline vessel diameter and nitroglycerin-induced endothelium-independent vasodilation. These findings suggest that vitamin C restores brachial artery endothelial dysfunction in response to an increase in the blood flow. Although the precise mechanisms of endothelial dysfunction and the beneficial effect of vitamin C in variant angina remain undetermined in this study, some possible explanations can be proposed. First, vitamin C is a potent water soluble antioxidant in human plasma, and this antioxidant effect helped to improve the endothelial dysfunction. Vitamin C has been shown to be an

TABLE 3 Effect of Vitamin C on Hemodynamics and Brachial Artery Parameters in Seventeen Patients With Variant Angina

	Vitamin C		Placebo	
	Before	After	Before	After
Heart rate (beats/min)	63 ± 9	62 ± 9	62 ± 8	61 ± 8
Mean blood pressure (mm Hg)	97 ± 8	92 ± 8	97 ± 8	96 ± 7
Baseline vessel diameter (mm)	3.98 ± 0.70	3.96 ± 0.71	4.01 ± 0.69	4.01 ± 0.70
Baseline blood flow (ml/min)	103 ± 35	97 ± 45	99 ± 36	92 ± 42
Relative increase in blood flow at hyperemia	4.1 ± 1.4	4.9 ± 2.7	4.1 ± 1.5	5.1 ± 1.9

Data are expressed as the mean ± SD.

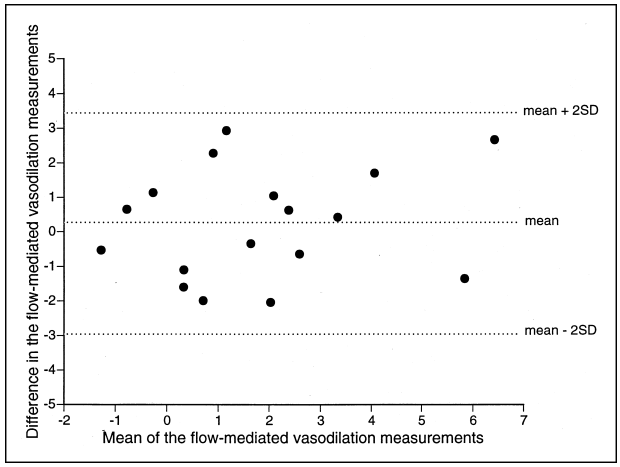

FIGURE 3. Relation between the differences in the measurements of FMD (the first time to the second time) and the mean of those measurements.

effective scavenger of free radicals, such as the superoxide anion, which rapidly reacts with and breaks down nitric oxide.[20] Second, the acute infusion of vitamin C resulting in supraphysiologic concentrations improves endothelial-dependent vasodilation in patients with diabetes,[21] hypertension,[22] a smoking habit,[23] or hypercholesterolemia[24] as well as coronary artery disease.[25] Again, it is possible that vitamin C ameliorates endothelial dysfunction in variant angina through the same mechanisms as those conditions described above. Lastly, vitamin C also plays an important role in regulating the intracellular redox state and it might improve the endothelial function through this mechanism. Vitamin C plays an important role in regulating the intracellular redox state together with glutathione. It can also prevent intracellular glutathione from oxidation.[26,27] As a result, preserved intracellular glutathione improves endothelial function probably through an increase in nitric oxide synthesis, an increased availability of essential cofactors for the enzyme, and/or through the stabilization of nitric oxide.[28]

However, increasing evidence still suggests that vascular oxidative stress might play an important role in the pathogenesis of variant angina. Recent studies have demonstrated a significantly lower plasma level of antioxidant vitamin E in patients with active variant angina while also showing oral vitamin E administration to improve impaired endothelial-dependent vasodilation in patients with variant angina.[17] Kugiyama et al[6] demonstrated that thiobarbituric acid reactive substances, a marker of lipid peroxidation, were produced in coronary circulation during intracoronary acetylcholine infusion and the production of thiobarbituric acid reactive substances is significantly higher in patients with variant angina than in control patients.

Quite recently, Hirashima et al[29] reported that the plasma levels of vitamin C were significantly lower in patients with variant angina than in control subjects and vitamin C improved impairment of FMD in the brachial artery. Our results agree with their findings. It is likely that oxidant stress plays a significant role in the pathogenesis of variant angina while vitamin C restores the peripheral endothelial dysfunction through the antioxidant action.

Study limitations: Our results showed that the acute administration of vitamin C improves endothelial dysfunction in the brachial artery in patients with variant angina. However, we did not study the effect of vitamin C on the frequency of anginal attacks or on the prognosis of variant angina. Quite recently, clinical studies have shown endothelial dysfunction in the coronary artery to be closely correlated with cardiac events in patients with coronary artery disease.[30] It is likely that an amelioration of endothelial dysfunction can improve the clinical outcome in patients with variant angina. In addition, the long-term effect of vitamin C should also be studied in patients with variant angina. A second limitation of this study is that we did not separate our study populations into active or inactive stages of variant angina. It is not clear whether the activity of variant angina influences the degree of endothelial dysfunction in patients with variant angina. This should be clarified in a future study. However, in 17 patients with variant angina, we measured the FMD in the brachial artery twice a week. As shown in Figure 3, the reproducibility of FMD in the brachial artery in the same patients proved to be sufficient. Over a short time period, FMD in patients with variant angina was stable.

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