Nitric Oxide-Mediated Vasodilatation is Decreased in Forearm Resistance Vessels in Patients With Coronary Spastic Angina

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It has been reported that coronary endothelial dysfunction is associated with the pathogenesis of coronary spasm, and that endothelial nitric oxide (NO) mediated vasodilatation was decreased in coronary epicardial arteries in patients with coronary spastic angina (CSA). However, there are few reports about the endothelial function in peripheral resistance vessels of patients with CSA, so the present study investigated the role of NO in forearm resistance vessels in such patients. The responses of forearm blood flow to acetylcholine (ACh; 8–24 μg/min) and sodium nitroprusside (SNP; 0.4–1.2 μg/ml) infusions was examined using plethysmography, and subsequently the responses to ACh after an infusion of NO-monomethyl-L-arginine (L-NMMA; 4 μmol/min, for 5 min) in 17 patients with CSA and 17 age- and sex-matched controls. The vasodilator responses to ACh and SNP were comparable between the 2 groups (p=NS). L-NMMA significantly suppressed the vasodilator responses to ACh in controls (p<0.05), but there was no significant difference in the responses to ACh before and after infusion of L-NMMA in patients with CSA (p=NS). These results indicate that endothelial NO-mediated vasodilatation is decreased in the forearm resistance vessels of patients with CSA. (Jpn Circ J 2001; 65: 81–86)

Key Words: Coronary spasm; Forearm; Nitric oxide; Resistance vessel

The endothelium plays a major role in determining vascular tone through the production and release of different vasodilator and vasoconstrictor substances that control the activity of the underlying smooth muscle layer. The most important endothelium-derived relaxing factor (EDRF) is nitric oxide (NO), which not only plays an important role in the normal regulation of vascular tone and blood pressure, but also helps to prevent atherosclerosis by maintaining vasodilatation and inhibiting platelet aggregation, leukocyte adhesion, and proliferation of smooth muscle cells. Impaired endothelial function appears to be an early sign of atherosclerosis, appearing long before the formation of atherosclerotic lesions. Recent studies have confirmed that endothelium-dependent and NO-mediated vasodilatation are impaired in patients with atherosclerotic risk factors.

Coronary spasm plays an important role in the pathogenesis of not only variant angina, but also ischemic heart disease, including other forms of angina pectoris, acute myocardial infarction and sudden death. We and others have shown that coronary endothelial dysfunction, including decreased NO activity, is related to the pathogenesis of coronary spasm.

Systemic vascular symptoms such as Raynaud’s phenomenon and migraine, which suggest generalized vascular dysfunction, are often seen in patients with variant angina. We and others have also reported that not only the spasm sites but also all the coronary arteries are hyperreactive to vasoconstrictor effects of acetylcholine (ACh) and that spasm frequently occurs in multiple coronary arteries in patients with coronary spastic angina (CSA). Moreover, we recently reported that flow-mediated vasodilatation of the brachial conduit arteries was also impaired in patients with CSA. However, the endothelial function in peripheral resistance vessels in patients with CSA has not been well reported. The present study used strain-gauge plethysmography to examine the endothelium-dependent and NO-mediated vasodilatation in forearm resistance vessels in patients with CSA and age- and sex-matched controls.

Methods

Subjects

The study population included 17 patients with CSA (mean age, 60±2, range, 40–72 years) and 17 age- and sex-matched controls (mean age, 60±2, range, 40–72 years). None of the patients with CSA had organic stenosis (>25%) on angiography and all showed angiographically documented coronary spasm associated with ST-segment changes after an intracoronary infusion of ACh, as previously reported. Eight patients showed transient ST-segment elevation and the remaining 9 patients showed ST-segment depression during the spontaneous attacks. The controls also underwent diagnostic cardiac catheterization for an evaluation of the chest pain. None of them had organic stenosis (>25%) in their coronary arteries nor did they show coronary spasm after an intracoronary injection of ACh. Female subjects were all postmenopausal. Because risk factors for atherosclerosis have been shown to be associated with the impairment of endothelium-dependent vasodilatation, the controls were selected to match the age and sex of the patients with CSA. None of the patients had previous myocardial infarction, congestive heart failure, or other...
We examined the responses of FBF to intra-arterial infusions of ACh and sodium nitroprusside (SNP) at graded doses (infusion rates 0.2, 0.4 and 0.6 ml/min), and we examined the effects of the infusion of NG-monomethyl-L-arginine (L-NMMA) on the responses to the same dose of ACh. This volume of infusion itself was previously reported not to alter FBF. ACh was used as an endothelium-dependent vasodilator that induces vasodilatation by stimulating the release of relaxing factors from the vascular endothelium. SNP is an endothelium-independent vasodilator, because its vasodilator effect is largely the result of its direct action on smooth muscle cells. ACh was infused at the rates of 8, 16 and 24 μg/ml, and SNP was infused at the rates of 0.4, 0.8 and 1.2 μg/min. Each dose was infused for 3 min, and FBF was measured during the last minute of each infusion. The sequence of the administration of ACh and SNP before the infusion of L-NMMA was randomized to avoid any bias related to the order of drug infusion. A 15-min rest period was allowed to corroborate the return to basal values, and basal measurements were repeated between the 2 drugs. After another 15-min rest period, flow measurements were obtained to corroborate the return to basal values. The L-NMMA was subsequently infused at a rate of 4 μmol/min (infusion rate, 0.1 ml/min) for 5 min, and the FBF was measured during the last minute of the infusion. L-NMMA is an arginine analogue that competitively antagonizes the synthesis of NO from L-arginine, and the dose used in the present study has previously been shown to effectively blunt in vivo synthesis of NO and thereby reduce the vasodilator effect of ACh in the human forearm. Subsequently, in all subjects, the responses of FBF to ACh were repeated using the same doses, infusion rates, and resting interval just described.

**Preparation of Drugs**

ACh was prepared by dissolving 100 mg of acetylcholine chloride (Daichi Chemicals, Tokyo, Japan) in physiological saline at a concentration of 40 μg/ml immediately before use. The SNP solution was prepared by dissolving 10 mg of SNP (Wako Chemicals, Osaka, Japan) in physiological saline at a concentration of 40 μg/ml and sterile-filtered before use. Special care was taken not to expose the SNP to light. L-NMMA was obtained from Wako Chemicals and dissolved in physiological saline to achieve a concentration of 4 μmol/ml, and was sterile-filtered before use.

**Statistical Analysis**

The hemodynamic values during the infusions of ACh and SNP at each dose were compared by one-way analysis of variance (ANOVA) for repeated measures to test treatment effects. The hemodynamic responses to ACh or SNP before and after the infusion of L-NMMA were compared by a 2-way ANOVA. To analyze relationships between variables, single or multiple linear regression analyses were performed. Presence or absence of coronary risk factors (sex, smoking and diabetes mellitus) and coronary spasm...
were coded as dummy variables (i.e., male = 0, female = 1 and absence = 0, presence = 1) in multiple linear regression analysis. Differences in the frequencies of risk factors between the patients with CSA and the control subjects were compared using the chi-square test. Differences between 2 mean values were compared by a paired and unpaired Student t test. All values are expressed as mean ± SEM, and a value of \( p<0.05 \) was considered significant.

**Results**

**Patient Characteristics**

The clinical characteristics of the study population are shown in Table 1. Although we did not match coronary risk factors other than the age and sex, there were no significant differences in smoking status, mean blood pressure, lipids profile, body mass index and the frequency of diabetes mellitus between the 2 groups.

**Vascular Responses to ACh and SNP**

The basal FBF were comparable between controls and patients (3.9±0.3 ml/min per 100 ml in controls vs 3.8±0.3 ml/min per 100 ml in CSA, p=NS). ACh produced a substantial vasodilator effect in both controls and patients, with comparable responses of FBF to ACh between the 2 groups (from 3.9±0.3 to 16.2±2.3 ml/min per 100 ml in controls vs from 3.8±0.3 to 18.5±2.5 ml/min per 100 ml in CSA, p=NS by ANOVA) (Fig 1). There was no significant difference in the responses of FBF to SNP between the 2 groups (from 4.0±0.4 to 11.7±1.0 ml/min per 100 ml in controls vs from 4.1±0.5 to 11.3±1.2 ml/min per 100 ml in CSA, p=NS by ANOVA) (Fig 1). The heart rate and mean blood pressure did not change in controls or patients during the protocol, and the responses to each drug were comparable between

Fig 1. Responses of forearm blood flow (FBF) to acetylcholine (ACh) (Left panel) and to sodium nitroprusside (SNP) (Right panel) in controls (open circles, n=17) and patients with coronary spastic angina (closed circles, n=17).

Fig 2. Responses of forearm blood flow (FBF) to acetylcholine (ACh) before (open circles) and after \( \text{NO}^- \)-monomethyl-L-arginine (L-NMMA) (closed circles) in controls (n=17) (Left panel) and in patients with coronary spastic angina (n=17) (Right panel).

Fig 3. The percent change in forearm blood flow (FBF) to acetylcholine (ACh) after the infusion of \( \text{NO}^- \)-monomethyl-L-arginine (L-NMMA) in controls (open circles, n=17) and patients with coronary spastic angina (closed circles, n=17).
Effect of L-NMMA on the Vascular Responses to ACh

In the controls, the responses of FBF to ACh significantly decreased after the infusion of L-NMMA (from 16.2±2.3 to 12.0±2.4 ml/min per 100 ml at 24 μg/min of ACh, p<0.05 by ANOVA) (Fig 2). In contrast, in the patients with CSA, there was no significant change in the responses of FBF to ACh before and after the infusion of L-NMMA (from 18.5±2.5 to 18.3±2.6 ml/min per 100 ml at the 24 μg/min of ACh, p=NS by ANOVA) (Fig 2). L-NMMA did not alter blood pressure or heart rate in either group. The percent decrease of the FBF response to ACh after L-NMMA was significantly less in patients than in controls (–30.3±5.0% by ANOVA) (Fig 3). The multiple linear regression analysis showed that the percent change of FBF after L-NMMA infusion at 24 μg/min of ACh was significantly and independently correlated with the presence of coronary spasm (Table 2).

Discussion

The present study showed that the response of FBF to both ACh and SNP in forearm resistance vessels was comparable between patients with CSA and controls. L-NMMA significantly suppressed the responses of FBF to ACh in controls, whereas there was no significant difference in responses of FBF to ACh before and after infusion of L-NMMA in patients with CSA. These results indicate that NO-mediated vasodilatation is decreased in forearm resistance vessels in patients with CSA.

In the present study, despite the decreased NO-mediated vasodilatation in the patients with CSA, there was no significant difference in the vasodilator response to ACh between the 2 groups. The mechanisms of this finding are undefined. In addition to NO, ACh induces other vasodilators such as prostacyclin or endothelium-derived hyperpolarizing factor (EDHF). The contribution of NO to endothelium-dependent vasodilatation of resistance vessels has been shown to be less than in large arteries and so it is possible that vasodilators other than NO may play a role in the preservation of forearm resistance vessels in patients with CSA. There is another possible mechanism. Vallance et al reported that L-NMMA caused a greater than 40% fall in both the basal blood flow and the response to ACh in healthy young subjects. However, in the present study the same dose of L-NMMA decreased the FBF by 20.8% in basal flow and by 30.3% at the maximum dose of ACh in controls, probably because they had some coronary risk factors comparable to those of the patients with CSA. On the other hand, in the patients with CSA, L-NMMA suppressed the FBF by only 3.0% at the maximum dose of ACh. Thus, the smaller contribution of NO to forearm resistance vessels in the population of the present study than in other studies may also explain our results.

Endothelium-dependent vasodilatation has been reported to be impaired in the presence of coronary risk factors such as age, sex, hypertension, diabetic mellitus, hypercholesterolemia and cigarette smoking. The present study was designed to match the age and sex of the controls with those of patients with CSA, and there was no significant difference in other risk factors between the 2 groups. Furthermore, in the present study multiple linear regression analysis demonstrated that the presence of coronary spasm was associated with decreased NO activity independently of sex, age and other coronary risk factors, which indicates that decreased NO-mediated vasodilatation in the forearm resistance vessels of patients with CSA is not caused by coronary risk factors.

In the present study, we demonstrated that the NO-mediated increase in FBF was impaired in patients with CSA. However, we previously reported that the increase in blood flow of brachial arteries during reactive hyperemia in patients with CSA was comparable with control subjects which is probably related to the greater role of adenosine in the increase in FBF than that of NO during reactive hyperemia.

An increased sensitivity to an exogenous nitrovasodilator in the setting of endothelial dysfunction after blockade of NO has been reported. We and others also reported that basal tone in the spasm arteries is increased and that spasm arteries were hyperresponsive to nitroglycerin because of deficient endogenous release of NO in the arteries. However, Anderson et al reported that sensitivity to an exogenous nitrovasodilator in the setting of endothelial dysfunction were not always found if basal tone were not increased. Moreover, the contribution of NO to resistance vessels has been shown to be less than in large arteries. In the present study, basal blood flow and response to SNP in forearm resistance vessels were comparable between patients with CSA and controls. Increased sensitivity to SNP was not found in forearm resistance vessels in patients with CSA in the present study, probably because their basal forearm blood flow was preserved and NO contribution was minimal in the forearm resistance vessels.

The mechanisms of the decrease in NO-mediated vasodilatation in patients with CSA are undefined. We recently
reported that mutation of the endothelial NO synthase (NOS) gene is associated with CSA. Impaired NO synthesis or release because of the disorder of the NOS gene or other mechanisms may be associated with the decreased endothelial NO-mediated vasodilatation in patients with CSA. In addition, we and others have suggested increased oxygen free radicals may play a role in the pathogenesis of coronary spasm. Thus, it is possible that the increased level of oxygen free radicals may cause an increased breakdown of NO in patients with CSA. All our studies, including the present one, suggest that NO-mediated vasodilatation is decreased not only in the coronary epicardial arteries, but also in forearm resistance vessels of patients with CSA. However, deficiency of endothelial NO activity does not completely explain the mechanism of coronary spasm. In addition to deficient endothelial NO activity, hyperreactivity of the coronary vascular smooth muscle has an important role.

In conclusion, we have demonstrated that NO-mediated vasodilatation is decreased in forearm resistance vessels in patients with CSA. Further studies are needed to explain the mechanisms by which ACh-induced vasodilatation is preserved in spite of the decreased NO-mediated vasodilatation.

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