Arteriosclerosis, Thrombosis, and Vascular Biology

CLINICAL AND POPULATION STUDIES

Marked Impairment of Endothelium-Dependent Digital Vasodilatations in Patients With Microvascular Angina

Evidence for Systemic Small Artery Disease

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OBJECTIVE: It remains to be elucidated whether and how endothelial functions are impaired in peripheral circulation of patients with coronary functional disorders, such as vasospastic angina (VSA) and microvascular angina (MVA). We simultaneously examined endothelial functions of peripheral conduit and resistance arteries in patients with coronary functional disorders, with a special reference to NO and endothelium-dependent hyperpolarization factors.

APPROACH AND RESULTS: Based on the results of invasive coronary acetylcholine testing and coronary physiological measurements, we divided 43 patients into 3 groups; VSA, MVA, and VSA+MVA. Endothelium-dependent vasodilatations of the brachial artery and fingertip arterioles to intra-arterial infusion of bradykinin were simultaneously evaluated by ultrasonography and peripheral arterial tonometry, respectively. To assess NO and endothelium-dependent hyperpolarization factors, measurements were repeated after oral aspirin and intra-arterial infusion of N^G-monomethyl-L-arginine. Additionally, endothelium-independent vasodilatations to sublingual nitroglycerin and plasma levels of biomarkers for endothelial functions were measured. Surprisingly, digital vasodilatations to bradykinin were almost absent in patients with MVA alone and those with VSA+MVA compared with those with VSA alone. Mechanistically, both NO- and endothelium-dependent hyperpolarization–mediated digital vasodilatations were markedly impaired in patients with MVA alone. In contrast, endothelium-independent vasodilatations to nitroglycerin were comparable among the 3 groups. Plasma levels of soluble VCAM (vascular cell adhesion molecule)-1 were significantly higher in patients with MVA alone compared with those with VSA alone.

CONCLUSIONS: These results provide the first evidence that both NO- and endothelium-dependent hyperpolarization–mediated digital vasodilatations are markedly impaired in MVA patients, suggesting that MVA is a cardiac manifestation of the systemic small artery disease.

VISUAL OVERVIEW: An online visual overview is available for this article.

Key Words: arterioles ■ aspirin ■ biomarkers ■ humans ■ manometry

Vasospastic angina (VSA) and microvascular angina (MVA) are major manifestations for nonobstructive coronary artery disease (CAD).[1,2] Previous studies showed that ≈50% of patients with chest pain had nonobstructive CAD and the number of patients with this clinical entity has been steadily increasing.[3,4] VSA is an important functional coronary vasomotor abnormality caused by epicardial coronary spasm. Mechanistically, Rho-kinase–induced myosin light-chain phosphorylation with resultant vascular smooth muscle cell hypercontraction is the

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Nonstandard Abbreviations and Acronyms

ACh	acetylcholine
BK	bradykinin
CAD	coronary artery disease
CFR	coronary flow reserve
COVADIS	Coronary Vasomotion Disorders International Study Group
EDH	endothelium-dependent hyperpolarization
hs-CRP	high-sensitivity C-reactive protein
ICAM	intercellular adhesion molecule
IMR	index of microcirculatory resistance
L-NMMA	N^G-monomethyl-L-arginine
MVA	microvascular angina
MVS	microvascular spasm
PAI	plasminogen activator inhibitor
PG	prostaglandin
VCAM	vascular cell adhesion molecule
VSA	vasospastic angina

Highlights

- Coronary functional abnormalities, including epicardial coronary spasm, enhanced microvascular vasoconstrictions, and impaired microvascular vasodilatations, frequently coexist in various combinations in patients with nonobstructive coronary artery disease.
- Dose-dependent vasodilatations to bradykinin of the brachial artery in the absence of inhibitors were significantly reduced in patients with vasospastic angina plus microvascular angina (MVA) compared with other 2 groups.
- Dose-dependent vasodilatations to bradykinin of fingertip arterioles in the absence of inhibitors were almost absent in patients with MVA alone and those with vasospastic angina plus MVA compared with those with vasospastic angina alone.
- Endothelium-dependent hyperpolarization–mediated vasodilatations of fingertip arterioles were markedly impaired in patients with MVA alone and those with vasospastic angina plus MVA.
- Endothelium-independent forearm and digital vasodilatations in response to nitroglycerine were comparable among the 3 groups.

central mechanism for coronary artery spasm, whereas the role of endothelial dysfunction may be minimal.[1] On the other hand, MVA can result from increased vasoconstrictive reactivity or impaired vasodilatations of coronary microvessels.[5–7] Previous studies showed that patients with angina-like chest pain and normal coronary arteries have coronary microvascular dysfunction and peripheral arterial dysfunction[8] and that those with MVA had peripheral small vascular abnormalities characterized by reduced endothelium-dependent relaxations and enhanced responses to vasoconstrictors.[9] Additionally, it has been proposed that MVA could be a small vessel disease that may manifest as a multisystem disorder affecting small vessels of the brain and other organs, such as dementia, renal dysfunction, and retinopathy.[10] However, the underlying mechanism(s) of peripheral small vascular abnormalities in MVA patients remains to be fully elucidated.

The endothelium plays important roles in the modulation of vascular tone by synthesizing and releasing several vasodilator substances, collectively named as endothelium-derived relaxing factors, including vasodilator prostaglandins (PGs), NO, and endothelium-dependent hyperpolarization (EDH) factors.[1,11,12] Since EDH-mediated responses are defined as the remaining responses after the blockade of those mediated by vasodilator PGs and NO, it is not easy to precisely evaluate the in vivo importance of EDH factors, especially in humans.[12,13] However, the existence of EDH factor–mediated responses has been repeatedly documented in isolated human arteries[13,14] and human forearm circulation in vivo.[15,16] Furthermore, it has been widely accepted that endothelium-derived NO mediates vascular relaxation of relatively large, conduit arteries (ie, aorta and epicardial coronary arteries), while the importance of EDH factors increases as the vessel size decreases in resistance arteries (eg, coronary microvessels).[1,16] Most of the previous studies using endothelial function testing in humans exclusively addressed NO-mediated responses but not EDH factor–mediated mechanisms. Moreover, recent randomized clinical trials have shown that the effects of isosorbide-5-mononitrate (an NO donor) were unexpectedly neutral in patients with microvascular myocardial ischemia despite successful coronary revascularization[17] and that the results of long-term administrations of inorganic nitrite (an NO donor) in patients with heart failure with preserved left ventricular ejection fraction were disappointing or even harmful despite the high prevalence and pathophysiological relevance of coronary microvascular dysfunction in patients with the disease.[18,19] Thus, we need to focus on EDH factor–mediated mechanisms in coronary functional abnormalities. However, the roles of each endothelium-derived relaxing factor, especially those of EDH, in the pathogenesis of VSA and MVA are still unclear.

In the present study, we tested our hypothesis that patients with coronary functional abnormalities (eg, VSA and MVA) also have peripheral vascular dysfunction, where NO- and EDH-mediated vasodilatations are impaired depending on the vessel size. To address this important issue, we simultaneously examined the roles of NO- and EDH-mediated vasodilatations of peripheral conduit (brachial artery) and resistance arteries (fingertip arterioles) for the first time in patients with VSA, MVA, or both of them in vivo.

MATERIALS AND METHODS

The present study was conducted following the ethical principles of the Declaration of Helsinki, and the protocol was approved by the Ethics Committees of Tohoku University Hospital (No. 2017-2-239). All patients provided written informed consensus before the study entry.

Study Population

From January 2015 to April 2019, a total of 484 patients underwent elective diagnostic cardiac catheterization for evaluation of rest angina or ECG abnormalities at Tohoku University Hospital, which is a tertiary advanced care hospital with >50 departments and >1300 beds. Among them, 366 patients had no significant coronary stenosis (luminal narrowing <70% or fractional flow reserve >0.80)[20] of the major coronary arteries on control coronary angiography, and 349 of them underwent coronary artery spasm provocation testing with acetylcholine (ACh) and coronary physiological measurements to evaluate coronary vasoconstricting responses and coronary microvascular vasodilatory functions, as described previously.[21–24] Cardiopulmonary exercise testing was performed on an upright cycle ergometer (BE-350 Well Bike; Fukuda Denshi, Tokyo, Japan) in the morning before coronary angiography. Patients were encouraged to perform a symptom-limited maximal test unless they met another indication for test termination (eg, dyspnea or leg fatigue). We excluded patients with acute coronary syndrome, acute decompensated heart failure, previous history of percutaneous coronary intervention, cardiomyopathy, collagen disease, chronic kidney disease (estimated glomerular filtration rate <60 mL/min per 1.73 m^2), atrial fibrillation, aspirin allergy, aspirin asthma, or oral administration of anticoagulants. Among 127 patients who fulfilled the inclusion criteria, we were unable to contact 48 patients and to obtain consensus from 32 patients. We also excluded 4 patients who showed normal responses due to the small number. Finally, we enrolled 43 patients in the present study (Figure I in the Data Supplement). We divided them into 3 groups based on the COVADIS (Coronary Vasomotion Disorders International Study Group) diagnostic criteria[6]: VSA alone, MVA alone, and both of them (VSA+MVA). Then, we simultaneously measured endothelial vasodilator functions of peripheral arteries by ultrasonography (brachial artery) and peripheral arterial tonometry (fingertip arterioles).

ACh Provocation Testing for Coronary Artery Spasm

ACh provocation testing for coronary artery spasm was performed as described previously.[21,25] Briefly, ACh was administrated into the coronary artery (20, 50, and 100 μg) with careful monitoring of arterial pressure, the 12-lead ECG, and serial coronary angiograms at 1-minute intervals.[21] Based on the guidelines of the Japanese Circulation Society,[26] transient >90% stenosis accompanied by chest pain or ischemic ECG changes was defined as a positive finding for epicardial coronary spasm. We defined microvascular spasm (MVS) as the presence of myocardial lactate production without angiographically demonstrable epicardial coronary spasm during ACh provocation testing associated with reproduction of patient's symptoms and ischemic ST-segment changes[2,25] in accordance with the COVADIS diagnostic criteria.[6]

Coronary Physiological Measurements

Coronary flow reserve (CFR) and the index of microcirculatory resistance (IMR) were measured in the left anterior descending artery in response to intravenous adenosine, as described previously.[22,23] Increased IMR (≥25) was representative of microvascular dysfunction.[6] CFR was calculated using thermodilution as resting mean transit time divided by hyperemic mean transit time (abnormal CFR was defined as <2.0).[6] Fractional flow reserve was calculated by the ratio of mean distal coronary pressure to mean aortic pressure at maximal hyperemia (abnormal fractional flow reserve was defined as ≤0.80).[20]

Peripheral Vascular Functions

Study patients lay in a supine position and in an air-conditioned room at 24 to 26°C. Under local anesthesia with 1% procaine, the right brachial artery was cannulated with a 22-gauge cannula for drug infusion. After placement of the cannula, at least 30 minutes were allowed for the patient to establish a stable baseline measurement before data collection. We evaluated the correlations between coronary functional abnormalities and endothelial functions of peripheral arteries in patients enrolled in the present study. We simultaneously measured endothelium-dependent vasodilatations of conduit and resistance arteries in an unblinded fashion. Endothelial functions of brachial (conduit) artery were assessed by measuring diameter changes of the artery using UNEXEF38G (UNEX Corporation, Nagoya, Japan).[27] Briefly, image of the right brachial artery was obtained using 10-MHz probe attached to a high-resolution ultrasonography. A mechanical support maintained the probe in a fixed position throughout the entire examination. The brachial artery diameter was measured throughout the test using a totally automated system.[27] Diameter changes were calculated as maximum percentage changes in the brachial artery diameter during intra-arterial infusion of bradykinin (BK; purchased from Clinalfa, Bubendorf, Switzerland) compared with the basal diameter.[27] Endothelial functions of fingertip (resistance) arterioles were assessed by measuring volume changes in the fingertip using Endo-PAT 2000 (Itamar Medical, Caesarea, Israel).[28] Endo-PAT 2000 comprises the 2 pneumatic probes that register arterial pulse wave amplitudes and are placed on both index fingers. Before measurements were started, the internal cuff was inflated to subdiastolic pressure to prevent venoarteriolar reflex vasoconstriction.[28] We measured arterial pulse wave at baseline in the right (A) and left (C) fingertip arterioles from the beginning of the test until 30 seconds before the infusion. Then, we measured arterial pulse wave of maximum vasodilatations in response to intra-arterial infusion of graded doses of BK in the right (B) and left (D) fingertip arterioles during 30 seconds. The volume changes in the fingertip were calculated as follows: ([B/A]/[D/C]−1)×100%.[28] An experienced sonographer, who had completed the training of image acquisition and analysis by ultrasonography for 2 years, performed measurements, as described previously.[27]

Protocols

As shown in Figure 1, the contributions of vasodilator PGs, NO, and EDH to BK-induced endothelium-dependent forearm and digital vasodilatations were evaluated. We performed the measurements for each patient for 4 protocols on the same day. We had confirmed the safety of

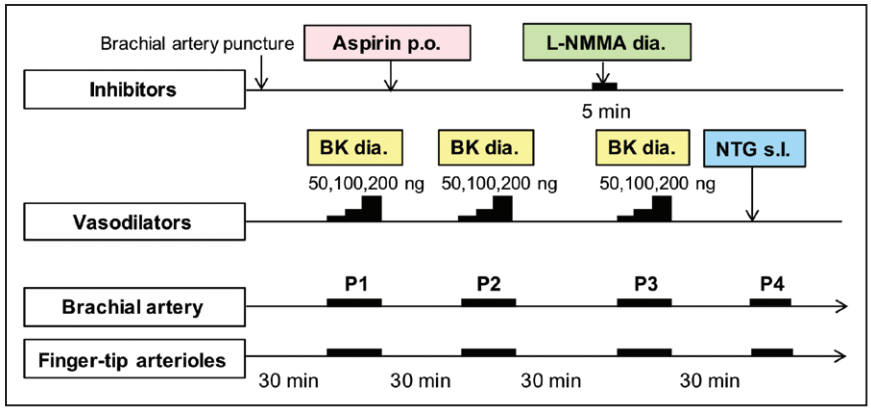

Figure 1. Study protocols.
BK indicates bradykinin; dia, drip infusion into the brachial artery; L-NMMA, N^G-monomethyl-L-arginine; NTG, nitroglycerine; P, protocol; p.o., per os; and s.l., sublingual.

the present protocol in the pilot study with 5 subjects (No. 2016-2-247).

Protocol 1: Entire Endothelium-Dependent Vasodilatations to BK

Graded doses (25, 50, 100 ng/min for 2 minutes each) of BK were infused into the brachial artery in the absence of any inhibitors and endothelial functions of the brachial artery and fingertip arterioles were simultaneously measured.

Protocol 2: NO- and EDH-Mediated Endothelium-Dependent Vasodilatations to BK

The patients were administered aspirin (486 mg) orally to inhibit PGs synthesis 30 minutes before starting the second measurement, and the measurements were repeated as in protocol 1. We previously demonstrated that this dose of oral aspirin inhibits PGs synthesis within 30 minutes before starting measurement.[29]

Protocol 3: EDH-Mediated Vasodilatations to BK

Following the protocol 2, an NO synthase inhibitor, N^G-monomethyl-L-arginine (L-NMMA; Clinalfa, Bubendorf, Switzerland), was infused into the brachial artery (8 μmol/min for 5 minutes) to examine EDH-mediated vasodilatations in response to BK.[29]

Protocol 4: Endothelium-Independent Vasodilatations to Nitroglycerin

Finally, the patients were administered sublingual nitroglycerine (0.3 mg). We calculated the maximum vasodilatations of the brachial artery and fingertip arterioles from the basal diameter during 10 minutes. The diameter (volume) changes of the fingertip arterioles were calculated as follows: ([D/C]−1)×100%.

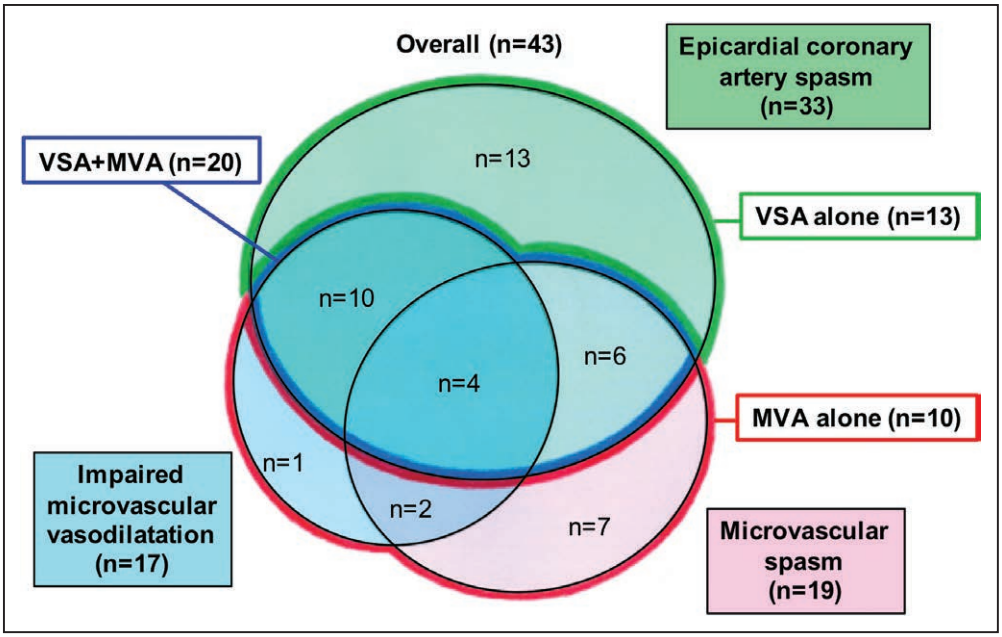

Figure 2. Marked overlaps among the coronary functional abnormalities.
There are 3 types of coronary functional abnormalities. Among the 43 patients, 33 (76.7%) were diagnosed as having vasospastic angina (VSA) by acetylcholine provocation testing, 17 (39.5%) had impaired microvascular vasodilatations based on low coronary flow reserve (<2.0) or high index of microcirculatory resistance (≥25), and 19 (44.2%) had microvascular spasm. Importantly, more than half of the VSA patients had microvascular functional abnormalities, including impaired microvascular vasodilatations and microvascular spasm (n=20; 60.6%). MVA indicates microvascular angina.

Biomarkers Related to Endothelial Functions

Before measurements of peripheral vascular functions, we obtained blood samples from the brachial artery. We measured plasma levels of biomarkers related to endothelial functions, including adiponectin, asymmetrical dimethylarginine, NOx, soluble ICAM (intercellular adhesion molecule)-1, soluble VCAM (vascular cell adhesion molecule)-1, and total PAI (plasminogen activator inhibitor)-1. All measurements were performed in a blinded and independent manner with respect to the clinical information at the SRL company (SRL, Tokyo, Japan).

Statistical Analysis

Continuous variables are presented as mean±SD or medians (interquartile ranges) and categorical variables as numerals and percentages (%). Jonckheere-Terpstra test was used for trend test of dose-responses to BK. Dose-response curves between groups were compared by repeated-measures 2-way ANOVA. Unpaired Student *t* test with unequal variances for normal distribution and Mann-Whitney *U* test for asymmetrical distribution were used to analyze differences in continuous variables. χ^2 test or Fisher exact test were used for categorical variables. Correlations between continuous variables were analyzed using a linear regression model. The Holm method was used for adjusting multiple comparisons. Statistical analysis was performed with JMP Pro 14.1.0 (SAS Institute, Cary, NC) and R, version 3.4.2 (R Foundation for Statistical Computing, Vienna, Austria). A value of $P<0.05$ was considered to be statistically significant.

RESULTS

Coronary Functional Disorders

We measured peripheral vascular functions in 47 eligible patients with rest angina or ECG abnormalities who

Table 1. Clinical Patient Characteristics at the Time of Measuring Peripheral Vascular Functions

	VSA Alone (n=13)	MVA Alone (n=10)	VSA+MVA (n=20)	*P* Value
Age, y	62.2±6.8	60.5±10.2	62.8±9.8	0.82
Male sex, n (%)	7 (54)	3 (30)	9 (45)	0.52
Current smoker, n (%)	8 (62)	4 (40)	9 (45)	0.53
Family history of CAD, n (%)	3 (23)	4 (40)	6 (30)	0.68
Diabetes mellitus, n (%)	0 (0)	2 (20)	0 (0)	0.03
Hypertension, n (%)	5 (38)	4 (40)	10 (50)	0.77
Dyslipidemia, n (%)	8 (62)	6 (60)	12 (60)	0.99
BMI, kg/m^2	23.5±2.8	22.6±3.0	23.5±2.6	0.66
Heart rate, beats per minute	61.8±6.2	69.0±17.3	67.4±13.3	0.36
Systolic BP, mm Hg (leg)	145.9±19.3	149.5±5.2	145.9±23.3	0.89
Diastolic BP, mm Hg (leg)	74.8±9.2	77.6±12.1	74.3±11.9	0.73
Laboratory tests				
WBC, ×10^3/μL	5.6±1.0	5.9±1.2	5.9±1.4	0.73
TG, mg/dL	118±64.9	121±77.9	128±44.7	0.89
T-CHO, mg/dL	205±31.9	196±39.4	186±34.7	0.26
LDL-C, mg/dL	113±22.0	98±17.1	95±28.6	0.13
HDL-C, mg/dL	59±14.5	71±24.2	63±14.9	0.26
hs-CRP, mg/mL	0.05 (0.02–0.09)	0.05 (0.02–0.08)	0.05 (0.02–0.09)	0.66
BNP, pg/mL	16 (9.6–32.0)	10.5 (5.8–35.3)	12.7 (7.6–21.3)	0.76
Echocardiography				
LVEF, %	68±4.7	68±5.3	69±4.1	0.82
E/A	1.04±0.22	0.93±0.33	1.04±0.42	0.70
E/e′	7.7±1.7	7.6±1.7	8.2±1.7	0.53
Medications, n (%)				
CCB	12 (92)	10 (100)	20 (100)	0.31
Nitrate	2 (15)	0 (0)	2 (10)	0.45
RAS inhibitor	1 (8)	2 (20)	5 (25)	0.45
β-Blocker	2 (15)	0 (0)	2 (10)	0.45
Statin	5 (38)	7 (70)	9 (45)	0.29
Antiplatelet	1 (8)	2 (20)	6 (30)	0.30
Diuretic	2 (15)	0 (0)	4 (20)	0.32

Results are expressed as mean±SD, n (%), or median (interquartile range).

BMI indicates body mass index; BNP, B-type natriuretic peptide; BP, blood pressure; CAD, coronary artery disease; CCB, calcium channel blocker; E/A, early diastolic filling velocity/atrial filling velocity; E/e′, early diastolic mitral flow velocity/tissue Doppler imaging velocity; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; MVA, microvascular angina; RAS, renin-angiotensin system; T-CHO, total cholesterol; TG, triglyceride; VSA, vasospastic angina; and WBC, white blood cell.

Table 2. Coronary Physiological Parameters

	VSA Alone (n=13)	MVA Alone (n=10)	VSA+MVA (n=20)	*P* Value
CFR	3.3±0.9	3.7±1.8	2.2±1.3	0.02
IMR	10.6±3.9	16.8±12.6	17.2±8.4	0.02
MVS, n (%)	0 (0)	9 (90)	10 (50)	<0.01

Results are expressed as mean±SD or n (%).

CFR indicates coronary flow reserve; IMR, index of microcirculatory resistance; MVA, microvascular angina; MVS, microvascular spasm; and VSA, vasospastic angina.

underwent elective diagnostic cardiac catheterization, ACh provocation testing for coronary artery spasm, and physiological measurements of coronary microvascular functions for IMR and CFR (Figure I in the Data Supplement). Only 4 (8.5%) patients showed normal coronary functions, and remaining 43 patients were divided into the 3 groups: VSA alone (n=13), MVA alone (n=10), and VSA+MVA (n=20). Among the 43 patients, 33 (76.7%) were diagnosed as having VSA by ACh provocation testing, 17 (39.5%) had impaired microvascular vasodilatations based on low CFR (<2.0) or high IMR (≥25), and 19 (44.2%) had MVS (Figure 2). Importantly, more than half of the VSA patients had microvascular functional abnormalities, including impaired microvascular vasodilatations and MVS (n=20; 60.6%), where marked overlaps were noted among the coronary functional abnormalities, in particular between VSA and others (Figure 2).

Clinical Patient Characteristics and Coronary Physiological Measurements

Clinical patient characteristics at the time of peripheral vascular function test are summarized in Table 1. Among the 3 groups, there were no significant differences in age, sex, or prevalence of coronary risk factors except for diabetes mellitus. Medications were also comparable among the 3 groups. Patients with MVA tended to have a higher prevalence (2-fold) of cold intolerance (4 of 10; 40%) as compared with those without (2 of 10; 20%); however, this difference did not reach a statistical significance because of the small number of patients (*P*=0.23). We also had performed cardiopulmonary exercise testing in a subset of 29 (67%) patients (8 VSA alone, 8 MVA alone, and 13 VSA+MVA) using an ergometer cycle with a standardized test protocol. Peak heart rate and chronotropic response were significantly lower in patients with VSA+MVA compared with those with MVA alone, although there was no significant difference in the peak oxygen consumption or anaerobic threshold value among the 3 groups (Table I in the Data Supplement). The results of coronary physiological measurements showed that mean IMR values were significantly higher in patients with MVA alone and those with VSA+MVA than in those with VSA alone, whereas mean CFR values were lower in patients with VSA+MVA than in those with VSA alone or MVA alone (Table 2).

Endothelium-Dependent Vasodilatations of Peripheral Conduit and Resistance Arteries

The interval between coronary physiological measurement and peripheral vascular examination varied individually (mean, 437±395 days). Finally, 40 patients (93%) completed both examinations. Blood pressure, heart rate, and baseline diameter of the brachial artery were comparable among the 3 groups in each protocol (Table 3). There was no interaction between each group and doses of BK. Thus, we performed repeated-measures 2-way ANOVA without

Table 3. Baseline Data in the 4 Protocols

		VSA Alone (n=13)	MVA Alone (n=10)	VSA+MVA (n=20)	*P* Value
Protocol 1	Systolic BP, mm Hg (leg)	144.8±19.9	160.0±24.7	142.5±19.7	0.14
	Diastolic BP, mm Hg (leg)	76.8±10.2	84.0±12.4	73.9±9.5	0.08
	Heart rate, beats per minute	57±4.5	64±12.9	62±11.9	0.23
	Brachial artery baseline diameter, mm	2.7±0.4	2.5±0.4	2.6±0.4	0.57
Protocol 2	Systolic BP, mm Hg (leg)	147.2±21.0	150.3±18.1	150.1±21.8	0.92
	Diastolic BP, mm Hg (leg)	78.9±13.2	74.8±11.0	76.7±10.9	0.71
	Heart rate, beats per minute	59±7.7	63±13.3	63±13.3	0.60
	Brachial artery baseline diameter, mm	2.8±0.6	2.6±0.4	2.7±0.4	0.46
Protocol 3	Systolic BP, mm Hg (leg)	149.5±17.4	148.1±22.2	142.0±23.5	0.61
	Diastolic BP, mm Hg (leg)	80.0±7.2	77.0±16.0	77.2±12.5	0.80
	Heart rate, beats per minute	59±4.8	58±10.8	63±13.5	0.67
	Brachial artery baseline diameter, mm	2.6±0.4	2.5±0.3	2.7±0.4	0.71
Protocol 4	Systolic BP, mm Hg (leg)	151.6±18.6	150.6±23.9	150.4±23.9	0.99
	Diastolic BP, mm Hg (leg)	82.2±7.4	81.9±14.7	78.1±12.0	0.54
	Heart rate, beats per minute	58±3.8	65±18.5	63±12.3	0.21
	Brachial artery baseline diameter, mm	2.7±0.5	2.5±0.3	2.7±0.4	0.32

Results are expressed as mean±SD.

BP indicates blood pressure; MVA, microvascular angina; and VSA, vasospastic angina.

interaction term for endothelium-dependent vasodilatation in combination of 3 dose levels of BK and our 3 patient groups. Following the results (Table II in the Data Supplement), we analyzed differences in continuous variables of maximum dose among the 3 groups by unpaired Student *t* test or Mann-Whitney *U* test. In the brachial artery, intra-arterial infusion of BK elicited endothelium-dependent vasodilatations in a dose-dependent manner, and L-NMMA similarly attenuated the responses among the 3 groups (Figure 3A). Of note, dose-responses to BK of the brachial artery in the absence of inhibitors were significantly reduced in patients with VSA+MVA compared with those with VSA alone or MVA alone (Figure 4A). Surprisingly, however, in the fingertip arterioles, dose-responses to BK in the absence of inhibitors were almost absent in patients with MVA alone and those with VSA+MVA compared with those with VSA alone (Figure 3B). Furthermore, EDH-mediated vasodilatations were also markedly impaired in patients with MVA alone and those with VSA+MVA (Figure 4B). These results indicate that BK-induced, EDH-mediated vasodilatations were abrogated when comorbid with MVA. To measure the effects of comorbid MVA on the 3 endothelium-derived relaxing factor components, we further evaluated the responses of the brachial artery and fingertip arterioles in response to the maximum dose of BK between the patients with and those without MVA. In the brachial artery, NO mainly contributed to endothelium-dependent forearm vasodilatations in patients with MVA compared with those without it (Figure 5A), whereas in the fingertip arterioles, both NO- and EDH-mediated digital vasodilatations tended to decrease in patients with MVA, especially EDH-mediated digital vasodilatations were abolished (Figure 5B).

Endothelium-Independent Vasodilatations of Conduit and Resistance Arteries

In both the brachial artery and fingertip arterioles, endothelium-independent vasodilatations in response to sublingual nitroglycerine were comparable among the 3 groups (Figure II in the Data Supplement).

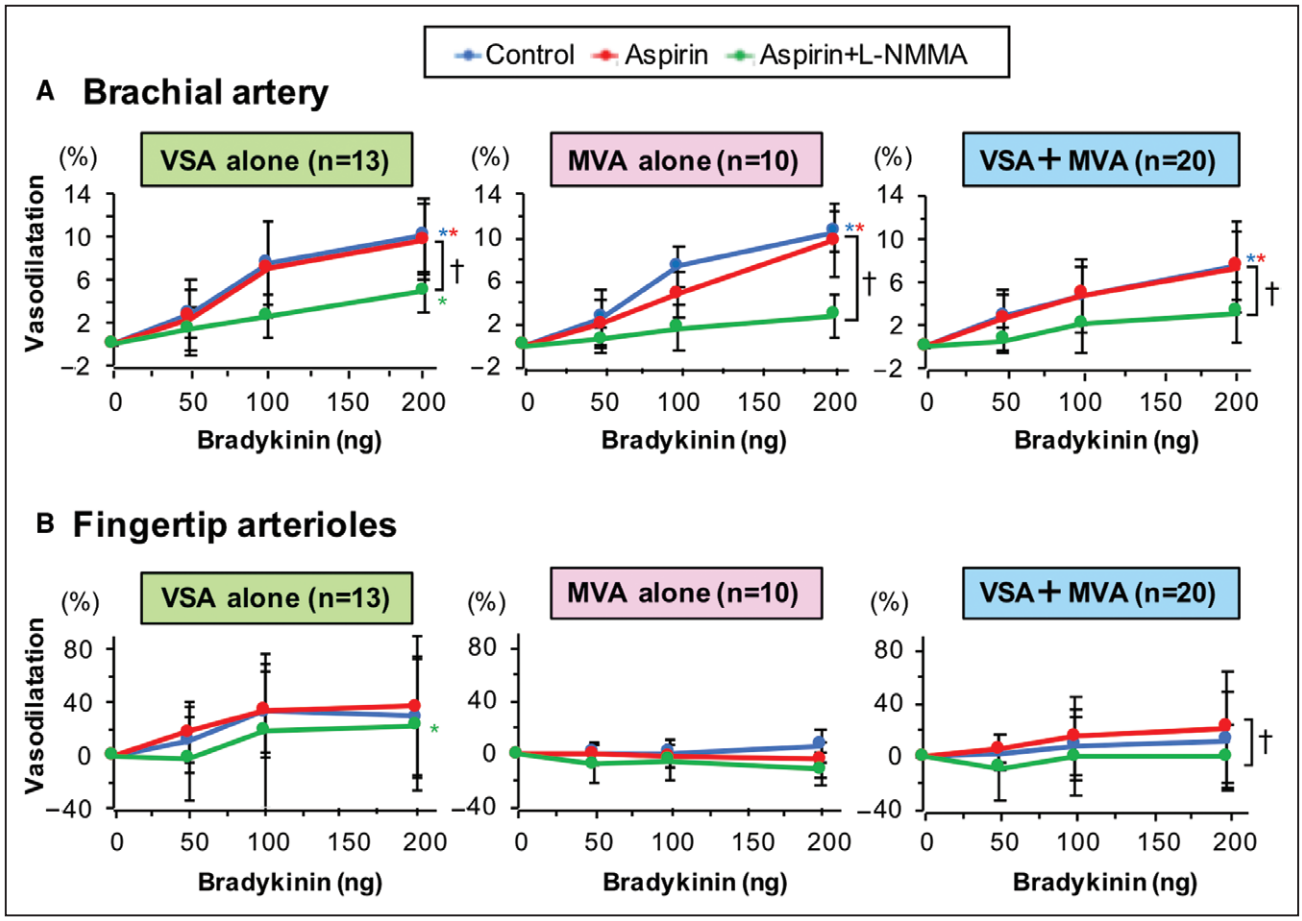

Figure 3. Relative contributions of endothelium-derived relaxing factors to bradykinin (BK)-induced vasodilatations in peripheral arteries among the 3 groups.
A, In the brachial artery, intra-arterial infusion of BK elicited endothelium-dependent vasodilatations in a dose-dependent manner, and N^G-monomethyl-L-arginine (L-NMMA) similarly attenuated the responses among the 3 groups. **B**, In fingertip arterioles, dose-responses to BK in the absence of inhibitors were almost absent in patients with microvascular angina (MVA) alone and those with vasospastic angina (VSA) plus MVA compared with those with VSA alone. *$P<0.05$ by Jonckheere-Terpstra test. †$P<0.05$ by unpaired Student *t* test and Holm method.

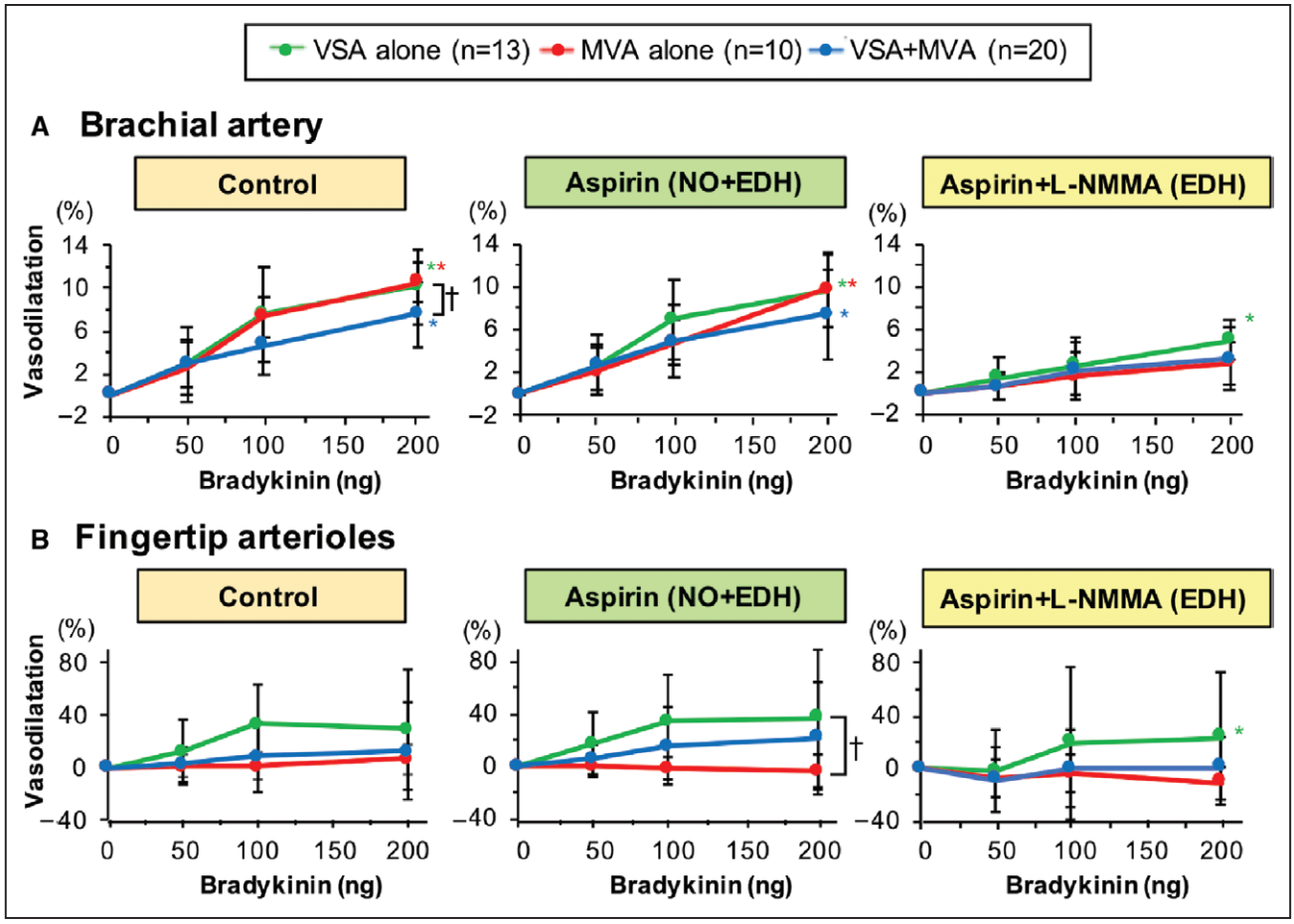

Figure 4. Comparison of bradykinin (BK)-induced vasodilatations in peripheral arteries among the 3 groups.
A, In the brachial artery, dose-responses to BK in the absence of inhibitors were significantly reduced in patients with vasospastic angina (VSA) plus microvascular angina (MVA) compared with those with VSA alone or MVA alone. **B**, In fingertip arterioles, endothelium-dependent hyperpolarization (EDH)–mediated vasodilatations were markedly impaired in patients with MVA alone and those with VSA+MVA. L-NMMA indicates N^G-monomethyl-L-arginine. **P*<0.05 by Jonckheere-Terpstra test. †*P*<0.05 by unpaired Student *t* test and Holm method.

Biomarkers Related to Endothelial Functions

Plasma levels of soluble VCAM-1 were significantly higher in patients with MVA alone compared with those with VSA alone, whereas no difference was noted for other biomarkers among the 3 groups (Table 4; Figure 6A). Furthermore, there were significant negative correlations between endothelium-dependent digital vasodilatations after oral aspirin and soluble VCAM-1 (Figure 6B).

DISCUSSION

The major findings of the present study were as follows: (1) coronary functional abnormalities, including epicardial coronary spasm, enhanced microvascular vasoconstrictions, and impaired microvascular vasodilatations, frequently coexist in various combinations in patients with nonobstructive CAD; (2) dose-dependent vasodilatations to BK of the brachial artery in the absence of inhibitors were significantly reduced in patients with VSA+MVA compared with other 2 groups; (3) dose-dependent vasodilatations to BK of fingertip arterioles in the absence of inhibitors were almost absent in patients with MVA alone and those with VSA+MVA compared with those with VSA alone; (4) EDH-mediated vasodilatations of fingertip arterioles were markedly impaired in patients with MVA alone and those with VSA+MVA; and (5) endothelium-independent forearm and digital vasodilatations in response to nitroglycerine were comparable among the 3 groups. To the best of our knowledge, this is the first study that evaluated endothelial functions of the brachial artery and fingertip arterioles by simultaneous ultrasonography and peripheral arterial tonometry in patients with nonobstructive CAD in vivo, providing the first evidence that both NO- and EDH-mediated digital vasodilatations are markedly impaired in MVA patients. Taken together, these results indicate that MVA is a cardiac manifestation of the systemic small artery disease (Figure 7).

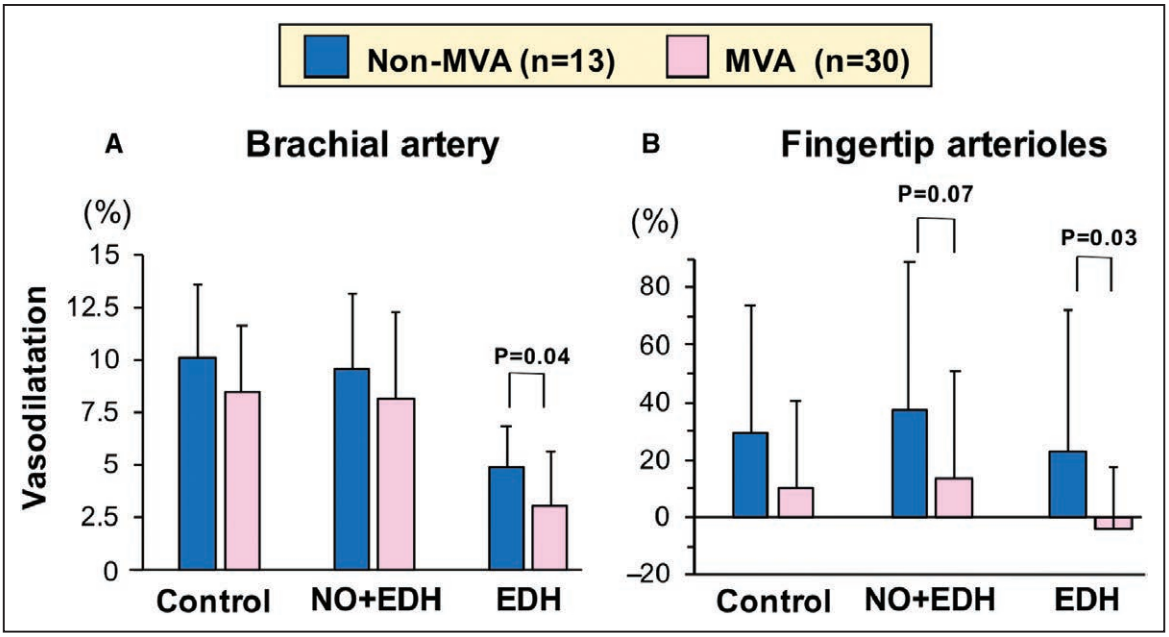

Figure 5. Responses of the brachial artery and fingertip arterioles in response to the maximum dose of bradykinin in patients with non-MVA and those with MVA.

A, In the brachial artery, NO mainly contributed to endothelium-dependent forearm vasodilatations in patients with MVA compared with those with non-MVA. **B**, In the fingertip arterioles, both NO- and endothelium-dependent hyperpolarization (EDH)–mediated digital vasodilatations tend to decrease in patients with MVA, especially EDH-mediated digital vasodilatations were markedly impaired in patients with MVA. MVA indicates microvascular angina.

Peripheral Vascular Functions in Patients With Nonobstructive CAD

We previously demonstrated that endothelium-derived NO mediates vascular relaxation of relatively large, conduit arteries, while EDH factors play important roles in modulating vascular tone of resistance arteries.[1,16,30] Furthermore, previous studies showed that EDH-mediated responses can be upregulated to compensate and maintain endothelium-dependent vasodilatations in pathological conditions where NO-mediated responses are compromised.[1,16] In the present study, since NO mainly contributed to endothelium-dependent vasodilatations of the brachial artery in MVA patients, the responses to BK of the conduit artery in MVA patients seem to be similar to healthy subjects. In contrast, in fingertip arterioles, dose-dependent vasodilatations to BK in the absence of aspirin and L-NMMA were almost absent in patients with MVA alone and those with VSA+MVA compared with those with VSA alone. Since endothelium-independent vasodilatations of both conduit and resistance arteries to nitroglycerine were comparable among the 3 groups, the absence of dose-responses to BK was attributed to impaired endothelial functions but not vascular smooth muscle cell dysfunction.

Recently, Ford et al[9] examined endothelium-dependent vasodilatations of isolated small gluteal arteries in response to ACh in patients with VSA or MVA ex vivo using wire myography. They demonstrated that the maximum relaxation to ACh was significantly lower in patients with VSA or MVA compared with controls.[9] The present study also demonstrates that dose-dependent vasodilatations to BK of resistance arteries in the absence of inhibitors were almost absent in patients with MVA alone and those with VSA+MVA compared with those with VSA alone. Taken together, these results indicate that MVA is a cardiac manifestation of the systemic small artery disease. However, there is a difference between the study by Ford et al and the present study. They showed that VSA patients had also peripheral microvascular abnormalities like MVA, whereas the present study showed that endothelium-dependent vasodilatations of resistance arteries were not impaired in VSA patients. The following 3 reasons could explain the difference between the 2 studies. First, they used relatively large isolated human

Table 4. Biomarkers Related to Endothelial Functions

	VSA Alone (n=13)	MVA Alone (n=10)	VSA+MVA (n=20)	*P* Value
Adiponectin, μg/mL	9.3±3.3	14.5±7.0	9.4±6.0	0.05
ADMA, μmol/L	0.38±0.04	0.39±0.05	0.38±0.06	0.98
NO_x, μmol/L	54.2±44.3	48.8±16.1	37.2±17.0	0.23
Soluble ICAM-1, ng/mL	158.2±28.5	171.1±68.8	177.7±62.8	0.68
Soluble VCAM-1, ng/mL	476.9±76.9	588.2±117.3	504.4±96.5	0.02
Total PAI-1, ng/mL	14.5±6.3	14.7±7.2	17.2±12.5	0.86

Results are expressed as mean±SD.

ADMA indicates asymmetrical dimethylarginine; ICAM, intercellular adhesion molecule; MVA, microvascular angina; PAI, plasminogen activator inhibitor; VCAM, vascular cell adhesion molecule; and VSA, vasospastic angina.

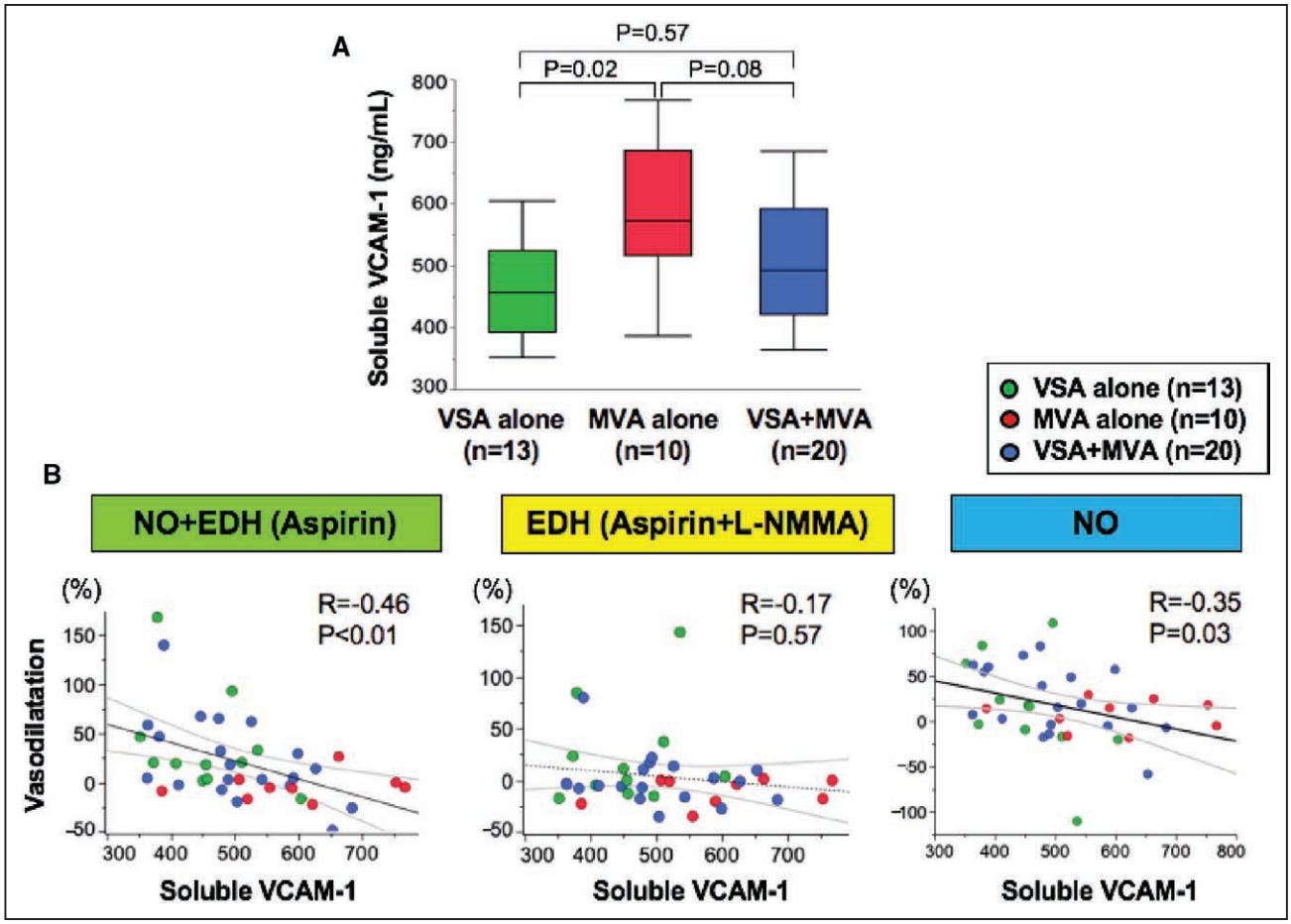

Figure 6. Correlations between soluble VCAM (vascular cell adhesion molecule)-1 and endothelium-dependent digital vasodilatations.

A, Plasma levels of soluble VCAM-1 were significantly higher in patients with microvascular angina (MVA) alone compared with those with vasospastic angina (VSA) alone. **B**, There were significant negative correlations between plasma levels of soluble VCAM-1 and endothelium-dependent digital vasodilatations after oral aspirin. EDH indicates endothelium-dependent hyperpolarization; and L-NMMA, N^G-monomethyl-L-arginine.

gluteal arteries (mean diameter ≥300 μm) for wire myography, whereas in the present study, we evaluated endothelial functions of fingertip arterioles in vivo.[24] Thus, it is conceivable that they evaluated endothelial functions of small arteries but not resistance arteries. Second, we used BK as a tool to examine the contribution of endothelium-derived relaxing factors, while they used ACh to examine endothelial relaxations of peripheral arteries. ACh is known to cause endothelium-dependent vasodilatations, as well as direct contractions of vascular smooth muscle cell, while BK is a pure endothelium-dependent vasodilator.[1] Third, their patients had higher prevalence of hypertension and higher body mass index than our Japanese patients who were thus considered to be at relatively lower risk. These differences in the methods of measurement and patient characteristics may, at least in part, explain the discrepancy between the 2 studies. In the present study, 90% of patients with MVA alone had MVS, suggesting that vascular smooth muscle cell hypercontraction mediated by activated Rho-kinase is involved. On the other hand, Rho-kinase activity in patients with VSA could be reduced by treatment with calcium channel blockers,[21] while that in those with MVA alone may not be sufficiently reduced. Taken together, the patients with MVA alone may have both endothelial dysfunctions and vascular smooth muscle cell hypercontraction, whereas those with VSA may only have the latter. Importantly, the central mechanism of epicardial coronary spasm is regarded as vascular smooth muscle cell hypercontraction rather than endothelial dysfunction.[1]

In the conduit (brachial) artery, the present study showed that EDH factors contributed to maintain vasodilatations in patients with VSA alone. By contrast, EDH-mediated forearm vasodilatations were markedly reduced in patients with MVA and those with VSA+MVA compared with those with VSA alone (Figure 4A). In the resistance (fingertip) arterioles, EDH-mediated vasodilatations were markedly reduced in patients with MVA and those with VSA+MVA (Figure 4B). Given that EDH is the predominant mechanism of endothelium-dependent vasodilatations in resistance arteries, MVA may be a cardiac manifestation of the systemic small artery disease. A previous study also

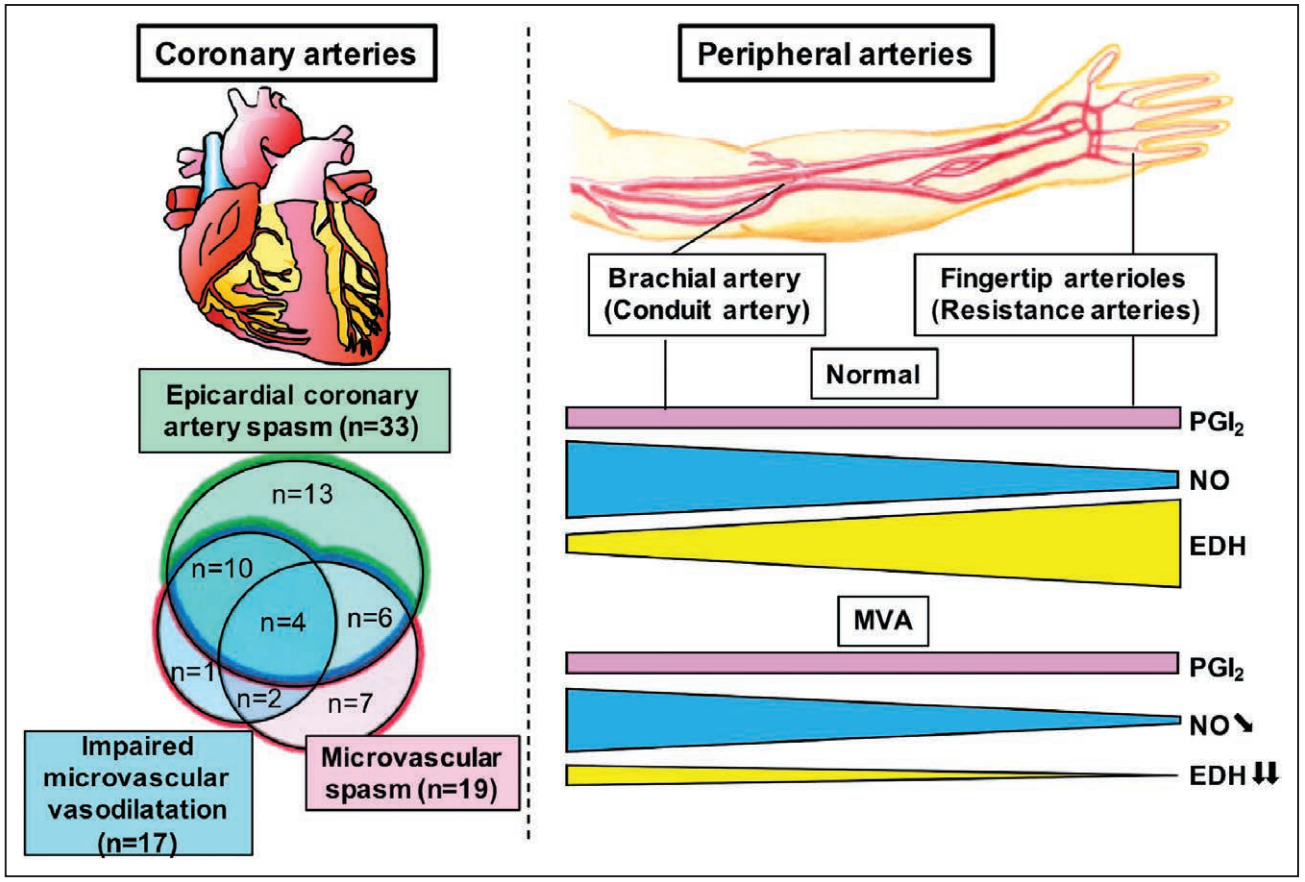

Figure 7. Summary of the present study.
Left, Coronary functional abnormalities frequently coexist in various combinations. **Right**, Endothelium-dependent vasodilatations of peripheral resistance arteries are markedly impaired in microvascular angina (MVA) patients, suggesting that MVA represents a systemic small vascular disease associated with endothelial dysfunction affecting both NO- and endothelium-dependent hyperpolarization (EDH)–mediated mechanisms (especially the latter). PG indicates prostaglandin.

suggested that endothelial dysfunction is a systemic disorder.[31] Furthermore, Berry et al[10] reported that MVA is a small vessel disease that may manifest as a multisystem disorder affecting small vessels of the brain and other organs such as dementia, renal dysfunction, and retinopathy, resulting in worse prognosis. In this context, our findings may provide a clue to the underlying mechanism of our recent observations that angina patients who have coronary vasomotor abnormalities at both epicardial and microvascular levels are at increased risk for future major adverse cardiovascular events.[32] In contrast, in fingertip arterioles, NO-mediated vasodilatations were greater in patients with VSA+MVA than in those with MVA alone (Figure 4B). Patients with MVA alone and those with VSA+MVA may have different kinds of coronary functional abnormalities, since 90% of patients with MVA alone had MVS but only 50% of patients with VSA+MVA had MVS. Thus, the difference in NO-mediated vasodilatations between patients with MVA alone and those with VSA+MVA may be caused, at least in part, by underlying coronary functional abnormalities. Further studies are needed to examine impaired vasodilatations and MVS separately in MVA patients.

Increased Plasma Levels of Soluble VCAM-1 in MVA Patients

Previous studies showed that NO- and EDH-mediated vasodilatations could be impaired by inflammation of vascular endothelial cells[33,34] and that elevated plasma levels of adhesion molecules are closely associated with endothelial dysfunction.[35,36] However, the relation between soluble VCAM-1 and endothelium-dependent vasodilatations in patients with VSA, MVA, or both of them remains to be fully elucidated. In the present study, plasma levels of soluble VCAM-1 were significantly elevated in patients with MVA alone, and there were significant negative correlations between soluble VCAM-1 and NO-mediated digital vasodilatations. These results suggest that soluble VCAM-1 could be a surrogate marker of impaired NO-mediated vasodilatations of resistance arteries in MVA patients.

Additionally, we consider that this explanation could be possible for VCAM-1 and ICAM-1. The expressions of VCAM-1 and ICAM-1, both of which are important factors for thrombus formation, are known to increase with inflammation.[37,38] However, there are several studies

reporting that plasma levels of VCAM-1, but not those of ICAM-1, increased in patients with CAD,[39,40] suggesting functional differences between them.

A previous study showed that increased expression of soluble adhesion molecules plays a direct role in the pathogenesis of atherosclerosis via endothelial dysfunction and inflammation.[35] However, in the present study, hs-CRP (high-sensitivity C-reactive protein) levels were comparable among the 3 groups, suggesting that plasma levels of soluble VCAM-1 more accurately reflect a low-grade inflammation than hs-CRP in patients with MVA. Thus, soluble VCAM-1 could be a useful and noninvasive biomarker for diagnosis of MVA. Additionally, soluble VCAM-1 may be not only a possible diagnostic tool for MVA but also a novel therapeutic target in MVA patients. Further studies are needed to elucidate the roles of soluble VCAM-1 in the pathogenesis of MVA.

Study Limitations

Several limitations should be mentioned for the present study. First, we were unable to analyze endothelial functions in control subjects without VSA or MVA because of their small numbers. Unfortunately, we were unable to continue the present protocols because the distributor had discontinued to produce the Good Manufacturing Practice grade BK and L-NMMA for human use anymore. Thus, the present findings need to be confirmed in future studies with a large number of patients. Second, the interval between coronary physiological measurement and peripheral vascular examination varied individually, which raises the possibility that change in medication following the coronary reactivity testing might have influenced the peripheral vascular functions. However, medications at the time of peripheral vascular function test were comparable among the 3 groups, and endothelium-dependent digital vasodilatations were almost absent only in patients with MVA. Third, we were unable to set a standard for discontinuation of medicine before peripheral vascular function test for ethical reasons. Fourth, we should consider the possibility that reduced responses to endothelium-dependent vasodilator agents could be due to the concomitant release of aspirin-insensitive endothelium-derived vasoconstrictors.[11]

Conclusions

The present study provides the first direct evidence that both NO- and EDH-mediated digital vasodilatations are markedly impaired in MVA patients, suggesting that MVA is a cardiac manifestation of the systemic small artery disease involving both NO and EDH.

ARTICLE INFORMATION

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Disclosures

None.

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