Original Article

Brachial Artery Flow-Mediated Vasodilation Is Correlated with Coronary Vasomotor and Fibrinolytic Responses Induced by Bradykinin

Yasuhiro TARUTANI, Tetsuya MATSUMOTO, Hiroyuki TAKASHIMA,
Tetsunobu YAMANE, and Minoru HORIE

Endothelium plays a key role in the regulation of not only vascular tone but also thrombosis and fibrinolysis. Brachial flow-mediated vasodilation (FMD) provides a noninvasive method of assessing coronary endothelial dysfunction. However, no data are available on the relationship between brachial FMD and coronary fibrinolytic activity. Thus, we examined the relationship between brachial FMD and coronary vasomotor and fibrinolytic function. Brachial FMD by reactive hyperemia was defined as a change in diameter relative to the baseline as measured using high-resolution ultrasound. Coronary blood flow (CBF) responses to bradykinin (BK) were analyzed using Doppler flow velocity measurement. Coronary release of tissue-type plasminogen activator (tPA) antigen was determined as the transcardiac tPA gradient $\times$ {CBF $\times$ (100 – hematocrit)/100}. In 77 patients with normal coronary arteries, BK caused dose-dependent increases in CBF, transcardiac tPA gradient, and coronary tPA release. Among them, brachial FMD, the BK-induced CBF increase, and the coronary tPA release induced by BK in 14 diabetic subjects were lower than those in 63 non-diabetic subjects ($p < 0.05$, respectively). Brachial FMD correlated with the CBF increase, transcardiac tPA gradient (0.2 $\mu\text{g}/\text{min}$: $r = 0.25$; 0.6 $\mu\text{g}/\text{min}$: $r = 0.43$; 2.0 $\mu\text{g}/\text{min}$: $r = 0.34$; $p < 0.05$, respectively), and coronary tPA release (0.2 $\mu\text{g}/\text{min}$: $r = 0.24$; 0.6 $\mu\text{g}/\text{min}$: $r = 0.44$; 2.0 $\mu\text{g}/\text{min}$: $r = 0.32$; $p < 0.05$, respectively) in response to BK. Brachial FMD correlated significantly with coronary endothelial function and fibrinolytic activity in response to BK. Type 2 diabetes impaired coronary and brachial endothelium-dependent vasodilation and coronary fibrinolytic activity. (*Hypertens Res* 2005; 28: 59–66)

Key Words: coronary, endothelial function, bradykinin, fibrinolytic activity

Introduction

Vascular endothelial cells, through the release of nitric oxide, tissue-type plasminogen activator (tPA) and plasminogen activator inhibitor type 1 (PAI-1), play an important role in the regulation of not only vascular tone but also thrombosis and fibrinolysis.

Recent studies have suggested that the endothelium-dependent coronary or peripheral vasomotor dysfunction was an independent predictor of cardiovascular disease events (1–3).

It has been accepted that reduced fibrinolytic activity is associated with an increased risk of myocardial infarction and cardiovascular mortality (4–6). Impaired fibrinolysis has also been observed in hypertension and impaired glucose tolerance in association with endothelial dysfunction (7). However, it remains to be established whether peripheral plasma concentrations of tPA or PAI-1 are indicators of endothelial function.

A noninvasive technique has been developed to evaluate flow-mediated vasodilation (FMD) of the brachial artery (8–10). The goal of measuring peripheral endothelial function is to predict the progression of coronary artery disease and future

From the Department of Cardiovascular and Respiratory Medicine, Shiga University of Medical Science, Otsu, Japan.

This study was supported by the Kimura Memorial Heart Foundation.

Address for Reprints: Tetsuya Matsumoto, M.D., Ph.D., Department of Cardiovascular and Respiratory Medicine, Shiga University of Medical Science, Seta Tsukinowa-cho, Otsu 520–2192, Japan. E-mail: tetsuyam@belle.shiga-med.ac.jp

Received August 30, 2004; Accepted in revised form September 29, 2004.

cardiovascular events. Early studies (11) demonstrated that a relationship exists between the coronary vasomotor response to acetylcholine and FMD of the brachial artery. Nevertheless, few studies have reported that the FMD of the brachial artery correlates with coronary endothelial function (12, 13).

Bradykinin (BK) is a vasoactive polypeptide that has cardioprotective effects (14). In our previous report, BK stimulated the release of tPA as well as nitric oxide in the human coronary circulation (15). However, no data are currently available on the relationship between brachial artery FMD and coronary fibrinolytic activity.

Thus, we examined the relationship between brachial artery FMD and coronary vasomotor and fibrinolytic function as estimated by the responses to BK in patients with suspected coronary artery disease.

Methods

Study Patients

The study protocols were approved by the Ethical Committee on Human Research at our institution, and written informed consent was obtained from all patients. The study population included 77 consecutive patients who underwent diagnostic cardiac catheterization, including coronary angiography, for the evaluation of chest pain and/or myocardial ischemia on ECG. Patients with myocardial infarction, congestive heart failure, cardiomyopathy, valvular heart disease, or coronary narrowing exceeding 25% of the luminal diameter of the left anterior descending coronary artery were excluded from this study.

All subjects fasted for at least 8 to 12 h, and did not ingest substances that might affect vasoreactivity, such as caffeine or vitamin C, for 4 to 6 h before the study. Smokers refrained from smoking for at least 7 days before the study to rule out the direct effects of oxidants contained in cigarette smoke. All vasoactive medications were withheld for at least 72 h before the study, and aspirin was discontinued for at least 7 days before the study. Subjects were considered to have hypertension if they had a blood pressure level of 140/90 mmHg or more or if they were being treated. Patients with secondary hypertension were excluded. Subjects were considered to be diabetic if they had a fasting blood glucose level of 126 mg/dl or more or if they were already being treated. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2).

Ultrasound of Brachial Artery

All individuals underwent an ultrasound examination according to the guidelines of the International Brachial Artery Reactivity Task Force (16) on the day before the cardiac catheterization.

The diameter of the right brachial artery (20 mm proximal to the antecubital fossa) was assessed using a high-resolution machine (SONOS 5500; Philips Medical Systems, Bothell,

USA) with a broadband (7 to 15 MHz) linear array transducer. To secure the ultrasound image and measurement position throughout the study, we used a stereotactic probe-holding device, while the subject's arm was positioned on a foam cast to inhibit movement. The ultrasound images were recorded on S-VHS videotape and analyzed off-line. The mean values of 3 measurements were averaged for each datum point.

A pressure cuff, placed on the forearm, was inflated and kept constant at 50 mmHg above systolic blood pressure to induce forearm ischemia. After 5 min the cuff was released, and the increase in blood flow caused increased shear stress, which served as the stimulus for FMD. FMD occurred maximally about 60 s after release of the occlusive cuff, and the maximum brachial artery diameter was measured. Brachial artery FMD was expressed as the change in the post-stimulus arterial diameter divided by the baseline diameter. Next, the subjects were given 15 min of rest to return to baseline conditions. Baseline diameter was re-determined. A single tablet of nitroglycerin (NTG) at a dose of 0.3 mg (Nihonkayaku Co., Tokyo, Japan) was then administered sublingually. After 5 min, the brachial artery diameter was determined again. NTG-induced brachial artery vasodilation was expressed in the same way as the percentage of baseline diameter.

Reproducibility was assessed in 10 individuals (five men; 39 ± 3 years) who were examined twice with a 2-week interval. The intra-observer intersession coefficients of variation were 2.8% for diameter, 2.5% for brachial artery FMD, and 1.6% for NTG-induced brachial artery vasodilation (the latter two were expressed as the absolute diameter change).

Protocol

After a diagnostic coronary angiography, a 0.014-inch Doppler-tipped guide wire (Jometrics FloWire, JoMed Inc., Rancho Cordova, USA) was advanced through the Judkins catheter, as previously reported (15, 17, 18). The wire tip was positioned at the area between the proximal and middle segments of the left anterior descending coronary artery that was free of any major branches within 1.0 cm from the tip. All drugs were infused directly into the left main coronary artery via a 2.6 F coronary-infusion catheter (Cordis Co., Miami Lakes, USA) at infusion rates ranging between 0.5 and 1.0 ml/min. A 6 F multipurpose catheter (GCS6; Goodtec, Gifu, Japan) was advanced via the right femoral vein into the coronary sinus (CS) for blood sampling.

Baseline coronary angiography and measurement of coronary blood flow (CBF) velocity were performed. BK-induced vasodilation responses were established by dose-response curves obtained with incremental 2-min intracoronary infusions of BK. Coronary angiography and measurement of CBF velocity were performed after each infusion. BK was started at 0.2 $\mu\text{g}/\text{min}$, and increased to 0.6 and 2.0 $\mu\text{g}/\text{min}$. Finally, this was followed by the intracoronary administration of papaverine at 12 mg for 20 s after an additional 10 min, by which time the CBF velocity and aortic pressure had returned

Table 1. Baseline Characteristics

Age (years)	62±1 (range 38–79)
Gender (male/female)	58/19
Body mass index (kg/m ²)	24.1±0.4 (range 15.8–33.6)
Hypertension (%)	36 (46.8%)
Diabetes mellitus (%)	14 (18.2%)
HbA1c (%)	7.1±0.3 (range 6.1–8.9)
Smoking status (%)	29 (37.7%)
Total cholesterol (mg/dl)	192±3 (range 106–261)
LDL cholesterol (mg/dl)	120±3 (range 56–189)
HDL cholesterol (mg/dl)	47±2 (range 15–105)
Triglycerides (mg/dl)	140±8 (range 33–410)
tPA antigens in Ao (ng/ml)	6.1±0.3 (range 2.3–17.4)
CS (ng/ml)	6.2±0.3 (range 2.2–16.9)
PAI-1 antigens in Ao (ng/ml)	5.8±0.5 (range 0.4–27.7)
CS (ng/ml)	6.0±0.5 (range 0.8–26.9)

Data are presented as the mean±SEM. Ao, aorta; CS, coronary sinus; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PAI-1, plasminogen activator inhibitor type 1; tPA, tissue-type plasminogen activator.

to the baseline. Maximal CBF velocity was measured after the infusion of papaverine. Coronary angiography was performed at 2 min after the infusion of papaverine. During the study, phasic and mean aortic blood pressure, heart rate, and 12-lead ECG were monitored continuously.

Quantitative Coronary Angiography and Measurements of CBF

The change in the diameter of the left anterior descending coronary artery was measured in a vessel segment 5 mm beyond the tip of the Doppler-wire. Coronary angiograms were analyzed by quantitative coronary angiography using a Cardiovascular Measurement System (QCA-CMS; MEDIS Medical Imaging Systems, Leiden, The Netherlands). Measurements were made 3 times, and the average value was used for analysis. Peak CBF velocity was monitored continuously using a fast Fourier transform-based spectral analyzer (FloMap; Cardiometrics Inc., Mountain View, USA). CBF was calculated as π average peak CBF velocity 0.125 (arterial diameter)² (19). Vessel diameter and CBF velocity were analyzed by investigators who were blinded to the sequence of investigations.

Blood Sampling and Biochemical Assays

Paired blood samples in the aorta (Ao) and CS were taken simultaneously before and after the infusion of BK and papaverine for the measurement of plasma tPA antigen and PAI-1 antigen. Blood samples were collected on ice and centrifuged immediately, and plasma was stored at -70°C until the time of assay. Blood for the measurement of tPA and PAI-1 was collected in tubes containing 0.105 mol/l sodium cit-

Table 2. Ultrasound Measurements of Brachial Artery

Brachial artery diameter (mm)	
Baseline	4.2±0.1 (range 3.1–5.2)
After hyperemia	4.5±0.1 (range 3.3–5.6)
After nitroglycerin	4.8±0.1 (range 3.5–6.1)
Flow-mediated dilation (%)	8.0±0.4 (range 1.8–14.3)
Nitroglycerin-induced dilation (%)	14.6±0.4 (range 8.0–24.8)

Data are presented as the mean±SEM.

rate. Antigen levels were determined using a two-site ELISA (Biopool, Umea, Sweden).

Arterio-venous concentration gradients were calculated by subtracting the plasma level measured in simultaneously collected CS venous and arterial blood. Thus, the net coronary release of tPA antigen at each time point was calculated as (concentration CS – concentration Ao) {CBF (100 – hematocrit)/100}.

Preparation of BK

BK (Sigma Chemical Co., St. Louis, USA) was diluted with physiological saline at a concentration of 2 g/ml and sterilized at the Pharmacy Department, Shiga University of Medical Science Hospital.

Statistical Analysis

Data are expressed as the means±SEM. Continuous variables are compared using the unpaired Student's *t*-test or a one-way analysis of variance (ANOVA) with Bonferroni's test. Linear regression analysis was used to determine the relationships between continuous variables. A value of $p < 0.05$ was considered statistically significant.

Results

Patient Characteristics

The baseline characteristics of the 77 patients are summarized in Table 1. The study population was predominantly male. All women were postmenopausal. All diabetic patients were type 2. HbA1c was $7.1 \pm 0.3\%$ in diabetic patients. BMI was higher in diabetic subjects (26.0 ± 0.7 kg/m², range 21.0 to 31.1 kg/m²) than in non-diabetic subjects (23.7 ± 0.4 kg/m², range 15.8 to 33.6 kg/m², $p = 0.027$). Clinical characteristics except for BMI did not differ between diabetic and non-diabetic subjects.

The plasma levels of tPA antigen and PAI-1 antigen did not differ between the Ao and the CS, respectively (Table 1). In simple linear regression analyses, the baseline plasma levels of tPA antigen in the Ao were significantly and positively correlated with age ($r = 0.25$, $p = 0.029$) and the plasma levels of PAI-1 antigen in the Ao ($r = 0.25$, $p = 0.033$), but not with BMI, or plasma levels of total-, low-density lipoprotein

Table 3. Correlations between the Changes in Brachial Artery Flow-Mediated Dilation (FMD), the Changes in Coronary Blood Flow (%CBF) and the Net tPA Release Induced by Bradykinin at 2.0 $\mu\text{g}/\text{min}$ and Other Parameters

Variables	FMD (%)		%CBF (%)		Net tPA release (ng/min)	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Age (years)	0.03	0.791	-0.11	0.331	0.12	0.289
Gender (male=1)		0.119		0.051		0.247
Body mass index (kg/m^2)	-0.12	0.301	-0.14	0.239	-0.02	0.864
Hypertension (yes=1)		0.315		0.205		0.763
Diabetes mellitus (yes=1)		0.032		0.008		0.026
Smoking (yes=1)		0.308		0.433		0.615
Total cholesterol (mg/dl)	0.002	0.987	0.09	0.462	-0.13	0.269
LDL cholesterol (mg/dl)	0.09	0.433	0.14	0.234	-0.02	0.858
HDL cholesterol (mg/dl)	0.04	0.772	0.04	0.806	-0.04	0.815
Triglycerides (mg/dl)	-0.03	0.795	-0.07	0.553	-0.26	0.025
tPA antigens in Ao (ng/ml)	0.16	0.161	-0.19	0.106	0.19	0.105
PAI-1 antigens in Ao (ng/ml)	-0.14	0.237	-0.12	0.296	-0.12	0.322

Ao, aorta; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PAI-1, plasminogen activator inhibitor type 1; tPA, tissue-type plasminogen activator.

Fig. 1. Changes in coronary blood flow responses (left), transcardiac gradients of tissue-type plasminogen activator (tPA) antigen concentration (middle), and net tPA release (right) induced by bradykinin (0.2, 0.6, and 2.0 $\mu\text{g}/\text{min}$). * $p < 0.0001$ and † $p < 0.01$ vs. baseline. ‡ $p < 0.0001$ vs. bradykinin at 0.2 $\mu\text{g}/\text{min}$.

(LDL)-, or high-density lipoprotein (HDL)-cholesterol, or triglyceride, and were independent of gender, smoking status, hypertension, and diabetes (data not shown). On the other hand, the plasma levels of PAI-1 antigen in the Ao were significantly and positively correlated with BMI ($r=0.35$, $p=0.002$) and the plasma levels of triglyceride ($r=0.41$, $p=0.0002$). BMI was positively correlated with the plasma levels of triglyceride ($r=0.27$, $p=0.016$), but not with age or the plasma levels of total-, LDL-, or HDL-cholesterol, and were independent of gender, smoking status, and hypertension (data not shown).

Brachial Artery FMD

Table 2 shows the results of brachial artery ultrasound. Brachial artery FMD was lower in diabetic subjects ($6.2 \pm 1.0\%$,

range 2.1 to 13.5%) than in non-diabetic subjects ($8.3 \pm 0.4\%$, range 1.8 to 14.3%, $p=0.032$). As shown in Table 3, brachial artery FMD was not correlated with age, BMI, or plasma levels of total-, LDL-, or HDL-cholesterol or triglyceride, and was independent of gender, smoking status and hypertension (hypertensive subjects: $7.6 \pm 0.5\%$ vs. normotensive subjects: $8.4 \pm 0.6\%$, $p=0.315$). Brachial artery FMD was not correlated with the plasma levels of tPA antigen or PAI-1 antigen in the Ao. NTG-induced brachial artery vasodilation was not correlated with any of these parameters (data not shown).

Coronary Vasomotor Responses to BK

The intracoronary infusion of BK did not affect the arterial pressure or heart rate in any of the patients. The infusion of graded doses of BK and papaverine at 12 mg significantly

Fig. 2. Relationship between brachial artery flow-mediated vasodilation (FMD) and the changes in coronary blood flow induced by bradykinin (BK; 0.2, 0.6, and 2.0 g/min).

Fig. 3. Relationship between brachial artery flow-mediated vasodilation (FMD) and the transcardiac gradients of plasma levels of tissue-type plasminogen activator (tPA) antigen induced by bradykinin (BK; 0.2, 0.6, and 2.0 g/min).

increased CBF (Fig. 1, left). As shown in Table 3, the increases in CBF induced by BK (2.0 g/min) were associated with diabetes (diabetic subjects: $161.8 \pm 21.7\%$ vs. non-diabetic subjects: $270.0 \pm 18.0\%$, $p = 0.008$), but were not correlated with age, BMI or plasma levels of total-, LDL-, or HDL-cholesterol or triglyceride, and were independent of gender, smoking status, and hypertension (hypertensive subjects: $228.6 \pm 23.4\%$ vs. normotensive subjects: $269.4 \pm 21.5\%$, $p = 0.205$). The increases in CBF induced by BK (2.0 g/min) were not correlated with the plasma levels of tPA antigen or PAI-1 antigen in the Ao.

As shown in Fig. 2, there were significantly positive correlations between brachial artery FMD and the increase in CBF in response to BK (0.2, 0.6, and 2.0 g/min). Brachial artery FMD was not correlated with the increase in CBF in response to papaverine at 12 mg ($p = 0.134$). In contrast, NTG-induced brachial artery vasodilation was not correlated with the increase in CBF in response to each dose of BK (data not shown).

Coronary Fibrinolytic Responses to BK

The levels of tPA antigen in the Ao were marginally increased by the intracoronary infusion of BK (0.2 g/min: 6.6 ± 0.4 ng/ml; 0.6 g/min: 6.8 ± 0.4 ng/ml; 2.0 g/min: 8.7 ± 0.5 ng/ml, respectively, $p < 0.05$ vs. baseline level). The levels of tPA antigen in the CS were significantly increased in a dose-dependent manner by the infusion of graded doses of BK (0.2 g/min: 10.0 ± 0.7 ng/ml; 0.6 g/min: 14.8 ± 1.0 ng/ml; 2.0 g/min: 16.7 ± 0.9 ng/ml, respectively, $p < 0.05$ vs. baseline level). Accordingly, transcardiac gradients of tPA antigen were significantly increased in response to the intracoronary infusion of BK (Fig. 1, middle). The net tPA release induced by BK was also significantly increased in a dose-dependent manner by the infusion of graded doses of BK (Fig. 1, right). Neither the transcardiac gradient of tPA antigen nor the net tPA release were influenced by the infusion of papaverine at 12 mg (Fig. 1, middle and right).

On the other hand, intracoronary infusion of BK (0.2, 0.6,

Fig. 4. Relationship between brachial artery flow-mediated vasodilation (FMD) and the net coronary release of tissue-type plasminogen activator (tPA) antigen induced by bradykinin (BK; 0.2, 0.6, and 2.0 g/min).

and 2.0 g/min) did not alter the plasma levels of PAI-1 antigen in either the Ao or CS. Intracoronary infusion of papaverine also did not alter the plasma levels of PAI-1 antigen in either the Ao or CS (data not shown).

As shown in Table 3, the net tPA release induced by BK (2.0 g/min) in diabetic subjects was significantly lower than that in non-diabetic subjects (diabetic subjects: 174.2 ± 33.9 ng/min vs. non-diabetic subjects: 360.1 ± 37.7 ng/min; $p = 0.026$). The net tPA release induced by BK (2.0 g/min) was significantly and inversely correlated with plasma levels of triglyceride ($r = -0.26$, $p = 0.025$), was not correlated with age, BMI or plasma levels of total-, LDL- or HDL-cholesterol, and was independent of gender, smoking status, and hypertension (hypertensive subjects: 315 ± 37.6 ng/min vs. normotensive subjects: 335.6 ± 51.7 ng/min, $p = 0.763$).

As shown in Table 3, the net tPA release induced by BK (2.0 g/min) was not correlated with the baseline plasma levels of tPA or PAI-1 antigen in the Ao. The increases in transcardiac gradients of tPA antigen in response to BK (0.2, 0.6 and 2.0 g/min) showed significant positive correlations with brachial artery FMD, respectively (Fig. 3). The net tPA release in response to BK (0.2, 0.6 and 2.0 g/min) also showed significant positive correlations with brachial artery FMD, respectively (Fig. 4). There were no significant correlations between either transcardiac gradients of tPA antigen or net tPA release and NTG-induced brachial artery vasodilation (data not shown).

Discussion

The present study is the first to demonstrate that the changes in FMD of the brachial arteries are significantly correlated with tPA release as well as vasodilative responses induced by BK in the coronary circulation. Furthermore, type 2 diabetes may not only deteriorate endothelium-dependent vasodilation in both the brachial and the coronary circulation, but also fibrinolytic activity in the coronary circulation.

FMD in the Brachial Arteries and Coronary Vaso-motor Response to BK

The most commonly used probes for recognizing vascular endothelial function are FMD of the brachial arteries and coronary artery response to acetylcholine (11). It has been shown that macro- and microvascular coronary endothelial dysfunction independently predict acute cardiovascular events in patients with and without coronary artery disease (2). Although the analysis of coronary endothelial function is certainly appropriate for cardiovascular events, this method is limited by the risk and expense of cardiac catheterization. Therefore, there has been great interest in the examination of endothelial function in more approachable vascular beds, such as the brachial arteries.

In the present study and our recent findings, brachial artery FMD correlated positively and significantly with epicardial and resistant coronary artery dilation induced by BK (20). On the other hand, it was previously reported that brachial artery FMD marginally correlated with the epicardial coronary artery response to acetylcholine (11).

It has been shown that the activation of this local vascular kallikrein-kinin system may underlie FMD in the arterial wall, and that this system contributes to the increased release of nitric oxide (21). In the coronary circulation, BK may play an important role in mediating the tonic baseline and flow-induced release of nitric oxide (22). In forearm circulation, Hornig *et al.* reported that the augmentation of FMD caused by the inhibition of angiotensin-converting enzyme was associated with an increase in endogenous BK levels (23). These previous findings may explain the close relationship between brachial artery FMD and coronary responses induced by exogenous BK.

In the present study, atherosclerotic risk factors other than diabetes did not deteriorate coronary or peripheral endothelial function. Type 2 diabetes may cause endothelial dysfunction through hyperglycemia, hyperinsulinemia and lipid disorders (24). Traditional coronary risk factors such as hypertension,

hypercholesterolemia, diabetes, increased age, obesity, and cigarette smoking have been shown to contribute to endothelial dysfunction in coronary and peripheral vessels (25, 26), but, except for diabetes, these effects were not observed in this study population. In the present study, many risk factors were not simply present or absent, treated or untreated, but rather were present to a greater or lesser degree. In addition, the duration affected by risk factors should be considered. In the future, it will be important to quantify these levels for each parameter, as well as to investigate the duration of their effects as risk factors.

Plasma Levels of tPA Antigen and PAI-1 Antigen

In the present study, the baseline levels of plasma tPA antigen were correlated with age, and the baseline levels of plasma PAI-1 antigen were correlated with BMI and triglyceride levels.

It has been reported that circulating levels of PAI-1 are increased in type 2 diabetic patients (27), and predict myocardial infarction (28, 29). The plasma levels of PAI-1 antigen are reported to be increased in subjects with hypertension, dyslipidemia, and hyperinsulinemia (30). Endothelial cells continuously secrete tPA by a constitutive pathway. An increase in circulating tPA levels has been shown to be due to responses to increased endogenous PAI-1, and predicts future myocardial infarction (6). The value of the plasma level of tPA or PAI-1 as an indicator of endothelial function remains to be established. In the present study, the baseline levels of circulating tPA or PAI-1 antigen did not serve as a marker of brachial or coronary endothelial function.

Intracoronary infusion of BK had no effects on PAI-1 levels at the Ao or CS, which was consistent with our previous findings (15).

FMD of the Brachial Arteries and Coronary Fibrinolytic Response Induced by BK

While the coronary arteries are prone to developing endothelial dysfunction, it has not been investigated whether or not the brachial arteries have a means of protecting against such dysfunction. In the present study, we first demonstrated that FMD of the brachial arteries may reflect not only coronary endothelium-dependent vasomotor function in response to BK but also coronary fibrinolytic function as estimated by an acute coronary release of tPA in response to BK. Therefore, impaired coronary fibrinolysis may be present in subjects with endothelium-dependent coronary vasomotor dysfunction who later develop coronary artery disease. Further studies are needed to examine whether the extent of the acute coronary release of tPA in response to BK is associated with the cardiovascular prognosis.

Zeiber *et al.* reported that chronic cigarette smoking impairs endothelium-dependent coronary vasodilation via increased oxidant stress (31). Thus, it is possible that coronary and brachial endothelial function and coronary fibri-

nolytic activity may have been normalized by the cessation of smoking prior to the present study.

In the present study, the presence of type 2 diabetes depressed FMD of the brachial arteries, the CBF response induced by BK, and the coronary release of tPA induced by BK. Although high plasma levels of tPA and PAI-1 reflect a state of fibrinolytic dysfunction, prior studies have not always demonstrated that parameters involved with the fibrinolytic system are abnormal in diabetic subjects. The present study was the first to demonstrate that endothelium-dependent vasomotor and fibrinolytic activity were impaired in the coronary circulation of diabetic patients. In patients with type 2 diabetes, disturbances in the fibrinolytic system and endothelial function in the coronary circulation may be associated with future coronary thrombotic events.

In conclusion, FMD of the brachial arteries correlated significantly with tPA release as well as vasodilative responses induced by BK in the coronary circulation. In patients without established ischemic heart disease, the estimation of brachial artery FMD may provide information on coronary endothelial function and fibrinolytic activity, which may predict future cardiovascular events. Prevention or correction of coronary endothelial dysfunction with drugs that target brachial artery FMD may be expected to reduce future cardiovascular events in patients with coronary risk factors, including type 2 diabetes.

Study Limitations

Many of our patients received medications such as statins, probucol, calcium antagonists, angiotensin-converting enzyme inhibitors, or angiotensin-1 receptor antagonists prior to the study. While these agents have been shown to have beneficial effects on endothelial function, the present study failed to address these beneficial effects (data not shown). Thus, we must further examine the effects of pharmacological intervention on vascular endothelial function and coronary fibrinolytic activity.

Furthermore, there may have been a potential selection bias in the present study, as only subjects undergoing coronary angiography were included. Therefore, our results may not be applicable beyond a subject group with chest pain.

Acknowledgements

We thank Dr. Nobuo Hoshino, Department of Pharmacy, Shiga University of Medical Science, for preparing BK.

References

1. Schachinger V, Britten MB, Zeiber AM: Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation* 2000; **101**: 1899–1906.
2. Halcox JP, Schenke WH, Zalos G, *et al*: Prognostic value of coronary vascular endothelial dysfunction. *Circulation* 2002; **106**: 653–658.

3. Fathi R, Haluska B, Isbel N, Short L, Marwick TH: The relative importance of vascular structure and function in predicting cardiovascular events. *J Am Coll Cardiol* 2004; **43**: 616–623.
4. Ridker PM, Hebert PR, Fuster V, Hennekens CH: Are both aspirin and heparin justified as adjuncts to thrombolytic therapy for acute myocardial infarction? *Lancet* 1993; **341**: 1165–1168.
5. Jansson JH, Olofsson BO, Nilsson TK: Predictive value of tissue plasminogen activator mass concentration on long-term mortality in patients with coronary artery disease. A 7-year follow-up. *Circulation* 1993; **88**: 2030–2034.
6. Thøgersen AM, Jansson JH, Boman K, *et al*: High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor. *Circulation* 1998; **98**: 2241–2247.
7. Tomiyama H, Kushiro T, Okazaki R, Yoshida H, Doba N, Yamashina A: Influences of increased oxidative stress on endothelial function, platelets function, and fibrinolysis in hypertension associated with glucose intolerance. *Hypertens Res* 2003; **26**: 295–300.
8. Laurent S, Lacolley P, Brunel P, Laloux B, Pannier B, Safar M: Flow-dependent vasodilation of brachial artery in essential hypertension. *Am J Physiol* 1990; **258**: H1004–H1011.
9. Celermajer DS, Sorensen KE, Gooch VM, *et al*: Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 1992; **340**: 1111–1115.
10. Sorensen KE, Celermajer DS, Spiegelhalter DJ, *et al*: Non-invasive measurement of human endothelium dependent arterial responses: accuracy and reproducibility. *Br Heart J* 1995; **74**: 247–253.
11. Anderson TJ, Uehata A, Gerhard MD, *et al*: Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol* 1995; **26**: 1235–1241.
12. Playford DA, Watts GF: Non-invasive measurement of endothelial function. *Clin Exp Pharmacol Physiol* 1998; **25**: 640–643.
13. Takase B, Uehata A, Akima T, *et al*: Endothelium-dependent flow-mediated vasodilation in coronary and brachial arteries in suspected coronary artery disease. *Am J Cardiol* 1998; **82**: 1535–1599.
14. Linz W, Wiemer G, Scholkens BA: Beneficial effects of bradykinin on myocardial energy metabolism and infarct size. *Am J Cardiol* 1997; **80**: 118A–123A.
15. Minai K, Matsumoto T, Horie H, *et al*: Bradykinin stimulates the release of tissue plasminogen activator in human coronary circulation: effects of angiotensin-converting enzyme inhibitors. *J Am Coll Cardiol* 2001; **37**: 1565–1570.
16. Corretti MC, Anderson TJ, Benjamin EJ, *et al*: Guidelines for the ultrasound assessment of endothelial dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002; **39**: 257–265.
17. Matsumoto T, Minai K, Horie H, *et al*: Angiotensin-converting enzyme inhibition but not angiotensin II type 1 receptor antagonism augments coronary release of tissue plasminogen activator in hypertensive patients. *J Am Coll Cardiol* 2003; **41**: 1373–1379.
18. Ohira N, Matsumoto T, Tamaki S, *et al*: Angiotensin-converting enzyme insertion/deletion polymorphism modulates coronary release of tissue plasminogen activator in response to bradykinin. *Hypertens Res* 2004; **27**: 39–45.
19. Doucette JW, Corl PD, Payne HM, *et al*: Validation of a Doppler guide wire for intravascular measurement of coronary artery flow velocity. *Circulation* 1992; **85**: 1899–1911.
20. Matsuo S, Matsumoto T, Takashima H, *et al*: The relationship between flow-mediated brachial artery vasodilation and coronary vasomotor responses to bradykinin: comparison with those to acetylcholine. *J Cardiovasc Pharmacol* 2004; **44**: 164–170.
21. Desta B, Vanhoutte PM, Boulanger CM: Inhibition of the angiotensin converting enzyme by perindoprilat and release of nitric oxide. *Am J Hypertens* 1995; **8**: 1S–6S.
22. Groves P, Kurz S, Just H, *et al*: Role of endogenous bradykinin in human coronary vasomotor control. *Circulation* 1995; **92**: 3424–3430.
23. Hornig B, Kohler C, Drexler H, Drexler H: Role of bradykinin in mediating vascular effects of angiotensin-converting enzyme inhibitors in humans. *Circulation* 1997; **95**: 1115–1118.
24. Makimattila S, Liu ML, Vakkilainen J, *et al*: Impaired endothelium-dependent vasodilation in type 2 diabetes. Relation to LDL size, oxidized LDL, and antioxidants. *Diabetes Care* 1999; **22**: 973–981.
25. Zeiher AM, Drexler H, Wollschlaeger H, Just H: Modulation of coronary vasomotor tone in humans. Progressive endothelial dysfunction with different early stages of coronary atherosclerosis. *Circulation* 1991; **83**: 391–401.
26. Furumoto T, Saito N, Dong J, Mikami T, Fujii S, Kitabatake A: Association of cardiovascular risk factors and endothelial dysfunction in Japanese hypertensive patients: implications for early atherosclerosis. *Hypertens Res* 2002; **25**: 475–480.
27. Sobel BE, Woodcock-Mitchell J, Schneider DJ, Holt RE, Marutsuka K, Gold H: Increased plasminogen activator inhibitor type 1 in coronary artery atherectomy specimens from type 2 diabetic compared with nondiabetic patients: a potential factor predisposing to thrombosis and its persistence. *Circulation* 1998; **97**: 2213–2221.
28. Meade TW, Ruddock V, Stirling Y, Chakrabarti R, Miller GJ: Fibrinolytic activity, clotting factors, and long-term incidence of ischaemic heart disease in the Northwick Park Heart Study. *Lancet* 1993; **342**: 1076–1079.
29. Thompson SG, Kienast J, Pyke SD, Haverkate F, van de Loo JC: Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. *N Engl J Med* 1995; **332**: 635–641.
30. Meigs JB, Mittleman MA, Nathan DM, *et al*: Hyperinsulinemia, hyperglycemia, and impaired hemostasis: the Framingham Offspring Study. *JAMA* 2000; **283**: 221–228.
31. Zeiher AM, Schachinger V, Minners J: Long-term cigarette smoking impairs endothelium-dependent coronary arterial vasodilator function. *Circulation* 1995; **92**: 1094–1100.