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Elevated Plasma Plasminogen Activator Inhibitor Type-1 is an Independent Predictor of Coronary Microvascular Dysfunction in Hypertension

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Background Elevated plasma plasminogen activator inhibitor-1 (PAI-1) is related to cardiovascular events, but its role in subclinical coronary microvascular dysfunction remains unknown. Thus, in the present study it was investigated whether elevated plasma PAI-1 activity is associated with coronary microvascular dysfunction in hypertensive patients.

Methods and Results Thirty patients with untreated essential hypertension and 10 age-matched healthy controls were studied prospectively. Myocardial blood flow (MBF) was measured by using ^{15}O-water positron emission tomography. Clinical variables associated with atherosclerosis (low-density lipoprotein-cholesterol, high-density lipoprotein (HDL)-cholesterol, triglyceride, homeostasis model assessment (HOMA-IR), and PAI-1 activity) were assessed to determine their involvement in coronary microvascular dysfunction. Adenosine triphosphate (ATP)-induced hyperemic MBF and coronary flow reserve (CFR) were significantly lower in hypertensive patients than in healthy controls (ATP-induced MBF: 2.77±0.82 vs 3.49±0.71 $ml \cdot g^{-1} \cdot min^{-1}$; $p<0.02$ and CFR: 2.95±1.06 vs 4.25±0.69; $p<0.001$). By univariate analysis, CFR was positively correlated with HDL-cholesterol ($r=0.46$, $p<0.02$), and inversely with HOMA-IR ($r=-0.39$, $p<0.05$) and PAI-1 activity ($r=-0.61$, $p<0.001$). By multivariate analysis, elevated PAI-1 activity remained a significant independent determinant of diminished CFR.

Conclusions Elevated plasma PAI-1 activity was independently associated with coronary microvascular dysfunction, which suggests that plasma PAI-1 activity is an important clue linking hypofibrinolysis to the development of atherosclerosis. (*Circ J* 2007; **71:** 348–353)

Key Words: Coronary circulation; Fibrinolysis; Hypertension; Plasminogen activator inhibitor type-1; Positron emission tomography

Hypertension is a major risk factor for coronary artery diseases! Coronary microvascular function decreases in parallel with the number of risk factors and is significantly associated with the risk of developing cardiovascular events in patients with coronary artery disease[2,3] Coronary microvascular dysfunction is caused not only by traditional risk factors such as hypertension, hyperlipidemia, diabetes mellitus, and smoking, but also by various atherogenic factors such as inflammation, oxidative stress, and thrombosis[4–7] Plasminogen activator inhibitor-1 (PAI-1), a fast-acting inhibitor of plasminogen activation, is produced by the vascular endothelium, but is also present in platelets and is considered to be an important regulator of fibrinolysis[5,8] Elevated plasma PAI-1 activity has been demonstrated to be associated with the impairment of flow-mediated dilatation, vessel wall thickening, and vascular wall stress[9–12] Elevated plasma PAI-1 activity was also related to the intima–media thickness of carotid arteries in a cross-sectional case–control study[11] Furthermore, elevated PAI-1 was associated with endothelial dysfunction, as well as glucose intolerance and hyperlipidemia, in patients with borderline and mild hypertension[12] Therefore, elevated plasma PAI-1 activity can be an important determinant of coronary microvascular dysfunction in hypertensive patients, but the pathophysiological significance of PAI-1 in subclinical coronary microcirculatory dysfunction remains unclear.

Noninvasive quantitative measurement of myocardial blood flow (MBF) and coronary flow reserve (CFR) by using oxygen-15 labeled (^{15}O-) water positron emission tomography (PET) and adenosine triphosphate (ATP)-induced hyperemia is useful for detecting coronary microvascular dysfunction and evaluating its severity in the clinical setting[13,14] Reproducibility of MBF measurement using ^{15}O-water PET is reported to be excellent[15] Therefore, the present prospective study was designed to identify the clinical variables, including plasma PAI-1 activity, in coronary microvascular dysfunction associated with hypertension.

Methods

Patients

Consecutive 30 untreated and uncomplicated hypertensive patients (16 males, 14 females; age 52.8±11.3 (SD) years)

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and 10 age-matched healthy controls were enrolled from December 2004 to March 2006 in the outpatient clinic of Hokkaido University Hospital. Hypertension was identified when systolic blood pressure (SBP) was over 140 mmHg and/or diastolic blood pressure (DBP) was over 90 mmHg by mercury sphygmomanometer, measured twice with an interval of 1 month. One patient was excluded because of an incomplete PET scan during ATP infusion. Therefore, remaining 29 hypertensive patients were analyzed. The mean duration from the onset of hypertension to the PET study was 2.9±3.1 years. Patients with a history or clinical evidence of recent infection, malignancies, bronchial asthma, coronary artery disease, peripheral vascular disease, cerebrovascular disease, secondary hypertension, diabetes mellitus with hemoglobin A1c >5.8%, hyperlipidemia with total cholesterol (TC) >260 mg/dl, wall motion abnormalities by echocardiography, aged more than 70 years, or on medications such as vasoactive agents, steroids, vitamins, estrogen, insulin, hypoglycemic agents, and statins were excluded.

All the subjects refrained from caffeine-containing beverages for at least 24 h before the PET study. Informed consent was given by each study subject. The study was approved by the institutional ethical committee, and the procedures were in accordance with institutional guidelines and the principles of Declaration of Helsinki.

Blood Chemical Analysis

Blood samples were obtained into EDTA tubes and 3.8% citric acid tubes at the time of PET scans at the fixed period of time after meal. They were centrifuged at 4°C at 2,500 rpm for 15 min and the supernatants were stored at –80°C. Enzyme-linked immunosorbent assay (ELISA) technique was used to measure levels of serum high-sensitivity C-reactive protein (hs-CRP) and malon-dialdehyde (MDA) low-density lipoprotein (LDL). The cytokines of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were measured by ELISA in duplicate. Plasma PAI-1 antigen level and PAI-1 activities were measured as described previously![16] Intra-assay variability was <9%. Overnight fasting levels of serum TC, LDL-cholesterol (C), high-density lipoprotein (HDL)-C, triglycerides (TG), blood sugar (BS) and insulin were measured. Homeostasis model assessment (HOMA-IR) was calculated as index for insulin resistance; HOMA-IR = fasting BS × insulin/405. Left ventricular mass index (LVMI) was measured by M-mode guided echocardiography recorded by cardiologists who were unaware of the patients' clinical data, according to the methods recommended by the American Society of Echocardiography. LVMI was derived from the formula described by Devereux et al![17]

PET Scans

MBF at rest and during infusion of ATP (inducing hyperemia) was measured noninvasively using ^{15}O-water and PET. All PET scans were performed with ECAT EXACT HR+ (Siemens/CTI). PET was performed as previously reported![18] A transmission scan was performed to correct the photon attenuation for 7 min with a ^{68}Ge source. Next, the subject inhaled 2,000 MBq ^{15}O-CO for 1 min to obtain blood volume image. After inhalation of the tracer 3 min were allowed for CO to combine with hemoglobin before a static scan for 5 min was started. A 12-min period was allowed for ^{15}O-CO radioactive decay before MBF measurement, 1,500 MBq ^{15}O-water was infused into an antecubital vein over 100 s. Simultaneously, a 24-frame dynamic PET scan was performed for 6 min consisting of 18×10-s and 6×30-s frames. Fifteen minutes after the first infusion of ^{15}O-water, intravenous infusion of ATP (0.16 mg·kg^{-1}·min^{-1}) was started until the end of the second PET scan using ^{15}O-water. The subject's motion was minimized by fastening a Velcro strap across the chest and abdomen. Heart rate (HR), blood pressure (BP), and a 12-lead ECG were recorded at rest and at 1-min intervals during and after the administration of ATP.

Reconstruction Methods

All emission sinograms were reconstructed with filtered back projection using a Hann filter (cutoff frequency 0.3). The in-plane resolution has 4.5 mm full width of half maximum in images reconstructed into a 128×128 matrix. All data were corrected for dead-time, decay, and measured photon attenuation.

Quantification of MBF

The left ventricular cavity time-activity curve was used as the input function. The myocardial time-activity curves were fitted by a single-compartment kinetic model that estimates MBF. The whole myocardial region of interest was set on the image obtained with ^{15}O-water according to the previously published methods![18] The entire left ventricle was divided into 3 major coronary territories (left anterior descending, left circumflex, and right coronary arteries) to quantify the regional MBF. No regional differences were found in the MBF of the patients. Therefore, to evaluate the relationship between CFR and clinical variables, the average blood flow of the global myocardium was calculated and used for the subsequent analysis. Each PET data was analyzed by 3 expert doctors who were unaware of the patients' clinical data. HR, DBP, and SBP were determined for each subject. The rate–pressure product (RPP) was calculated as HR×SBP. CFR was calculated by dividing ATP-induced MBF by resting MBF. ATP-induced MBF and CFR were used as markers of coronary microvascular function.

Statistical Analyses

Results are expressed as means ± SD. The changes in hemodynamic parameters between resting and during ATP infusion in all subjects were compared with a paired t-test. MBF at rest, ATP-induced MBF, and CFR in both groups were compared with an unpaired t-test. For observed variables, univariate analysis was used to determine the relationship to ATP-induced MBF and CFR. For multivariate analysis, variables associated with ATP-induced MBF and CFR at $p<0.20$ were entered in the linear regression model to determine whether these variables were independently associated with ATP-induced MBF or CFR. A p-value <0.05 was considered significant.

Results

Clinical Characteristics of the Study Subjects

Table 1 shows the clinical characteristics of both the patients with hypertension and the healthy controls. Both SBP and DBP were higher in the hypertension group than in the control group. The BP values for the hypertensive patients were mildly to moderately severe according to the Japanese Society of Hypertension classification. Other clinical variables including age, body mass index (BMI), LVMI, TC,

Table 1 Clinical Characteristics of the Study Subjects

	Healthy control (n=10)	Hypertension (n=29)	p value
Age (years)	50.1±9.7	52.8±11.5	NS
Sex (M/F)	6/4	15/14	NS
BMI (kg/m²)	24.1±2.5	25.1±4.2	NS
Smoking [no (%)]	2 (20)	4 (14)	NS
HR (beats/min)	63±10	64±8	NS
SBP (mmHg)	118±11	150±13	<0.001
DBP (mmHg)	66±8	100±12	<0.001
LVMI (g/m²)	86.5±11.5	98.1±19.3	NS
TC (mg/dl)	208.7±32.1	202.6±32.3	NS
HDL-C (mg/dl)	56.9±12.8	63.1±15.2	NS
LDL-C (mg/dl)	126.1±26.8	122.3±29.7	NS
MDA-LDL (U/L)	126±54	142±64	NS
TG (mg/dl)	130.3±73.4	130.8±120.0	NS
BS (mg/dl)	96.2±12.5	99.9±8.0	NS
Insulin (mU/L)	7.25±4.17	6.53±4.25	NS
HOMA-IR	1.80±1.33	1.63±1.07	NS
IL-6 (pg/ml)	0.72±0.44	1.54±1.34	NS
TNF-α (pg/ml)	1.03±0.41	1.18±0.44	NS
hs-CRP (ng/ml)	300±283	1,012±1,740	NS
PAI-1 activity (AU/ml)	7.11±1.98	8.35±4.11	NS
PAI-1 antigen (ng/ml)	21.3±20.7	20.7±9.5	NS

BMI, body mass index; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVMI, left ventricular mass index; TC, total cholesterol; HDL, high-density lipoprotein; C, cholesterol; LDL, low-density lipoprotein; MDA, malondialdehyde; TG, triglycerides; BS, blood sugar; HOMA-IR, homeostasis model assessment; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; hs-CRP, high-sensitivity C-reactive protein; PAI-1, plasminogen activator inhibitor-1.
Data are mean±SD.

Table 2 Hemodynamic Parameters and MBF by PET Scan

	Healthy control (n=10)	Hypertension (n=29)	p value
Rest			
SBP	118±11	143±12	<0.001
DBP	66±8	80±9	<0.001
mBP	83±9	101±9	<0.001
HR	63±10	64±8	NS
RPP	7,520±1,503	9,178±1,393	<0.01
ATP			
SBP	115±14	133±19	<0.01
DBP	62±10	71±12	<0.05
mBP	80±11	92±14	<0.02
HR	85±12	84±9	NS
RPP	9,738±1,701	11,205±1,961	<0.05
MBF			
Rest (ml·g⁻¹·min⁻¹)	0.83±0.18	0.98±0.19	<0.05
Corrected by RPP (ml·g⁻¹·min⁻¹)	0.75±0.11	0.72±0.12	NS
ATP (ml·g⁻¹·min⁻¹)	3.49±0.71	2.77±0.82	<0.02
CFR	4.25±0.69	2.95±1.06	<0.001

MBF, myocardial blood flow; PET, positron emission tomography; mBP, mean blood pressure; RPP, rate–pressure product; ATP, adenosine triphosphate; CFR, coronary flow reserve. See Table 1 for other abbreviations.
Data are mean±SD.

LDL-C, HDL-C, TGs, BS, and insulin were comparable between groups. Plasma PAI-1 activity tended to be higher in the hypertensive patients than in the controls although statistically not significant.

Relationship Between MBF and Clinical Variables

Hemodynamic and MBF data of the patients and controls are shown in Table 2. SBP, DBP, mean BP, and RPP at rest and during ATP infusion in the hypertensive patients were significantly higher than in the control group. Resting MBF was elevated in patients as compared with healthy controls, corresponding to the higher RPP (r=0.53, p<0.01). Therefore, resting MBF corrected by RPP was comparable between the 2 groups. ATP-induced MBF and CFR were significantly lower in the hypertensive patients than in the controls.

By univariate analysis, ATP-induced MBF and CFR in the patients positively correlated with HDL-C (ATP-induced MBF: r=0.51, p<0.01 and CFR: r=0.46, p<0.02), and inversely with HOMA-IR (ATP-induced MBF: p=0.22 and CFR: r=–0.39, p<0.05) and PAI-1 activity (ATP-induced MBF: r=–0.62, p<0.001 and CFR: r=–0.61, p<0.001) (Fig 1), but not with age, BP, BMI, LVMI, LDL-C, MDA-LDL, IL-6, or TNF-α. Resting MBF did not correlate with any clinical variables except RPP.

By multivariate analysis, elevated plasma PAI-1 activity

was the single independent predictor of a reduction of ATP-induced MBF and CFR (Table 3).

Discussion

This is the first study to demonstrate that coronary microvascular function assessed by ^{15}O-water PET during ATP-induced hyperemia is associated positively with serum HDL-C and inversely with HOMA-IR and plasma PAI-1 activity in hypertensive patients. Importantly, plasma PAI-1 activity was independently associated with coronary microvascular dysfunction, suggesting that increase in plasma PAI-1 activity may attenuate coronary microvascular function in patients with mild to moderate hypertension.

Coronary Microvascular Dysfunction in Hypertension

The present study has clearly demonstrated by ^{15}O-water PET that coronary microvascular dysfunction is present in hypertensive patients. The reduction of CFR is caused by impaired endothelium-dependent coronary vasodilation, structural abnormalities of coronary artery such as perivascular fibrosis and myocardial fibrosis, increased left ventricular end-diastolic pressure, and elevated resting MBF in response to the higher RPP![9] In the present study, ATP-induced MBF itself decreased in the hypertensive subjects, indicating that blunted CFR was mainly caused by reduction of ATP-induced increase in MBF, despite elevated resting MBF. Elevated left ventricle end-diastolic pressure may not contribute to this phenomenon because the study subjects did not have symptoms of heart failure or left ventricular contractile dysfunction. Accordingly, in the present study subjects coronary microvascular dysfunction may be caused by endothelial dysfunction and perivascular fibrosis. In the present study, BP and LVMI values did not correlate with the degree of coronary microvascular dysfunction, clearly suggesting that BP and LVMI have only limited value in the early detection of coronary microvascular dysfunction in hypertensive patients with a normal to modest increase of LVMI. These results are in agreement with a previous study that showed that the blunted CFR was not correlated with 24-h ambulatory BP values in borderline hypertension without left ventricular hypertrophy![20] In contrast, low HDL-C and high HOMA-IR levels were significantly correlated with coronary microvascular dysfunction, suggesting that metabolic biomarkers may have potentially more value. HDL-C could have an antiatherogenic effect on coronary microvasculature because it activates endothelial

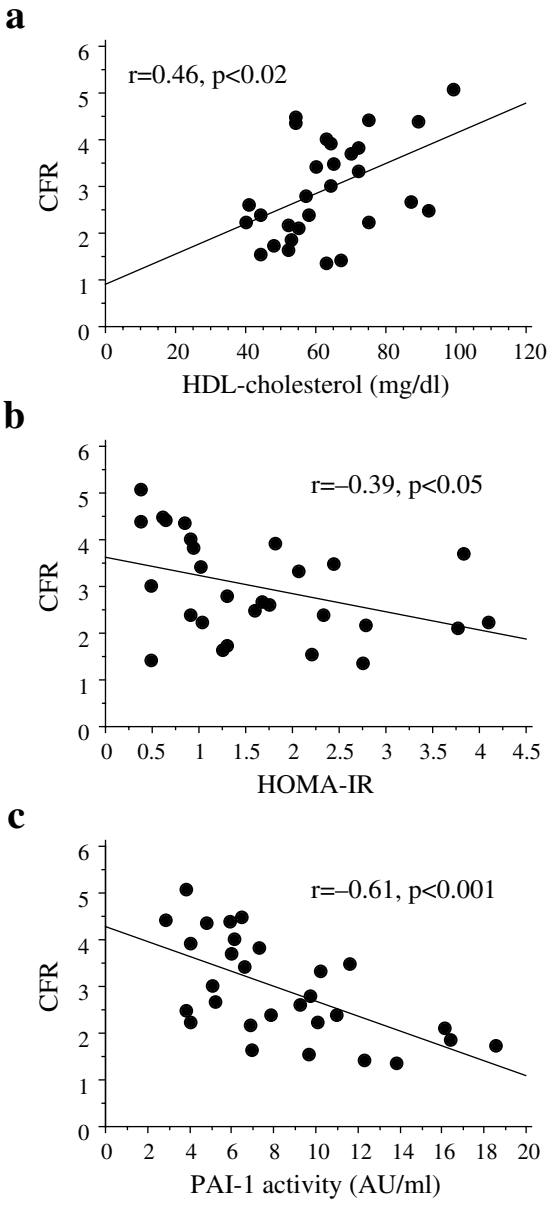

Fig 1. Relationships among coronary flow reserve (CFR) and high-density lipoprotein (HDL)-cholesterol (a), homeostasis model assessment (HOMA-IR) (b), and plasma plasminogen activator inhibitor-1 (PAI-1) activity (c) in 29 untreated hypertensive patients.

Table 3 Determinants of ATP-Induced MBF and CFR in Hypertensive Patients

	ATP-induced MBF		*CFR*	
	Standardized coefficient	*p value*	*Standardized coefficient*	*p value*
PAI-1 activity (AU/ml)	*–0.54*	*0.04*	*–0.60*	*0.02*
Age (years)	*0.08*	*0.67*	*0.26*	*0.17*
Sex	*0.15*	*0.44*	*0.40*	*0.04*
Smoking	*0.14*	*0.54*	*0.01*	*0.96*
BMI (kg/m^2)	*0.22*	*0.28*	*0.26*	*0.18*
RPP (mmHg/min)	*0.21*	*0.31*	*–0.01*	*0.96*
HDL-C (mg/dl)	*0.36*	*0.07*	*0.30*	*0.11*
TG (mg/dl)	*–0.05*	*0.81*	*–0.04*	*0.83*
HOMA-IR	*–0.05*	*0.84*	*–0.15*	*0.48*
hs-CRP (ng/ml)	*–0.17*	*0.31*	*–0.08*	*0.62*
Adjusted R2	*0.96*		*0.94*	
Significance (ANOVA)		*<0.0001*		*<0.0001*

ANOVA, analysis of variance. See Tables 1,2 for other abbreviations.

NO synthesis, leading to increased production of NO?[21] On the other hand, CFR is blunted in obesity with hyperinsulinemia?[22] Acute hyperhomocysteinemia induced by hyperinsulinemia impairs microvascular dilation as a result of reduced NO bioavailability?[23] Hypertriglyceridemia is also a significant factor for reduced myocardial vasodilation?[24]

Elevated Plasma PAI-1 and Coronary Microvascular Dysfunction

To the best of our knowledge this is the first report that elevated plasma PAI-1 is a major independent predictor of coronary microvascular dysfunction. PAI-1 is a major regulator of fibrinolysis and its production is regulated by vasoactive molecules?[25] Increased PAI-1 activity is implicated in atherothrombosis, insulin resistance and obesity, and troponin-positive acute coronary syndrome?[26–28] Overexpression of PAI-1 can induce intimal growth?[29] PAI-1 can stimulate cell migration[30] and enhance matrix accumulation, which contributes to the intimal thickening?[30–33] Alterations of fibrinolytic activity may destabilize plaque?[34] In addition to its role in atherogenesis PAI-1 may be involved in vascular tone regulation. High BP and chronic systemic inflammation damages the coronary endothelium and depletes endothelial tissue plasminogen activator (t-PA) storage. Mutant t-PA administration before thrombectomy improves Thrombolysis in Myocardial Infarction myocardial perfusion grade in acute myocardial infarction?[35] Because PAI-1 inhibits t-PA-mediated vasoactivity?[36] reduced coronary vasoreactivity mediated by hypofibrinolysis may be an additional mechanism by which PAI-1 contributes to vasculopathy?[37] The association of PAI-1 with vessel wall damage and clinical cardiac events[11,38,39] may be at least partly caused by reduced vascular tone by PAI-1. Our results provide an important direct biological link of hypofibrinolysis to vasculopathy. Plasma PAI-1 levels could identify the high risk subgroup for cardiovascular events among hypertensive subjects. In the future, evaluating the effect of antihypertensive medications on CFR and PAI-1 will further clarify the mechanism of PAI-1 mediated coronary microvascular dysfunction.

Study Limitations

First, the number of the study patients was small. However, the relationship between CFR and PAI-1 activity proved to be significant and the number of patients was enough to perform this analysis. Second, the presence of coronary artery disease was not completely excluded because coronary angiography was not performed in any of the patients. However, the contribution of myocardial ischemia could be excluded because none of the subjects had a history of angina or left ventricular wall motion abnormality on echocardiography, and CFR measured by PET showed no regional abnormalities. Third, no outcome information was provided because uncomplicated hypertensive patients with normal to modestly increased LVMI were treated properly after this study and a good clinical course was expected.

Conclusions

Elevated plasma PAI-1 activity is a major independent predictor of coronary microvascular dysfunction, as measured by ^{15}O-water PET with ATP infusion in hypertensive patients. The present study adds evidence that diminished fibrinolysis contributes to coronary microvascular dysfunction and the pathobiological pathways of subclinical early coronary atherosclerosis in the setting of hypertension. Plasma PAI-1 measurement may help to identify hypertensive patients at high risk of developing coronary atherosclerosis.

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