Original Article

Plasma Interleukin-6 and Tumor Necrosis Factor-α Can Predict Coronary Endothelial Dysfunction in Hypertensive Patients

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Coronary endothelial function is impaired in hypertension; however, the severity of this impairment varies among patients. We aimed to identify the predictors of coronary endothelial dysfunction among clinical variables related to hypertension and atherosclerosis. Twenty-seven untreated, uncomplicated essential hypertensive patients and 10 age-matched healthy controls were studied prospectively. Myocardial blood flow (MBF) was measured by using ^{15}O-water positron emission tomography (PET) at rest and during a cold pressor test (CPT). Coronary vascular resistance (CVR) during CPT was used as a marker of coronary endothelial function. Serum low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, malondialdehyde-LDL, homeostasis model assessment, high-sensitivity C-reactive protein (hs-CRP), and plasma interleukin-6 (IL-6) and tumor necrosis factor (TNF)-α were also measured. CVR during CPT was significantly higher in hypertensive patients than in healthy controls (114±26 *vs.* 94±12 mmHg/[mL/g/min]; $p<0.05$). By univariate analysis, CVR during CPT was correlated with LDL cholesterol ($r=0.38$, $p<0.05$), IL-6 ($r=0.46$, $p<0.02$), and TNF-α ($r=0.39$, $p<0.05$) in hypertensive patients. By multivariate analysis, IL-6 and TNF-α were significant independent predictors of CVR during CPT. Elevated plasma IL-6 and TNF-α levels were independent predictors of coronary endothelial dysfunction in hypertensive patients. These results suggest that plasma IL-6 and TNF-α might be useful for identifying the high risk subgroup of hypertensive patients with coronary endothelial dysfunction and provide an important clue to link systemic inflammation to the development of coronary atherosclerosis. (*Hypertens Res* 2007; 30: 541–548)

***Key Words*: interleukin-6, tumor necrosis factor-α, coronary endothelial dysfunction, hypertension, positron emission tomography**

Introduction

Hypertension is a major risk factor of coronary artery disease (*1*). In hypertensive patients, coronary vasomotion is impaired even in patients without coronary artery stenosis (*2*, *3*), which might be related not only with the elevation of blood pressure (BP) but also with lipoprotein(a), vascular inflammation and oxidative stress (*4–7*). Because the impairment of coronary endothelial function is significantly associated with the risk of developing cardiovascular events (*8–12*), it is of importance to evaluate the severity and, moreover, identify predictors of this dysfunction. Proinflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor (TNF)-α can induce the hepatic synthesis of C-reactive protein (CRP). IL-6 is secreted by endothelial cells and smooth

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Table 1. Clinical Characteristics of the Study Subjects

	Healthy control (n=10)	Hypertension (n=27)	p-value
Age (years)	50.1±9.7	53.1±10.8	n.s.
Sex (M/F)	6/4	14/13	n.s.
BMI (kg/m^2)	24.1±2.5	25.1±4.3	n.s.
Smoking (n (%))	2 (20)	4 (15)	n.s.
HR (bpm)	63±10	64±8	n.s.
SBP (mmHg)	118±11	150±13	<0.001
DBP (mmHg)	66±8	100±12	<0.001
LVMI (g/m^2)	86.5±11.5	98.5±19.0	n.s.
Total cholesterol (mg/dL)	208.7±32.1	201.9±33.2	n.s.
HDL cholesterol (mg/dL)	56.9±12.8	63.7±15.4	n.s.
LDL cholesterol (mg/dL)	126.1±26.8	121.2±30.5	n.s.
MDA-LDL (U/L)	126±54	144±65	n.s.
Triglycerides (mg/dL)	130.3±73.4	130.8±120.0	n.s.
BS (mg/dL)	96.2±12.5	100.4±8.0	n.s.
Insulin (mU/L)	7.25±4.17	6.27±4.30	n.s.
HOMA-IR	1.80±1.33	1.57±1.09	n.s.
IL-6 (pg/mL)	0.72±0.44	1.54±1.34	0.07
TNF-α (pg/mL)	1.03±0.41	1.18±0.44	n.s.
hs-CRP (ng/mL)	300±283	864±1,542	n.s.

M, male; F, female; BMI, body mass index; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVMI, left ventricular mass index; HDL, high density lipoprotein; LDL, low density lipoprotein; MDA, malondialdehyde; BS, blood sugar; HOMA-IR, homeostasis model assessment of insulin resistance; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; hs-CRP, high sensitivity C-reactive protein. Data are expressed as mean±SD.

muscle cells and increases inflammatory activity in atheroma. Elevated plasma IL-6 and TNF-α levels have been shown to be associated with peripheral endothelial dysfunction and increase the risk of myocardial infarction (*13–15*). These results suggest a close relationship between cytokine-mediated inflammation and vascular endothelial dysfunction. However, the relationship between IL-6 or TNF-α and the endothelial dysfunction remains unestablished in the coronary artery.

Oxygen-15 labeled (^{15}O-) water positron emission tomography (PET) can provide noninvasive, quantitative measurement of myocardial blood flow (MBF). The reproducibility of MBF measurement using ^{15}O-water PET is reported to be excellent (*16*). The cold pressor test (CPT) activates sympathetic release of norepinephrine and epinephrine (*17*). Direct β-adrenergic activation and an increase of shear stress on endothelial cells induce coronary vasodilation in healthy subjects (*18*). However, abnormal myocardial flow response to CPT was observed in patients with early atherosclerosis. Therefore, ^{15}O-water PET with CPT allows us to detect early coronary atherosclerosis and evaluate its severity (*19–21*). In the present study, we aimed to identify the predictors associated with coronary endothelial dysfunction assessed by using ^{15}O-water PET in patients with hypertension.

Methods

Patients

Twenty-seven consecutive untreated and uncomplicated essential hypertensive patients (14 male and 13 female; age 53.1±10.8 [mean±SD] years) and 10 age-matched healthy controls were enrolled from December 2004 to March 2006 in the outpatient clinic of Hokkaido University Hospital for this study. Patients could be included when their systolic BP (SBP) was over 140 mmHg and/or their diastolic BP (DBP) was over 90 mmHg by mercury sphygmomanometer. Subjects 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 HbA1c >5.8%, hyperlipidemia with total cholesterol >260 mg/dL, left ventricular wall motion abnormalities by echocardiography, of age older than 70 years, or on medications such as vasoactive agents, steroids, vitamins, estrogen, and statins were excluded. The mean duration from the onset of hypertension to the PET study was 3.0±3.1 years.

All the subjects refrained from caffeine-containing beverages for at least 24 h and smoking for at least 12 h before the PET study. Informed consent was obtained from each study subject. The study was approved by the institutional ethical

Table 2. Hemodynamic Parameters during PET Scans

	Healthy control (n=10)	Hypertension (n=27)	p-value
Rest			
SBP (mmHg)	118±11	143±13	<0.001
DBP (mmHg)	66±8	80±9	<0.001
mBP (mmHg)	83±9	102±9	<0.001
HR (bpm)	63±10	64±8	n.s.
RPP (mmHg/min)	7,520±1,503	9,127±1,413	<0.01
CPT			
SBP (mmHg)	139±25	163±17	<0.01
DBP (mmHg)	80±13	91±12	<0.02
mBP (mmHg)	100±17	115±13	<0.01
HR (bpm)	76±12	73±8	n.s.
RPP (mmHg/min)	10,733±3,494	11,935±1,713	n.s.
ΔRPP to CPT (mmHg/min)	3,213±2,948	2,807±1,350	n.s.

PET, positron emission tomography; SBP, systolic blood pressure; DBP, diastolic blood pressure; mBP, mean blood pressure; HR, heart rate; RPP, rate pressure product; CPT, cold pressor test. Data are expressed as mean±SD.

committee, and the procedures were in accordance with institutional guidelines and the principles of the Declaration of Helsinki.

Blood Chemical Analysis and Left Ventricular Mass Index

Blood samples were obtained at the time of PET scans. They were centrifuged at 4°C at 2,500 rpm for 15 min and their supernatant was stored at −80°C. High-sensitivity (hs)-CRP and malondialdehyde (MDA)–low density lipoprotein (LDL) were measured by ELISA. The cytokines, IL-6 and TNF-α, were measured in duplicate by ELISA. In addition, the overnight fasting levels of serum total cholesterol, LDL cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, blood sugar (BS), and insulin were also measured.

Left ventricular mass index (LVMI) was measured on the M-mode guided echocardiogram by expert doctors according to the methods recommended by the American Society of Echocardiography. LVMI was derived from the formula described by Devereux *et al*. (*22*).

PET Scans

MBF at rest and during CPT was measured noninvasively using ^{15}O-water and PET. All PET scans were performed with ECAT EXACT HR+ (Asahi Siemens, Tokyo, Japan). 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 a 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 the flow 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/min) was started until the end of the second PET scan using ^{15}O-water. Finally, CPT was performed as follows. The patient's right foot was immersed in ice water up to the ankle. Sixty seconds later, PET scanning of ^{15}O-water was started, and the CPT was continued for 4 min. The subject's motion was minimized by fastening a Velcro strap across the chest and abdomen.

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 (*19*). 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 (*19*). The entire left ventricle was divided into three major coronary territories (left anterior descending, left circumflex, and right coronary arteries) to quantify the regional MBF. No regional differences were found in the hyperemic MBF of each subject. Therefore, the average blood flow of the global myocardium at rest and during CPT was calculated and used for the subsequent analysis. All PET data

were analyzed by expert doctors who were blind to the patients' clinical data.

Calculation of the Rate Pressure Product and Coronary Vascular Resistance

Heart rate (HR), DBP, and SBP were determined for each subject in the supine position during PET scans. The rate pressure product (RPP) was calculated as HR × SBP. Coronary vascular resistance (CVR) during CPT was used to assess coronary endothelial function in the present study. That CVR was greater during CPT indicates an abnormal vasoreactivity to the sympathetic stimulation induced by CPT. The mean BP was calculated as (2 × DBP + SBP)/3 and the average during dynamic PET scans was obtained.

Statistical Analyses

Data were expressed as the means±SD. The changes in hemodynamic parameters between at rest and during CPT in all groups were compared with a paired *t*-test. MBF at rest and MBF during CPT in both groups were compared with an unpaired *t*-test.

Univariate analysis was used to determine the relationship between each observed parameter and CVR during CPT.

For multivariate analysis, confounding factors (age, sex, body mass index [BMI], and smoking) and variables associated with CVR during CPT at $p<0.2$ were entered into the linear regression model to determine whether these variables were independently associated with CVR during CPT. Values of $p<0.05$ were considered statistically significant.

Results

Clinical Characteristics

Table 1 shows the clinical characteristics of the hypertensive patients and healthy controls. BP was significantly higher in the hypertensive patients. BMI, LVMI, BS, LDL cholesterol, HDL cholesterol, and triglycerides were not different between the two groups. Plasma IL-6 tended to be higher in hypertensive patients than healthy controls, but the difference did not reach the level of statistical significance ($p=0.07$).

MBF, ΔMBF, and CVR during CPT Assessed by ^{15}O-Water PET

SBP, DBP, mean BP, and RPP at rest and during CPT were significantly higher in hypertensive patients than in the control groups (Table 2). The changes of RPP in response to CPT were comparable between the two groups.

MBF at rest was elevated in hypertensive patients as compared with healthy controls (0.98±0.19 *vs*. 0.83±0.18 mL/g/min; $p<0.05$), corresponding to the higher RPP at rest ($r=0.54$, $p<0.01$). MBF during CPT did not differ in hypertensive patients compared with healthy controls. Thus ΔMBF from rest to CPT was significantly decreased in hypertensive patients (0.07±0.22 *vs*. 0.23±0.21 mL/g/min; $p<0.05$) (Fig. 1).

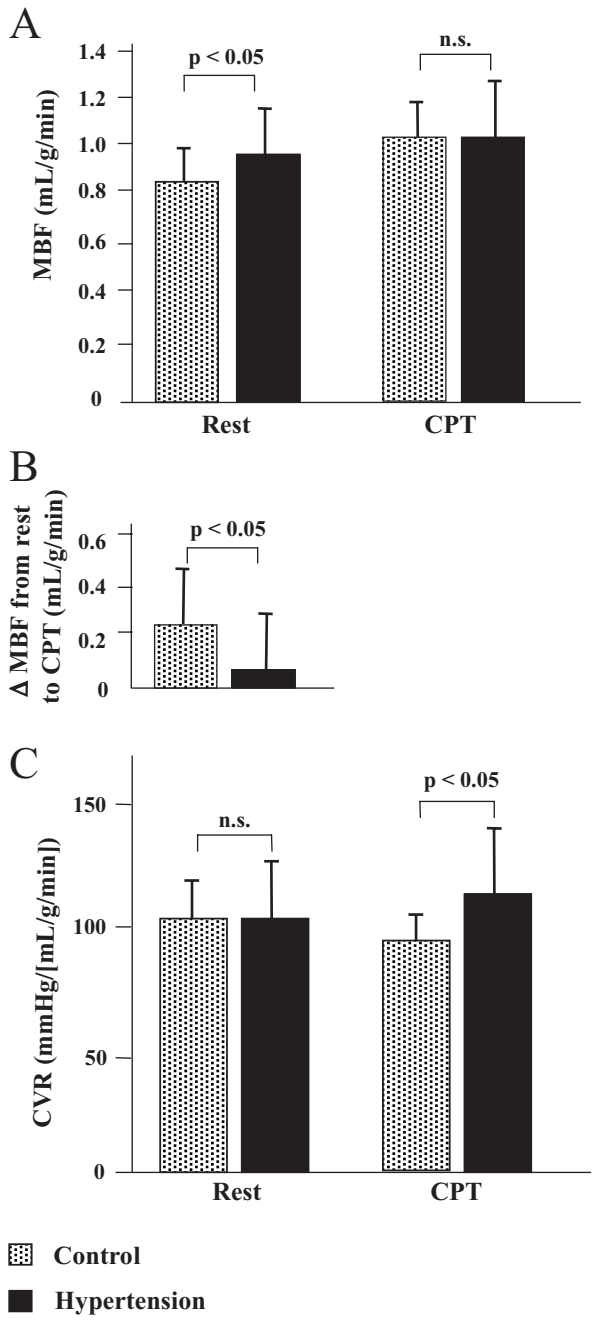

Fig. 1. *MBF (A), ΔMBF (B), and CVR (C) at rest and during CPT in healthy controls and hypertensive patients. MBF, myocardial blood flow; CVR, coronary vascular resistance; CPT, cold pressor test.*

CVR at rest was similar between the two groups (107±22 *vs*. 104±18 mmHg/[mL/g/min]; n.s.), whereas CVR

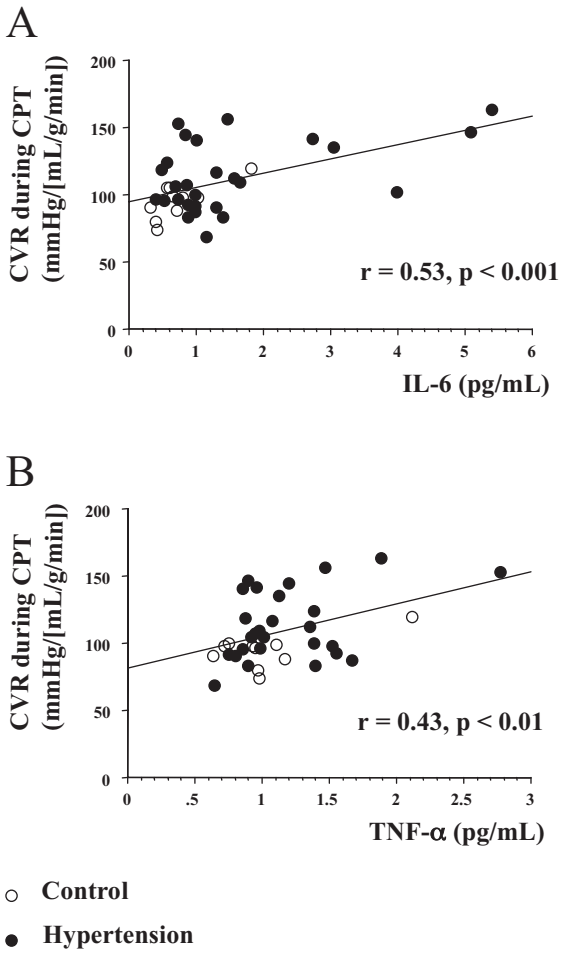

Fig. 2. *Relationship between IL-6 (A) or TNF-α (B) and CVR during CPT in both healthy controls (n = 10) and hypertensive patients (n = 27). IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; CVR, coronary vascular resistance; CPT, cold pressor test.*

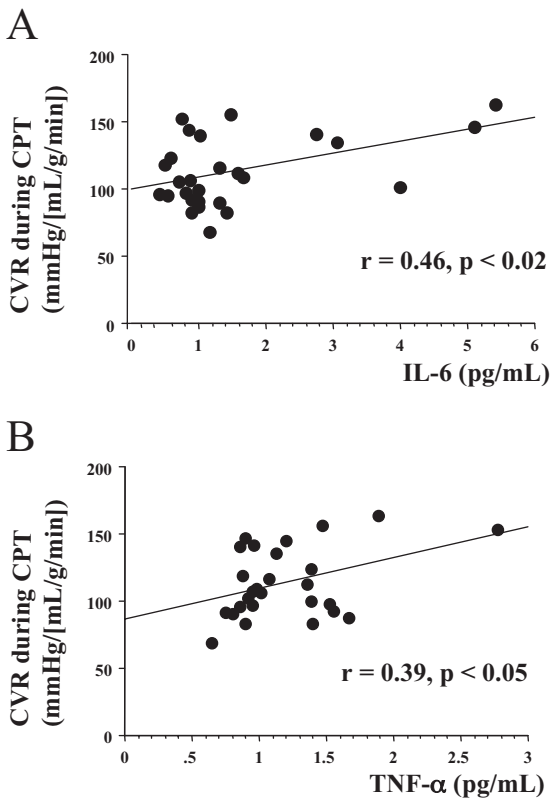

Fig. 3. *Relationship between IL-6 (A) or TNF-α (B) and CVR during CPT in only hypertensive patients (n = 27). IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; CVR, coronary vascular resistance; CPT, cold pressor test.*

during CPT was higher in hypertensive patients than in healthy controls (114±26 *vs*. 94±12 mmHg/[mL/g/min]; $p<0.05$) (Fig. 1).

Relationship between Clinical Variables and CVR during CPT

By univariate analysis, in both healthy controls and hypertensive patients, CVR during CPT was significantly correlated with IL-6 and TNF-α (Fig. 2), but not with the other clinical variables, *i.e.*, BMI, BP, LVMI, LDL cholesterol, HDL cholesterol, triglycerides, MDA-LDL, homeostasis model assessment of insulin resistance (HOMA-IR), and hs-CRP. When the analysis was limited to hypertensive patients, CVR during CPT was also significantly correlated with LDL cholesterol ($r=0.38$, $p<0.05$), IL-6 ($r=0.46$, $p<0.02$), and TNF-α ($r=0.39$, $p<0.05$) (Fig. 3). A nonsignificant trend of correlation was found between CVR during CPT and MDA-LDL ($r=0.34$, $p=0.08$), but not between CVR during CPT and the other clinical variables ($p>0.2$). In healthy controls, CVR during CPT was correlated with IL-6 ($r=0.80$, $p<0.01$) and a trend of correlation was found between CVR during CPT and LDL cholesterol ($r=0.62$, $p=0.055$) and TNF-α ($r=0.57$, $p=0.086$).

By multivariate analysis, only plasma IL-6 and TNF-α were independent predictors of CVR during CPT in hypertensive patients (Table 3).

Discussion

This is the first study to demonstrate that coronary endothelial dysfunction is independently associated with elevation of the plasma proinflammatory cytokines IL-6 and TNF-α in patients with essential hypertension. These results provide intriguing insights into the contribution of the systemic inflammatory process to coronary atherogenesis. Furthermore, elevated plasma IL-6 and TNF-α levels might be useful for identifying the high risk subgroup of hypertensive patients with coronary endothelial dysfunction.

Table 3. Predictors of CVR during CPT in Hypertensive Patients

	CVR during CPT standardized coefficient	*p*-value
Age (years)	0.04	0.84
Sex	0.40	0.03
Smoking	−0.17	0.41
BMI (kg/m^2)	0.15	0.29
LDL cholesterol (mg/dL)	0.29	0.31
MDA-LDL (U/L)	−0.04	0.87
IL-6 (pg/mL)	0.50	<0.01
TNF-α (pg/mL)	0.52	<0.01
Adjusted r^2	0.97	
Significance (ANOVA)		<0.0001

n=27. CVR, coronary vascular resistance; CPT, cold pressor test; BMI, body mass index; LDL, low density lipoprotein; MDA, malondialdehyde; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α.

Coronary Endothelial Dysfunction in Hypertension

The severity of coronary endothelial dysfunction was assessed by CVR during CPT using ^{15}O-water PET in the present study. We used CVR calculated from MBF measured by using PET as a marker of coronary endothelial function, because MBF is primarily regulated by mean BP as coronary perfusion pressure, and CVR during acetylcholine infusion has been shown to indicate endothelium-dependent function and independently predict cardiovascular events in patients with and without coronary artery disease (*12*). Although CVR at rest was comparable between hypertensive patients and healthy controls, CVR during CPT was higher in hypertensives, indicating that abnormal vasoconstriction of the coronary conduit vessels and microvasculature was induced in response to sympathetic stimulation. Risk factors for atherosclerosis such as hypertension can damage the vascular endothelium, resulting in vascular dysfunction *via* reduced bioavailability of NO. More importantly, coronary endothelial dysfunction has been shown to be associated with cardiac events independent of classical coronary risk factors (*9–12*). Therefore, the quantitative measurement of coronary vasomotor dysfunction may provide important information to assess the severity and to elucidate the potential mechanisms of coronary atherosclerosis in humans.

Relationship between IL-6, TNF-α, and Coronary Endothelial Dysfunction

The present study demonstrated that plasma levels of IL-6 and TNF-α, but not hs-CRP, were major independent biomarkers for identifying coronary endothelial dysfunction, suggesting that proinflammatory cytokines lead to early coronary atherosclerosis in uncomplicated hypertension. Furthermore, IL-6 was correlated with CVR during CPT in healthy controls, suggesting that IL-6 may be involved in the regulation of coronary endothelial function under physiological conditions. The severity of hypertension, as indicated by parameters such as BP and LVMI, did not correlate with coronary endothelial dysfunction, indicating that these factors may not influence coronary endothelial dysfunction. Moreover, proinflammatory cytokines, but not LDL cholesterol, were independently associated with endothelial dysfunction, suggesting that inflammation rather than LDL cholesterol itself directly impairs coronary vasomotion.

Experimental studies have shown that the production of IL-6 is increased in bovine aortic endothelial cells exposed to shear stress due to mechanical stress (*23*). IL-6 can induce an up-regulation of angiotensin II type 1 receptor gene expression, which leads to angiotensin II–mediated vasoconstriction as well as oxygen radical production and the development of endothelial dysfunction (*14*). Furthermore, IL-6 was identified as an independent predictor of flow-mediated dilatation in patients with stable coronary artery disease (*24*). IL-6 is also a strong independent risk factor for cardiovascular events in healthy men (*13*) as well as in patients with coronary artery disease (*25*).

TNF-α induces the expression of NAD(P)H oxidase subunit and abrogates endothelium-dependent vascular dilation (*26*). These results support a close relationship between cytokines and vascular endothelial dysfunction in the development of coronary atherosclerosis. Moreover, IL-6 and TNF-α have been reported to be independent risk factors for high BP in apparently healthy subjects (*27*). Taken together, these findings indicate that anti-inflammatory therapy to target circulating proinflammatory cytokines in addition to lowering BP might prevent the progression of coronary atherosclerosis in hypertension.

On the other hand, the present study demonstrated that hs-CRP was not elevated in hypertensive patients compared with healthy controls and that there was no association between hs-CRP and coronary endothelial dysfunction. This observation indicates that IL-6 and TNF-α, rather than hs-CRP, are useful for identifying coronary vascular dysfunction in hypertensive patients.

Study Limitations

There are several limitations that should be recognized in this study. First, the number of study patients was small. However, the relationship between coronary endothelial dysfunction and cytokines proved to be significant and the number of patients was sufficient to perform this analysis. Second, we cannot completely rule out the possible presence of coronary artery disease, because coronary angiography was not performed in any of the study patients. However, the contribu-

tion of myocardial ischemia could be excluded because none of the subjects had a history of angina, and coronary flow reserve measured by PET showed no regional abnormalities. Third, clinical outcome data were not available in the study patients.

Conclusions

The plasma proinflammatory cytokines IL-6 and TNF-α were major independent predictors of coronary endothelial dysfunction in hypertensive patients. Therefore, plasma IL-6 and TNF-α might be useful for identifying high risk patients with coronary endothelial dysfunction and provide an important clue to link systemic inflammation to the development of coronary atherosclerosis.

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