Symposium

Association of Cardiovascular Risk Factors and Endothelial Dysfunction in Japanese Hypertensive Patients: Implications for Early Atherosclerosis

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Although hypertension, hyperlipidemia, diabetes and smoking are known risk factors of atherosclerosis in Caucasians, their relative contributions to early atherosclerosis among Japanese are unknown. Decrease in flow-mediated dilation (FMD) of the brachial artery is a useful marker of endothelial dysfunction and early atherosclerosis. To evaluate the relative contribution of hypertension to early atherogenesis, we determined FMD, as well as plasma levels of tissue-type plasminogen activator (t-PA; a sensitive index of endothelial damage) and tumor necrosis factor (TNF)- α and interleukin (IL)-6 (established markers of inflammation) in normotensive and hypertensive patients under treatment. FMD was significantly reduced as the number of risk factors increased, suggesting that accumulations of risk factors were related to endothelial dysfunction. FMD was reduced in hypertensives (9.9 ± 5.8 (SD) %) compared to normotensives (14.6 ± 7.6 , $p < 0.01$) despite good blood pressure control ($139 \pm 20/80 \pm 14$ mmHg in hypertensives). Nitroglycerine-induced endothelium-independent vasodilation was not altered in hypertensives ($16.0 \pm 6.3\%$) as compared to normotensives (16.7 ± 5.8). Plasma t-PA, TNF- α , and IL-6 levels were increased in hypertensives despite good blood pressure control. Thus, hypertension alone is a high risk for early atherosclerosis. Persistent endothelial damage and moderate inflammation may increase the risk of early atherosclerosis synergistically under the presence of hypertension in Japanese. (*Hypertens Res* 2002; 25: 475–480)

Key Words: inflammation, fibrinolysis, endothelial function, risk factors, atherosclerosis

Introduction

Although hypertension, hyperlipidemia, diabetes and smoking are known risk factors of atherosclerosis in Caucasians, their relative contributions to early atherosclerosis among Japanese have not been thoroughly investigated (1). Endothelial cells maintain vascular tone and structure (2). In response to shear stress derived from flowing blood, endothelium can release nitric oxide (NO), which causes smooth mus-

cle cell relaxation (3). Flow-mediated dilation (FMD) of the brachial arteries, a useful noninvasive method to determine peripheral endothelial functions, is reduced in patients with hypertension and cardiovascular risk factors (2, 4).

With respect to other risk factors, inflammation has been implicated in vasculopathy (5). Plasma levels of tumor necrosis factor (TNF)- α and interleukin (IL)-6 are known to be elevated in atherosclerotic cardiovascular diseases (6), and the plasma level of tissue-type plasminogen activator (t-PA), which plays a crucial role in modulating thrombosis

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and thrombolysis (7), is a sensitive index of endothelial damage (8) and is associated with risk of accelerated atherosclerosis (9).

Endothelial dysfunction may be reversed by antihypertensive therapy in experimental models of hypertension and in humans (10, 11). To evaluate the relative contribution of hypertension to early atherogenesis, we determined FMD, as well as plasma levels of t-PA (a sensitive index of endothelial damage) and TNF- α and IL-6 (established markers of inflammation) in normotensive and hypertensive patients under treatment.

Methods

Study Population

Patients were recruited from the Hokkaido University Hospital ($n = 119$). Written informed consent was obtained from all patients in accordance with the institutional ethical guidelines. Patients with a history of essential hypertension (pretreatment systolic blood pressure ≥ 140 mmHg and/or pretreatment diastolic blood pressure ≥ 90 mmHg) that resulted in antihypertensive treatment (calcium blocker, $n = 47$; β blocker, $n = 17$; angiotensin converting enzyme inhibitor, $n = 17$) were included in the hypertension group ($n = 67$). Control subjects were matched to hypertensive subjects according to age and gender ($n = 52$). Blood pressure was expressed as the mean of 3 different sphyngomanometric measurements, each performed on 3 separate days. The possibility of secondary hypertension was excluded by clinical and laboratory tests. Exclusion criteria also included heart failure, valvular heart disease, congenital heart disease, acute coronary syndrome and liver disorders. Clinical data, including risk factor assessment for coronary heart disease, biochemical data, and results of the present investigations, were recorded. A known history of smoking (current smokers included individuals who stopped smoking < 5 years before the enrollment) was recorded. Body mass index (BMI) was calculated as the ratio of weight (kg) to the square of height (m^2). Routine lipid estimation was performed, including estimation of total cholesterol, triglyceride (TG) and high-density lipoprotein (HDL) levels. Subjects with an elevated serum total cholesterol (> 220 mg/dl) or subjects with a history of elevated serum total cholesterol that resulted in cholesterol-lowering treatment were considered to have a positive history of hyperlipidemia. No subject had documented familial hyperlipidemia. Subjects with an elevated fasting plasma glucose (> 126 mg/dl) or subjects with a history of elevated fasting plasma glucose that resulted in anti-diabetes treatment were considered to have a positive history of diabetes mellitus.

Sonographic Measurements

Sonographic measurements were performed in 62 patients

(25 normotensives and 37 hypertensives). A 5–10 MHz high-resolution linear phased array ultrasound transducer (ATL HDI 3000, Tokyo, Japan) was used to image the dominant arm brachial artery longitudinally above the antecubital fossa. After an overnight fast, patients rested for 10 min in a quiet room. All vasodilating drugs except sublingual nitroglycerin spray were discontinued at least 18 h before the study. After the baseline image was obtained, a blood pressure cuff was inflated to 200 mmHg on the forearm for 5 min and then released. Images were obtained every 30 s after cuff dilation and FMD was used as a measure of endothelium-dependent vasodilation. The brachial artery was then allowed to return to normal (10 min), and repeated baseline images were obtained. After administration of 0.3 mg sublingual nitroglycerin spray, the brachial artery was imaged for the ensuing 5 min for measurement of maximal vasodilation. Images were coded and recorded on VHS videotape for subsequent blinded analysis. The arterial diameter was measured in mm as the distance between the anterior and the posterior wall media-adventitial interface at end-diastole coincident with the R wave on the electrocardiogram at 2 sites along the artery and for 3 cardiac cycles, and expressed as the average of these 6 measurements. The average arterial diameter was used to calculate the percent diameter change of the brachial artery in response to reactive hyperemia or nitroglycerin. The intraobserver and interobserver variabilities for repeated measurements were 3.5%.

Blood Sampling and Laboratory Assays

Morning blood samples were taken after an overnight fast. The samples were drawn into plastic tubes containing 1: 9 volumes of 0.103 mol/l trisodium citrate and centrifuged at $3,000 \times g$ for 15 min. Aliquots of plasma were stored at -80°C until analysis. Personnel blinded to the clinical data performed the laboratory measurements. Fasting plasma glucose was measured by the glucose dehydrogenase method, and immunoreactive insulin (IRI) was measured by enzyme-linked immunosorbent assay (ELISA). Homeostasis model assessment (HOMA), defined as $\text{IRI } (\mu\text{U/ml}) \times \text{fasting plasma glucose (mg/dl)} / 405$, was used as an index of insulin sensitivity. The plasma level of t-PA antigen was measured by ELISA (Biopool, Umeå, Sweden). Plasma TNF- α was measured by high sensitivity immunoassay (R&D Systems, Minneapolis, USA). The sensitivity of the assay is 0.11 pg/ml. The plasma concentration of IL-6 was measured by enzyme-linked immunoassay (R&D Systems), which can detect values as low as 0.094 pg/ml. The cytokine levels in the present study were within the detection limit of the assay. All assays were performed in duplicate and the intra- and interassay variabilities were less than 10%.

Statistical Evaluation

Data were expressed as the mean $\pm$ SD. A χ^2 analysis was

Table 1. Characteristics of Patients

	Hypertensives (n = 67)	Normotensives (n = 52)
Age (years)	63.7 ± 10.9	59.4 ± 14.8
Male (%)	62.7	73.1
Blood pressure (mmHg)		
Systolic	139 ± 20	118 ± 13**
Diastolic	80 ± 14	71 ± 11**
Total cholesterol (mg/dl)	186 ± 32	196 ± 39
TG (mg/dl)	153 ± 75	123 ± 60*
HDL (mg/dl)	51 ± 14	56 ± 17
BMI (kg/m ²)	25.1 ± 4.0	23.3 ± 2.9**
Diabetes (%)	55.2	20.3**
HOMA index	1.66 ± 1.48	1.39 ± 2.02
Smoking (%)	40.3	50
Brachial artery diameter (mm)	4.26 ± 0.67	4.20 ± 0.67

TG, triglyceride; HDL, high-density lipoprotein; BMI, body mass index; HOMA, homeostasis model assessment. * $p < 0.05$, ** $p < 0.01$ compared to hypertensives.

used to determine differences in categorical variables. An unpaired Student's *t*-test was used to assess differences in continuous variables. Values of $p < 0.05$ were considered to indicate statistical significance.

Results

Brachial Artery FMD and Nitroglycerin-Induced Dilation

The characteristics of the normotensive and hypertensive patients are summarized in Table 1. The blood pressure in the hypertensive group was well controlled. No difference in brachial artery diameter was noted between the two groups. FMD was significantly lower in hypertensives ($9.9 \pm 5.8\%$) than in normotensives ($14.6 \pm 7.6\%$, $p < 0.05$) (Fig. 1A). Endothelium independent vasodilation was not significantly lower in hypertensives ($16.0 \pm 6.3\%$) than in normotensives ($16.7 \pm 5.8\%$).

Markers of Fibrinolysis and Inflammation

The plasma t-PA antigen level was higher in hypertensives than in normotensives (10.4 ± 4.2 vs. 8.4 ± 4.2 ng/ml, $p < 0.05$), indicating continued endothelial damage despite blood pressure reduction (Fig. 2A). The plasma TNF- α level was higher in hypertensives than in normotensives (2.6 ± 1.5 vs. 2.1 ± 1.4 pg/ml, $p < 0.05$), indicating persistent inflammation (Fig. 2A). However, the plasma IL-6 level was not significantly different between hypertensives and normotensives (1.8 ± 1.2 vs. 1.5 ± 1.2 pg/ml).

Fig. 1. (A) Comparison of flow mediated dilation (FMD) in normotensives (N: n = 52) and hypertensives (HT: n = 67). (B) Comparison of FMD in normotensives (N: n = 52), low hypertensives (L-HT: systolic blood pressure 130 mmHg and diastolic blood pressure 85 mmHg, n = 22) and high hypertensives (H-HT: systolic blood pressure > 130 mmHg and/or diastolic blood pressure > 85 mmHg, n = 45). Values are expressed as the mean ± SD.

Control of Blood Pressure and FMD

The hypertensives were divided into the following two subgroups based on their level of blood pressure control. Blood pressure was insufficiently controlled in high hypertensives (H-HT; systolic blood pressure > 130 mmHg and/or diastolic blood pressure > 85 mmHg), and well controlled in low hypertensives (L-HT; systolic blood pressure 130 mmHg and diastolic blood pressure 85 mmHg). The clinical characteristics in terms of age, gender, cholesterol, TG, HDL, BMI, HOMA, brachial artery diameter and prevalence of diabetes and smoking did not differ between the H-HT and L-HT groups (results not shown). Calcium blocker was used in 67% of H-HT and 71% of L-HT subjects. Angiotensin converting enzyme inhibitor was used in 28% of H-HT and 19% of L-HT and β blocker in 22% of H-HT and 33% of L-HT subjects. The average blood pressure in the L-HT group ($119 \pm 8/70 \pm 8$ mmHg) was not significantly higher than that in normotensives ($118 \pm 13/71 \pm 11$ mmHg). However, the blood pressure of the H-HT group was significantly higher than that of either normotensives or L-HT subjects ($149 \pm 17/85 \pm 14$, $p < 0.01$). Despite the good blood pressure control in the L-HT group, the FMD of this group was significantly lower than that in normotensives (Fig. 1B). And the plasma level of TNF- α in the L-HT group was significantly higher than that in normotensives (Fig. 2B).

Fig. 2. (A) Comparison of plasma levels of t-PA, TNF- α and IL-6 levels in normotensives (N: n = 52) and hypertensives (HT: n = 67). (B) Comparison of plasma levels of t-PA, TNF- α and IL-6 in normotensives (N: n = 52), low hypertensives (L-HT: systolic blood pressure 130 mmHg and diastolic blood pressure 85 mmHg, n = 22) and high hypertensives (H-HT: systolic blood pressure > 130 mmHg and/or diastolic blood pressure > 85 mmHg, n = 45). Values are expressed as the mean $\pm$ SD.

Fig. 3. Relationship between flow mediated dilation (FMD) and total number of risk factors. Risk factors include hypertension, hyperlipidemia, diabetes mellitus and smoking. Values are expressed as the mean $\pm$ SD.

Number of Risk Factors and FMD

FMD was significantly reduced as the total number of risk factors increased (Fig. 3). Furthermore, hypertensives without hyperlipidemia, diabetes or a smoking habit exhibited significantly lower FMD compared to normotensives (Fig. 4). Among hypertensives, addition of one or two other risk factors did not further reduce FMD (Fig. 4). There was no difference in endothelium-independent relaxation between normotensives and hypertensives regardless of the number of risk factors. Among low-risk hypertension patients, in whom the only risk factors were hypertension or hypertension plus one other risk factor, plasma levels of t-PA, TNF- α and IL-6 were moderately elevated in hypertensives as compared to

Fig. 4. Relationship between flow mediated dilation (FMD) and total number of risk factors (hyperlipidemia, diabetes and smoking) in subjects with or without hypertension. Values are expressed as the mean $\pm$ SD.

normotensives (Fig. 5).

Discussion

FMD is known to be reduced in hypertensives (12, 13). In this study, FMD was lower in hypertensives despite good control of blood pressure, suggesting that a reduction of shear stress as a result of a decline in blood pressure may not be sufficient to fully restore endothelial function. There was no difference in endothelium-independent vasodilation between hypertensives and normotensives, suggesting that there may be no disruption of endothelium-independent

Fig. 5. Comparison of plasma levels of t-PA, TNF- α and IL-6 in normotensives (N) and low-risk hypertensives (HT), in whom the only risk factors were hypertension or hypertension plus one other risk factor (hyperlipidemia, diabetes or smoking). Values are expressed as the mean $\pm$ SD.

vasodilation in hypertension.

In addition to decreased FMD, hypertensives exhibited higher plasma TNF- α levels than normotensives. Secretion of proinflammatory cytokines is upregulated in monocytes from hypertensives (14). Although TNF- α and IL-6 are the major determinants of acute phase protein production (15), their roles have not been thoroughly investigated in hypertensives. Inflammatory cytokines impair endothelium-dependent dilatation in human veins *in vivo* (16), and vascular inflammatory responses are implicated in hypertension-induced end-organ damage (17). Thus, mild inflammatory reaction in hypertensives might be a useful index of impairment in vascular reactivity.

The level of t-PA antigen was increased in hypertensives in the present study, reflecting endothelial damage. TNF- α modulates the fibrinolytic system in the endothelium (18) and IL-6 induced by TNF- α has procoagulant properties (19). The results of a previous study indicate that there may be a close link between fibrinolysis and atherogenic metabolic derangement (20). An increased t-PA level may reflect chronic endothelial stimulation by thrombin and endothelial damage during vasculopathy. The results of a recent study by Newby *et al.* indicate that upregulation of basal t-PA release and subsequent depletion of endothelial t-PA stores play a role in coronary atherosclerosis (21).

Even a mild inflammatory reaction coupled with an alteration of fibrinolysis may disturb endothelial regulation of vascular tone in the arterial circulation in essential hypertension. In the present study, although both groups had similar blood pressure levels, the L-HT group had lower FMD than the normotensive subjects, suggesting that FMD might not have been fully improved by medication. TNF- α is linked to hypertension and angiotensin II is implicated in TNF- α regulation (22). Further, the use of angiotensin enzyme inhibitors is reported to improve FMD (23). The frequent use of angiotensin converting enzyme inhibitors in the H-HT group may partly explain the tendency for H-HT subjects to show

slightly higher FMD and slightly lower plasma TNF- α levels compared to L-HT subjects. Alterations in endothelial function may provide a link between systemic inflammation and hypertension.

In this study, the presence of hypertension alone—*i.e.*, without hyperlipidemia, diabetes or smoking—was associated with reduced FMD, suggesting that hypertension *per se* is a very important risk factor among Japanese. Furthermore, addition of other risk factors only moderately reduced FMD in hypertensives, suggesting that future treatment of hypertension may need to include anti-inflammatory therapeutic strategies directed at reducing inflammation (24). Because the cross-sectional nature of the present study did not permit analysis of causal inferences, further studies on fibrinolytic factors and inflammatory reactions will be needed to clarify the pathogenesis of early atherosclerosis in hypertension and to determine alternative preventive measures against clinical cardiovascular events. Accurate noninvasive evaluation of peripheral vascular function (25, 26) and morphology (27) may become useful for predicting future cardiovascular events in Japanese hypertensives.

In conclusion, impairment of FMD reflects a risk factor burden, and hypertension alone indicates a high-risk status for early atherosclerosis. Persistent endothelial damage and moderate inflammation may increase the risk of early atherosclerosis synergistically under the presence of hypertension in Japanese.

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