Original Article

Collagen Metabolism in Extracellular Matrix May Be Involved in Arterial Stiffness in Older Hypertensive Patients with Left Ventricular Hypertrophy

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Collagen metabolism in the extracellular matrix (ECM) is related to the pathogenesis of cardiovascular stiffness and remodeling in hypertension. We evaluated the association between collagen metabolism markers and the newly developed parameter, brachial-ankle pulse wave velocity (baPWV), in older hypertensive patients with left ventricular hypertrophy (LVH). We performed echocardiography and baPWV measurement using a new device, form PWV/ABI (Colin Medical Technology, Komaki, Japan), and measured plasma levels of markers of collagen metabolism such as procollagen type I C-terminal propeptide (PICP: a marker of collagen synthesis), collagen type I pyridinoline cross-linked C-terminal telopeptide (ICTP: a marker of collagen type I degradation), matrix metalloproteinase-1 (MMP-1: a marker of collagen degradation) and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) in 46 hypertensive patients with LVH. BaPWV was correlated with the plasma level of PICP (r=0.33, p=0.03) and ICTP (r=0.29, p=0.05) and the total TIMP-1/MMP-1 ratio (an index of collagen turnover; r=0.30, p=0.04). BaPWV was negatively correlated with the *E*/*A* ratio of left ventricular inflow (r=−0.36, p<0.05), while baPWV was not correlated with left ventricular mass index (LVMI; r=−0.175, p=0.25) or deceleration time of the mitral E wave (DCT; r=0.15, p=0.31). The measures of hypertensive heart disease, such as the *E*/*A* ratio, DCT or LVMI were not correlated with any collagen markers in this study. In multiple regression analysis adjusted for confounding factors such as age, sex, pulse pressure, mean blood pressure, pulse rate, LVMI, *E*/*A* ratio and DCT, the positive correlation between baPWV and total TIMP-1/MMP-1 ratio remained significant (p<0.05). In conclusion, arterial stiffness in high-risk older hypertensive patients may involve ECM collagen metabolism. (*Hypertens Res* 2005; 28: 995–1001)

***Key Words*: extracellular matrix, hypertension, brachial-ankle pulse wave velocity**

Introduction

Atherosclerosis is one of the most important factors for the development of hypertension and leads to target organ damage and cardiovascular events. Progression of atherosclerosis increases arterial stiffness and pulse wave velocity (PWV) in large elastic arteries. In previous studies, carotid-femoral PWV (cfPWV), a parameter of central arterial stiffness, was reported to be related to cardiovascular mortality (*1*), fatal stroke events (*2*) and cardiovascular diseases (*3*).

Recently, it has become possible to measure brachial-ankle PWV (baPWV) automatically using a newly developed machine, and baPWV has been reported to increase with the severity of hypertension (*4*). BaPWV is reported to be related to a parameter of target organ damage such as microalbumin-

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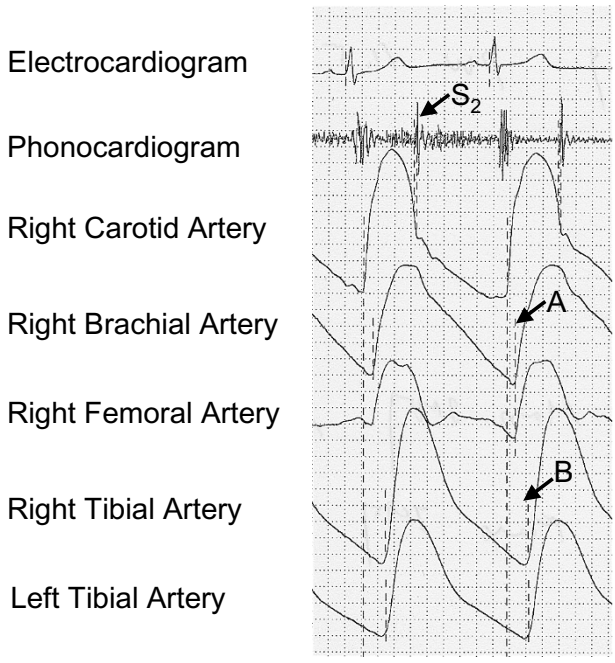

Fig. 1. *Simultaneous measurement of pulse waveform using form PWV/ABI. S_2, the second heart sound; A, the starting point of right brachial arterial waveform; B, the starting point of right tibial arterial waveform.*

uria and intima-media thickness (*5*, *6*) and the Framingham risk score (*7*). These facts suggest that it may substitute baPWV for cfPWV; however, the pathophysiological significance of baPWV has been unclear.

Fibrillar collagen type I and III are major constituents of the intima, media and adventitia (*8*). Atherosclerosis is characterized by abnormal accumulation of types I and III fibrillar collagens in the adventitia. Perivascular fibrosis extends into the contiguous interstitium and creates interstitial fibrosis (*9*). An increase of extracellular matrix (ECM) may be related to arterial stiffness in hypertensive patients and left ventricular (LV) hypertrophy (LVH).

Procollagen type I C-terminal propeptide (PICP: a marker of collagen type I synthesis) and collagen type I pyridinoline cross-linked C-terminal telopeptide (ICTP: a marker of collagen type I degradation) were reported to be increased in patients with hypertension and LVH (*10*). In addition, hypertensive accumulation of ECM proteins in resistance arteries may be facilitated by diminished matrix metalloproteinase (MMP) activities (*11*). Matrix metalloproteinase-1 (MMP-1: a marker of collagen degradation) and tissue inhibitor of MMP-1 (TIMP-1) are also reported to be related to hypertension and LVH (*12*).

Therefore, we studied the relationship between arterial stiffness measured using baPWV and parameters of collagen metabolism in hypertensive patients with LVH.

Table 1. Patients' Background (*N*=46)

Age (years)	71.3±8.4 (48–87)
Sex (male %)	63.0
Body mass index (kg/m^2)	24.8±3.0 (19.7–33.2)
Waist-hip ratio	0.86±0.06 (0.72–0.97)
Duration of hypertension (years)	10.1±10.0 (0.0–48.0)
Systolic blood pressure (mmHg)	138.9±19.3 (111–191)
Diastolic blood pressure (mmHg)	73.0±8.7 (56–95)
Pulse pressure (mmHg)	65.9±17.3 (45–112)
Mean blood pressure (mmHg)	95.0±10.4 (77–127)
Pulse rate (beat/min)	64.8±10.1 (48–87)
BaPWV (cm/s)	1,895±328 (1,391–2,751)
PICP (ng/ml)	84.0±26.6 (37–154)
ICTP (ng/ml)	3.6±1.1 (2.2–6.8)
MMP-1 (ng/ml)	5.3±3.4 (1.3–15.7)
TIMP-1 (ng/ml)	156.7±28.3 (111–240)

Data are shown as mean±SD (minimum value–maximum value). BaPWV, brachial-ankle pulse wave velocity; PICP, procollagen type I C-terminal propeptide; ICTP, collagen type I pyridinoline cross-linked C-terminal telopeptide; MMP-1, matrix metalloproteinase-1; TIMP-1, tissue inhibitor of matrix metalloproteinase-1.

Methods

Patients

Forty-eight medicated hypertensive patients with LVH detected by electrocardiography ($SV_1 + RV_5 > 35$ mm with strain pattern) and/or by echocardiography (LV mass index (LVMI) ≥120 g/m^2 for males, LVMI ≥110 g/m^2 for females, as measured previously) were enrolled consecutively in this study. The exclusion criteria were atrial fibrillation, history of heart failure, severe valve disease, an ankle brachial index of less than 0.9, and recent history (within 6 months) or symptoms of ischemic heart disease.

The patients were instructed to visit the hospital in a fasting state without having smoked or ingested caffeine or antihypertensive medication in the morning before the study.

The institutional review board of Jichi Medical School approved this study and informed consent was obtained from all the study patients.

Blood Samples

Fasting blood samples were drawn from the cubital vein after at least 30 min of rest with the subject in a supine position in the morning. The plasma samples were stored at −40°C in a refrigerator until the measurement of markers. Plasma PICP and ICTP levels were measured using radioimmunoassays (Orion Diagnostic, Espoo, Finland). The plasma MMP-1 level was measured using a 2-site enzyme immunoassay (Dai-

ichi Fine Chemicals Co., Ltd., Toyama, Japan). The plasma TIMP-1 level was measured using an enzyme-linked immunosorbent assay (Fuji Chemical Industries, Ltd., Toyama, Japan). The coefficients of variation were 5.0% for plasma PICP, 4.0% for ICTP, 8.1% for MMP-1, and 11.3% for TIMP-1. The ratio of the TIMP-1 to the MMP-1 level (total TIMP-1/MMP-1 ratio) was calculated afterward as a parameter of collagen turnover.

Brachial-Ankle Pulse Wave Velocity

BaPWV was measured by the volume plethysmographic method, using the form PWV/ABI (Colin Medical Technology, Komaki, Japan). The reliability of the equipment and the reproducibility of obtained measures have been well validated in various clinical studies (*4*, *7*, *13–21*). Cuffs were connected to both plethysmographic and oscillometric sensors and were placed around both the arms and ankles with the subject in the supine position. The electrocardiogram was taken in lead I. A phonocardiography was placed on the right second parasternal border to detect second heart sounds.

The path length between the right arm and ankle was calculated automatically according to the patient's height and anthropomorphic data for the Japanese population. The pulse transit time between the right brachial arterial wave and both tibial arterial waves (ΔT_a) was determined by the foot-to-foot method (Fig. 1). The path lengths from the suprasternal notch to the arm (ΔD_a), from the suprasternal notch to the femur (ΔD_b), and from the femur to the ankle (ΔD_c) were calculated to be $0.2195 \times H - 2.0734$, $0.5643 \times H - 18.381$, and $0.2486 \times H + 30.709$, respectively, where H is the patient's height in cm. The baPWV was calculated using the following formula: $(\Delta D_b + \Delta D_c - \Delta D_a)/\Delta T_a$.

In this study, right baPWV was used for the analyses.

Echocardiography

One operator, who did not know the patient's background, performed echocardiography and another operator reviewed each measurement. The two-dimensional M-mode image was recorded using an echocardiography machine (Vivid 7; GE Medical Systems, Milwaukee, USA) according to the guidelines of the American Society of Echocardiology (*22*, *23*). The LV mass was obtained using the formula validated by Deveraux and Reichek (*24*, *25*) and LVMI (LV mass/body surface area) was calculated. Relative wall thickness (RWT) was calculated using the formula: [diastolic interventricular septal diameter (IVSd) + diastolic posterior wall diameter (PWd)]/[diastolic LV dimension (LVDd)]. Doppler assessment of LV inflow was performed using pulsed-wave Doppler through an apical 4-chamber view. Peak early velocity (E wave) and its deceleration time (DCT), and peak atrial velocity (A wave) were measured at the mitral valve leaflet. The value of the peak E wave divided by the peak A wave (E/A ratio) was calculated as a measure of the LV diastolic function. The lack of apparent asynergy in the LV wall motion was confirmed during echocardiography in all patients.

Blood Pressure

Blood pressure (BP) and pulse pressure (PP) were measured using the validated semiautomatic cuff-oscillation method (HEM-907; Omron Healthcare, Kyoto, Japan) (*26*). Right brachial BP and pulse rate were measured in a sitting position just after baPWV evaluation. BP measurements were repeated more than twice until the differences in two consecutive measurements of systolic BP were less than 10 mmHg. The average of the last two measurements was used for evaluation. The lack of laterality of BP of both brachial arteries was confirmed.

Statistical Analysis

Two patients were excluded because of paroxymal supraventricular tachycardia (1 patient) and the taking of antihypertensive medications just before the study (1 patient). Statistical analyses were thus conducted for 46 patients. Data are shown as the mean±SD. Correlations between markers and clinical or echocardiographic variables were analyzed using Pearson's correlation. BaPWV and collagen markers were related to some variables; therefore, analysis of covariance and partial correlation coefficients were examined to rule out their effects on these parameters in multiple linear regression analysis. Computer software, SPSS version 11.0J (SPSS Inc., Chicago, USA) was used for the analyses and probability values <0.05 were considered statistically significant.

Results

Patients' Characteristics

The patients' characteristics are shown in Table 1. The average number of antihypertensive drug classes used was 1.43±0.94. Percentage of antihypertensive drug that had been used in the patients were 39.1% for calcium channel blockers (CCB), 69.6% for angiotensin II receptor blockers (ARB), 4.3% for α-blockers, 8.7% for β-blockers and 21.7% for diuretics. The percentages of patients reporting a history of angina pectoris, myocardial infarction and stroke were 10.9%, 4.3% and 15.2%, respectively; however, there were no apparent neurological signs of stroke or abnormal Q waves on electrocardiography. Hyperlipidemia and diabetes mellitus were seen in 30.4% and 21.7% of the patients, and 13.0% and 14.8% of the patients were regular smokers and alcohol drinkers, respectively.

LVMI was 157.4±37.0 g/m^2. The E/A ratio and DCT of the LV inflow were 0.72±0.21 and 274±88 ms, respectively. All of the patients had preserved LV ejection fraction (≥40%) and no apparent asynergy in the LV wall motion.

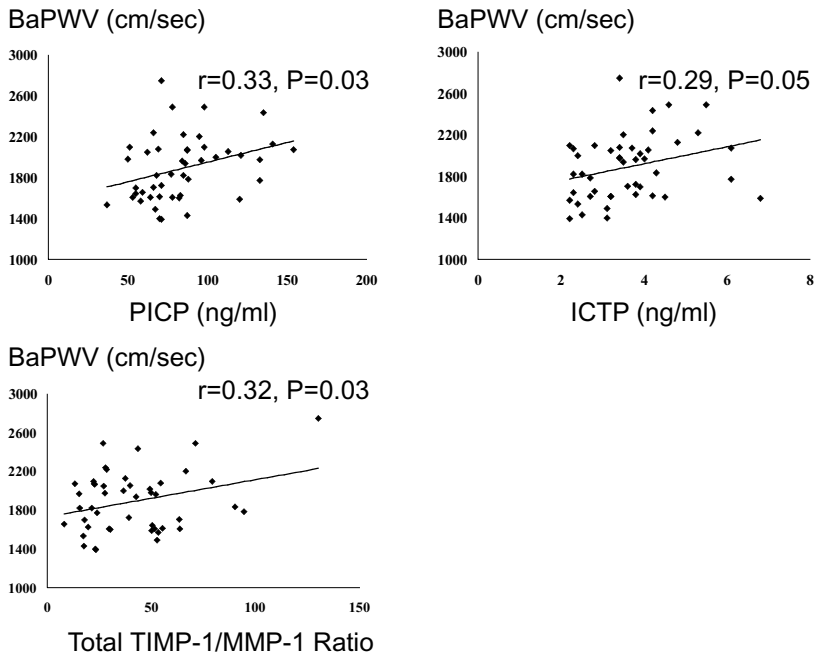

Fig. 2. *Correlations between collagen metabolism markers and brachial-ankle pulse wave velocity (baPWV). PICP, procollagen type I C-terminal propeptide; ICTP, collagen type I pyridinoline cross-linked C-terminal telopeptide; MMP-1, matrix metalloproteinase-1; TIMP-1, tissue inhibitor of matrix metalloproteinase-1.*

Table 2. Simple Correlation between ECM, baPWV and Variables

	PICP	ICTP	MMP-1	TIMP-1	Total TIMP-1/ MMP-1 ratio
Age (years)	0.31*	0.46**	−0.17	0.07	0.24
Pulse pressure (mmHg)	0.13	0.29	0.29*	0.23	−0.10
Mean blood pressure (mmHg)	0.22	0.22	0.40**	0.17	−0.41**
Pulse rate (beat/min)	0.12	−0.001	−0.01	−0.06	−0.13
BaPWV (cm/s)	0.33*	0.29*	−0.14	0.21	0.30*

Pearson, *$p<0.05$, **$p<0.01$. ECM, extracellular matrix; baPWV, brachial-ankle pulse wave velocity; PICP, procollagen type I C-terminal propeptide; ICTP, collagen type I pyridinoline cross-linked C-terminal telopeptide; MMP-1, matrix metalloproteinase-1; TIMP-1, tissue inhibitor of matrix metalloproteinase-1.

Brachial-Ankle Pulse Wave Velocity

BaPWV was significantly and positively correlated with age ($r=0.52$, $p<0.01$) and PP ($r=0.33$, $p<0.05$) and negatively correlated with the E/A ratio of the LV inflow ($r=-0.36$, $p<0.05$).

Collagen Markers

The PICP and ICTP levels were significantly correlated with the baPWV (PICP: $r=0.33$, $p=0.03$; ICTP: $r=0.29$, $p=0.05$; Fig. 2, Table 2). The significance of the correlations of baPWV with the PICP and ICTP levels disappeared after adjusting for age, sex, PP, mean BP (mBP) and pulse rate in the multiple regression analyses (Table 3).

The total TIMP-1/MMP-1 ratio, an index of collagen turnover, was significantly correlated with the baPWV ($r=0.32$, $p=0.03$; Fig. 2), and mBP level ($r=-0.41$, $p<0.01$; Table 2). In the multiple linear regression analysis for baPWV, the correlation between baPWV and the total TIMP-1/MMP-1 ratio remained significant after adjustment by age, sex, PP, mBP, pulse rate, CCB use and ARB use, and presence of hyperlipidemia and diabetes mellitus ($r=0.38$, $p=0.01$; Table 3). Moreover, even after other confounding parameters of echocardiography, such as LVMI, E/A ratio and DCT of the LV inflow, were added to the model, this correlation

Table 3. Multiple Linear Regression Analysis for baPWV

	Model 1	Model 2	Model 3-1	Model 3-2
Age (years)	0.52*	0.61**	0.51**	0.52**
Sex (female=0, male=1)	0.12	0.11	0.02	0.06
Pulse pressure (mmHg)	0.25	0.23	0.17	0.13
Mean blood pressure (mmHg)	0.11	0.16	0.32	0.38*
Pulse rate (beat/min)	0.35	0.38*	0.35*	0.31
PICP (ng/ml)	0.09			
ICTP (ng/ml)		−0.07		
TIMP-1/MMP-1			0.38**	0.38*

BaPWV, brachial-ankle pulse wave velocity; PICP, procollagen type I C-terminal propeptide; ICTP, collagen type I pyridinoline cross-linked C-terminal telopeptide; TIMP-1, tissue inhibitor of matrix metalloproteinase-1; MMP-1, matrix metalloproteinase-1. *$p<0.05$, **$p<0.01$. Model 1, 2 and 3-1 were adjusted by calcium channel blocker use, angiotensin II receptor blocker use and presence of hyperlipidemia and diabetes mellitus. In the model 3-2, left ventricular mass index relative wall thickness, E/A ratio of mitral inflow and deceleration time of E wave of mitral inflow were added to the adjustments.

remained significant ($r=0.38$, $p<0.05$; Table 3).

The correlations of baPWV with the plasma MMP-1 ($r=-0.14$, $p=0.36$) and TIMP-1 levels ($r=0.21$, $p=0.15$) were not significant.

Blood Pressure Levels, Left Ventricular Hypertrophy and Collagen Markers

The plasma MMP-1 level was significantly correlated with PP and mBP levels (PP: $r=0.29$, $p<0.05$; mBP: $r=0.40$, $p<0.01$), while the plasma TIMP-1 level did not show a significant correlation with these levels (PP: $r=0.23$, $p=0.13$; mBP: $r=0.17$, $p=0.27$) (Table 2). Therefore, the total TIMP-1/MMP-1 ratio was negatively correlated with mBP ($r=-0.41$, $p<0.01$). In the present study, the plasma TIMP-1 level, plasma MMP-1 level and total TIMP-1/MMP-1 ratio were not correlated with LVMI, RWT, E/A ratio or DCT.

There were no significant differences of baPWV between the users and non-users of CCB (1,823±266 cm/s *vs*. 1,932±335 cm/s, n.s.) or ARB (1,892±271 cm/s *vs*. 1,884±401 cm/s, n.s.). There were also no significant differences of the total TIMP-1/MMP-1 ratio between the users and non-users of CCB (37.5±17.9 *vs*. 43.6±28.1, n.s.) or ARB (41.2±21.3 *vs*. 41.4±31.8, n.s.).

Discussion

Arterial stiffness assessed by baPWV was significantly correlated with markers of collagen metabolism independently of age, sex, BP levels, and pulse rate in hypertensive patients with LVH. This result may show that baPWV is a parameter of arterial stiffness related to pathophysiological mechanisms.

BaPWV was significantly correlated with PP. PP and mBP levels were significantly correlated with the plasma MMP-1 level. BaPWV is reported to be associated with microalbuminuria (*27*) and coronary artery disease (*28*) independently of BP levels. The correlations of baPWV and collagen markers may show that baPWV is a vascular risk predictor independent of BP levels.

BaPWV was correlated with the E/A ratio of LV inflow in this study. Yamabe *et al*. (*21*) previously reported that baPWV was related to both the E/A ratio of LV inflow and the plasma brain natriuretic peptide level in patients with hypertension. In our study, we evaluated hypertensive patients with more advanced target organ damage, such as LVH, than Yamabe's patients, and our results showed that baPWV is not only a marker of arterial stiffness, but also a marker of cardiac afterload and diastolic function. Lindsay *et al*. (*10*) retorted that TIMP-1 was significantly elevated in never-treated hypertensive patients with diastolic dysfunction and was correlated with markers of diastolic filling such as E/A ratio and DCT of mitral inflow. Thus, the correlation between the baPWV and collagen metabolism markers might be derived from both cardiac and vascular overload. The relation between baPWV and collagen turnover may therefore be useful as a marker of cardiac burden.

BaPWV was correlated with the PICP and ICTP levels in this study; however, these correlations disappeared after adjustments for age. BaPWV is reported to increase with age and severity of hypertension (*19*), and the correlations of baPWV with PICP and ICTP might have been the result of normal ageing. Querejeta *et al*. (*29*) reported that coronary and peripheral PICP levels were increased in patients with a history of hypertensive heart disease. Thus, it is possible that the relation between baPWV level and PIPC level is a predictor of high-risk hypertension with LVH.

MMP-1 and TIMP-1 exist in a bound form in circulating blood, and the balance of MMP-1 and TIMP-1 is considered to be important (*12*). Laviades *et al*. (*12*) reported that the free TIMP-1/MMP-1 ratio was increased in hypertensive patients and decreased by lisinopril, an angiotensin-converting enzyme inhibitor. Hirono *et al*. (*30*) also reported that benidipine improved the free TIMP-1/MMP-1 ratio. In the present

study, we used the total TIMP-1/MMP-1 ratio, and the clinical implications of which remain to be clarified. In order to evaluate the free form, we would need to measure the TIMP-1/MMP-1 complex. However, Laviades *et al*. (*12*) also reported in patients with hypertension that total MMP-1, free MMP-1, and the TIMP-1/MMP-1 complex level were diminished, and that the total TIMP-1 level was increased. Differences of the TIMP-1/MMP-1 ratio between free form and total form would be smaller in hypertensive patients.

The problem with the clinical use of baPWV is that there are little prognostic data about baPWV as a prognostic factor for the risk of cardiovascular events. A cohort study to clarify the risk of baPWV may show the real utility of the baPWV as a marker of arterial stiffness.

Study Limitation

The effects on baPWV have been reported to differ depending on the antihypertensive drug class (*18*). Myocardial interstitial matrix metalloproteinase activity is reported to be altered by mechanical changes in LV load, and the mechanism of this effect was shown to be partly mediated by angiotensin II type 1 receptors in an experimental model (*31*). In addition, renin-angiotensin-aldosterone system blockers and CCBs are reported to have preferential effects on the levels of collagen metabolism markers (*12*, *30*, *32*). Even though we adjusted by ARB and CCB use, the patients in this study were medicated patients and we could not exclude the interaction of antihypertensive drugs completely.

Conclusion

In hypertensive patients with LVH, arterial stiffness was significantly associated with a parameter of diastolic function and ECM collagen metabolism. Management of hypertension that targets the baPWV level through the use of certain classes of antihypertensives may be useful for controlling collagen turnover in ECM in hypertensive patients with LVH.

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