Original Article

Impact of the Metalloproteinase-9/Tissue Inhibitor of Metalloproteinase-1 System on Large Arterial Stiffness in Patients with Essential Hypertension

Jing TAN[1)], Qi HUA[1)], Xiurong XING[1)], Jing WEN[1)], Rongkun LIU[1)], and Zheng YANG[1)]

The extracellular matrix is vital for maintaining tissue integrity, and the matrix metalloproteinases/tissue inhibitors of metalloproteinases (MMPs/TIMPs) system is involved in the regulation of extracellular matrix metabolism. Extracellular matrix turnover plays an important role in the change of large arterial mechanical properties in hypertension. However, the association of the metalloproteinase-9/tissue inhibitor of metalloproteinase-1 (MMP-9/TIMP-1) system and arterial stiffness is not straightforward and existing data are rather limited. Our objective is to explore the impact of the MMP-9/TIMP-1 system on large arterial stiffness in patients with essential hypertension. An automatic pulse wave velocity (PWV) measuring system was used to examine carotid-femoral PWV (CFPWV) and carotid-radial PWV (CRPWV) as the parameters reflecting central elastic large arterial and peripheral muscular medium-sized arterial elasticity, respectively; and serum MMP-9 and TIMP-1 levels, along with a number of other established biomarkers, were measured by enzyme-linked immunosorbent assay (ELISA) in 202 essential hypertensive patients and 54 age and gender-matched control subjects. Compared with the control subjects, hypertensive patients exhibited higher levels of MMP-9 ($p=0.001$) and TIMP-1 ($p=0.002$). Spearman's correlation analysis showed that serum levels of MMP-9 ($p=0.014$) and TIMP-1 ($p=0.005$) were significantly and positively correlated with CFPWV in hypertensive patients. A stepwise multiple regressive analysis demonstrated that age, systolic blood pressure, heart rate and TIMP-1 were independent predictors of CFPWV in patients with essential hypertension (adjusted $r^2=0.458$). In conclusion, our results imply that the MMP-9/TIMP-1 system may play an important role in the determination of arterial function, and these findings may have implications for the involvement of MMP-9/TIMP-1 system in the pathophysiology of cardiovascular disease. (*Hypertens Res* 2007; 30: 959–963)

***Key Words*: hypertension, pulse wave velocity, large arterial stiffness, metalloproteinase-9, tissue inhibitor of metalloproteinase-1**

Introduction

There is accumulating evidence that hypertension is a chronic low-grade inflammatory disease, and inflammation reaction plays a pivotal role in the formation and progress of cardiovascular remodeling, which has been shown to be characterized by an increase in extracellular matrix (*1*). Large arterial mechanical properties are primarily dependent on the quantity but also on the arrangement and interactions between arterial wall components, *i.e.*, extracellular matrix proteins, such as collagen and elastin, and smooth muscle cells. Matrix metalloproteinases (MMPs) and their natural inhibitors, the tissue inhibitors of metalloproteinases (TIMPs) determine a major part of matrix composition. Under normal circumstances, the TIMPs are in delicate balance with the MMPs, and the matrix is digested in a highly regulated fashion. However, during certain disease states, including hypertension and

From the [1)]Department of Cardiology, Xuanwu Hospital, Capital Medical University, Beijing, P.R. China.

Address for Reprints: Qi Hua, M.D., Department of Cardiology, Xuanwu Hospital, Capital Medical University, No. 45 Changchun Street, Xuanwu district, Beijing 100053, P.R. China. E-mail: huaqi5371@medmail.com.cn

Received March 31, 2007; Accepted in revised form June 7, 2007.

atherosclerosis, there is an imbalance between the activities of these two families of proteins that leads to tissue destruction, and that may contribute to the change of large arterial structure and function. However, the association of the MMP-9/TIMP-1 system and arterial stiffness is not straightforward and existing data are rather limited. Aortic pulse wave velocity (PWV) offers a noninvasive assessment of arterial stiffness (*2*–*4*). Accordingly, in the present study we sought to investigate the association between circulating levels of MMP-9 and TIMP-1 and arterial elastic properties as measured by PWV in hypertensive patients.

Methods

Subjects

The study group was comprised of 202 essential hypertensive patients aged 33–83 years (159 males and 43 females; mean age, 60.17±11.74 years) and 54 age- and gender-matched healthy controls. The hypertensive patients had either never been treated with antihypertensive medications ($n=67$) or had been off treatment for 2 weeks ($n=135$) with a median duration of hypertension of 5 (interquartile range: 1–12) years. Hypertension was defined as a seated systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg on at least three separate clinic visits or previously diagnosed hypertension, regardless of the levels of their blood pressure at the study entry.

Furthermore, the present study did not include patients with a medical history of secondary hypertension, diabetes mellitus, stroke, coronary artery disease, heart failure, hematological, neoplastic, renal, liver or thyroid disease, or patients receiving treatment with anti-inflammatory drugs. Patients with acute or chronic infections, or autoimmune disease were also excluded from the study. The study protocol was approved by the ethics committee of our institution, and written informed consent was obtained from all subjects.

Clinic Blood Pressure

The clinic blood pressure was measured on the right upper arm after 5 min of seated rest using a standard cuff mercury sphygmomanometer with the patients in a sitting position. Phase I and V Korotkoff sounds (disappearance) were taken as the SBP and DBP, respectively. The mean of the two measurements was used for analysis.

Body Mass Index and Waist-to-Hip Ratio

Body mass index (BMI) was calculated from measured height and body mass using the following equation: BMI=body mass (kg)/height^2 (m^2). Waist circumference was measured at a point midway between the costal margin and iliac crest and in line with the mid-axilla. To measure hip circumference, the greater trochanter was located as the widest part of the hips at the level of the buttock line. The waist-to-hip ratio was calculated at the waist circumstance divided by hip circumstance.

Table 1. Baseline Characteristics of the Study Population

Variables	Hypertension ($n=202$)	Control ($n=54$)
Age (years)	60.17±11.74	59.54±12.60
Gender (male) (*n* (%))	159 (78.7)	38 (70.4)
Waist-to-hip ratio	0.89±0.06	0.87±0.07
Body mass index (kg/m^2)	25.56±3.01*	24.01±3.31
Heart rate (bpm)	67.45±11.91	64.63±6.26
Systolic blood pressure (mmHg)	144.37±19.97**	124.11±11.17
Diastolic blood pressure (mmHg)	89.32±12.65**	78.84±7.93
Carotid-femoral PWV (m/s)	11.44±2.14*	10.34±1.48
Carotid-radial PWV (m/s)	10.90±1.57	10.78±1.65

Data are presented as mean±SD or number (percentage). *$p<0.05$, **$p<0.001$ *vs*. control. PWV, pulse wave velocity.

Pulse Wave Velocity

Carotid-femoral pulse wave velocity (CFPWV) and carotid-radial pulse wave velocity (CRPWV) as the parameters reflecting central elastic large arterial and peripheral muscular medium-sized arterial elasticity, respectively, were calculated from measurements of pulse transit time and the distance traveled between two recording sites (PWV=distance [m]/transit time [s]) using a validated noninvasive device (Complior, Artech Medical, France), which allows online pulse wave recording and automatic calculation of PWV (*5*, *6*). This apparatus simultaneously and automatically recorded CFPWV and CRPWV. The validation and reproducibility of this automatic method have been described previously, with an interobserver repeatability coefficient of 0.890 and an intraobserver repeatability coefficient of 0.935 (*5*). This is similar to findings from other studies (*7*).

Blood Collection and Assay

In every subject peripheral venous blood was drawn after an overnight fast. After clotting, the sample was centrifuged at 2,500 rpm for 10 min, and the serum was frozen and stored at −70°C until analyzed. A sandwich enzyme-linked immunosorbent assay (ELISA) was performed to measure the concentrations of serum interleukin-6 (IL-6), MMP-9 and TIMP-1 using Quantikine R&D Systems commercial kits and of serum soluble CD40 ligand (sCD40L) using Bender Medsystems commercial kits. The lower detection limits were 0.7 pg/mL for IL-6, 0.156 ng/mL for MMP-9, 0.08 ng/mL for TIMP-1 and 0.095 ng/mL for sCD40L.

Table 2. Concentrations of IL-6, sCD40L, MMP-9, TIMP-1 and MMP-9/TIMP-1 Ratio in the Study Groups

Variables	Hypertension (n=202)	Control (n=54)
IL-6 (pg/mL)	3.07 (2.60–3.57)**	2.55 (2.33–3.07)
sCD40L (ng/mL)	1.53 (0.74–3.16)	1.84 (0.96–4.82)
MMP-9 (ng/mL)	839.50 (514.25–1,310)*	584.00 (403.75–786.75)
TIMP-1 (ng/mL)	248.00 (151.00–384.50)*	190.00 (146.00–245.00)
MMP-9/TIMP-1 ratio	3.25 (2.07–5.17)	3.04 (1.97–3.91)

Data are presented as median (interquatile range). *p<0.01, **p<0.001 *vs*. control. IL-6, interleukin-6; sCD40L, soluble CD40 ligand; MMP-9, metalloproteinase-9; TIMP-1, tissue inhibitor of metalloproteinase-1.

Table 3. Spearman's Correlation Analysis between Assessed Biomarkers and PWV in Hypertensive Patients (n=202)

Variables	sCD40L (ng/mL)	MMP-9 (ng/mL)	TIMP-1 (ng/mL)	CFPWV (m/s)	CRPWV (m/s)
IL-6 (pg/mL)	−0.089	0.057	0.196*	0.120	−0.081
sCD40L (ng/mL)		0.221**	0.214**	−0.010	−0.029
MMP-9 (ng/mL)			0.556***	0.198*	0.100
TIMP-1 (ng/mL)				0.240**	0.049

*p<0.05, **p<0.01,*** p<0.001, figures are Spearman's correlation coefficients r. IL-6, interleukin-6; sCD40L, soluble CD40 ligand; MMP-9, metalloproteinase-9; TIMP-1, tissue inhibitor of metalloproteinase-1; CFPWV, carotid-femoral pulse wave velocity; CRPWV, carotid-radial pulse wave velocity.

Statistical Analysis

Data were expressed as the means±SD for normally distributed variables, and serum IL-6, sCD40L, MMP-9, TIMP-1 and the MMP-9/TIMP-1 ratio were expressed as median and interquartile ranges, since these values were non-normally distributed. Qualitative data were presented as numbers (percentages). The comparison of the IL-6, sCD40L, MMP-9, TIMP-1 and MMP-9/TIMP-1 ratio levels between the controls and hypertensive patients were performed using the non-parametric Mann-Whitney *U*-test, and the differences of normally distributed variables between the two groups were evaluated by unpaired *t*-test. For categorical variables, χ^2 tests were performed. Correlations of serum biomarkers and PWV were assessed by Spearman's correlation analysis, and multiple stepwise regression analysis was used to analyze the relationship between these variables and CFPWV. A value of p<0.05 was considered statistically significant. All calculations were performed using SPSS statistical software for Windows (version 11.5).

Results

Clinical Characteristics

The clinical characteristics of the study population are summarized in Table 1. SBP (p<0.001), DBP (p<0.001), BMI (p=0.039) and CFPWV (p=0.032) were significantly higher in the hypertension group than in the control group. There were no significant differences between the two groups in terms of age, gender, waist-to-hip ratio, or heart rate. CRPWV was also similar in the two groups (Table 1).

Serum Concentration of IL-6, sCD40L, MMP-9, and TIMP-1

The concentrations of IL-6 (p<0.001), MMP-9 (p=0.001) and TIMP-1 (p=0.002) were significantly higher in the hypertension group than in the control group. There were no significant differences in sCD40L or the MMP-9/TIMP-1 ratio between the two groups (Table 2).

Correlation Analysis

Spearman's correlation analysis showed that the serum levels of MMP-9 (p=0.014) and TIMP-1 (p=0.005) were significantly and positively correlated with CFPWV. No significant correlations between the biomarkers and CRPWV were found in hypertensive patients (Table 3).

Multiple Stepwise Regression Analysis

Using multiple stepwise regression analysis of CFPWV as a dependent variable, and age, SBP, DBP, BMI, waist-to-hip ratio, heart rate, MMP-9, TIMP-1, IL-6 and sCD40L as independent variables, only age, SBP, heart rate and TIMP-1 were found to be independent predictors of CFPWV in hypertensive patients (adjusted r^2=0.458) (Table 4).

Discussion

The present study showed that the serum levels of MMP-9

Table 4. Multiple Stepwise Regression Analysis of Relations of Risk Factors to CFPWV in Hypertensive Patients (n=202)

Variables	Standard β	t value	p value
Age (years)	0.330	4.883	<0.001
Systolic blood pressure (mmHg)	0.306	4.507	<0.001
Heart rate (bpm)	0.426	6.481	<0.001
TIMP-1 (ng/mL)	0.165	2.546	0.012

TIMP-1, tissue inhibitor of metalloproteinase-1; CFPWV, carotid-femoral pulse wave velocity.

and TIMP-1 were both elevated in hypertensive patients. In addition, MMP-9 and TIMP-1 were positively associated with large arterial stiffness as measured by CFPWV, whereas they were not related to muscular arterial elasticity as evaluated by CRPWV in patients with essential hypertension. Importantly, the relationship between TIMP-1 and CFPWV remained significant even after various confounders were adjusted. Our findings suggest that the MMP-9/TIMP-1 system may be a key mediator of large artery function through ventricular matrix composition.

Matrix metalloproteinases constitute a family of zinc-dependent endopeptidases that mediate the degradation of collagen, elastin and other components of the cardiovascular extracellular matrix (*8*). The substance MMP-9 belongs to the gelatinase family and degrades native collagen type IV, V, VII and X as well as denatured collagens and elastin and may therefore have profound effects on the mechanical properties of the vessel wall. MMP-9 is mainly expressed in macrophages, is induced in the vascular wall, and may be upregulated under an inflammatory condition (*9*). Continued excessive MMP-9 production and activation result in increased degradation of aortic wall elastin rather than an increase in its synthesis, with alteration of the mechanical properties of the aortic wall, leading to elevated large arterial stiffness. Our finding that the MMP-9 level is increased and positively correlated with CFPWV in hypertensive patients is in accord with the literature and is consistent with the evidence implying that the composition of the extracellular matrix plays an important role in genesis of vascular changes in hypertension (*10*, *11*).

The activity of MMPs is regulated at three levels: gene expression and protein secretion; activation of proenzymes by other MMPs or specific inducers; and inactivation by TIMPs or other inhibitors (*12*). An important mechanism for the regulation of the activity of the MMP-9 is binding to TIMP-1. MMPs and TIMPs may play a central role in the modulation of the extracellular matrix. Although there are many important elements making up the tissue matrix of cardiovascular tissues, collagen types I and III predominate. The former has high tensile strength and rigidity because of its thicker strands, whereas the latter has a weave-type pattern and contributes to tissue elastance and compliance. TIMP-1 is thought to increase tissue concentrations of collagen type I by preventing its breakdown by MMPs, thereby increasing the type I/III collagen ratio. These changes are macroscopically manifested as increased tissue stiffness and reduced compliance. Our findings are consistent with previous studies showing that circulating TIMP-1 levels are higher in hypertensive patients than controls, and that TIMP-1 levels are associated with left ventricular diastolic dysfunction (*13*, *14*). In addition, they add weight to the hypothesis that TIMP-1 is a key mediator of large arterial stiffness.

In the present study, the MMP-9/TIMP-1 ratio was identical between normotensives and hypertensives, and we did not find any significant correlation between the MMP-9/TIMP-1 ratio and CFPWV, although a close correlation (r=0.556, p<0.001) between MMP-9 and TIMP-1 was observed. One possible explanation could be that TIMP-1 is thought to increase tissue concentrations of collagen type I by preventing its breakdown by MMPs, and the interstitial collagenase family is involved in the degradation of collagen type I. Therefore, the imbalance between the interstitial collagenase (*i.e.*, MMP-8, MMP-1) and TIMP-1 may be more important than the imbalance between gelatinase family members (*i.e.*, MMP-9) and TIMP-1, at least with respect to chronic changes in the mechanical properties of the vessel wall. Our study failed to detect a significant correlation between the MMP-9/TIMP-1 system and CRPWV, suggesting that the contribution of the MMP-9/TIMP-1 system is less crucial in the remodelling of muscular arteries. This is consistent with a previous study which suggested that the remodelling process depends on MMPs in large elastic arteries, but not in small muscular arteries (*15*), and as well as with the finding that age-related changes are non-uniform throughout the vascular tree and affect large elastic arteries such as aorta to a greater extent than muscular arteries (*16*). We also failed to find any significant correlations between circulating levels of IL-6, sCD40L and PWV. Thus, we speculate that in contrast to an unstable and subsequently ruptured atherosclerotic coronary plaque, in which the contributions of proinflammatory cytokines are manifest (*17*), the contributions of IL-6 and sCD40L in the process of arterial stiffening in hypertension might be rather small. However, we found that serum IL-6 was significantly elevated and positively correlated with TIMP-1 (r=0.196, p=0.013), suggesting that inflammatory processes are participants in the pathophysiology of hypertension. Hypertension has been suggested to exert pro-inflammatory actions through the increased expression of several mediators, including angiotensin. Chua *et al*. (*18*) reported that angiotensin II increased the TIMP-1 level in cultured rat endothe-

lial heart cells. However, the exact sources and the determinant factors of TIMP-1 and MMP-9 in hypertension remain to be determined.

One of the limitations of the current study was the lack of tissue samples to directly link circulating and tissue concentrations of MMP-9/TIMP-1. Serum levels of MMP-9/TIMP-1 cannot be directly related to tissue concentration; therefore, circulating levels of MMP-9/TIMP-1 do not necessarily reflect large arterial matrix degradation. Ideally, correlations should have been established between aortic PWV and aortic tissue rather than circulating MMP-9/TIMP-1 levels. Furthermore, the activity of MMP-9 and TIMP-1 should have been determined by gelatin zymogram, rather than merely by protein levels, so as to explore the relationship of the MMP-9/TIMP-1 system and arterial stiffness in greater detail.

In summary, our study showed that the serum levels of MMP-9 were positively associated with large artery stiffness in patients with essential hypertension. In addition, we demonstrated that TIMP-1 was an independent predictor of large artery stiffness. Our findings imply that the MMP-9/TIMP-1 system, especially TIMP-1, may be a key mediator of large artery dysfunction. It has been established that aortic arterial stiffness assessed by CFPWV is an independent predictor of cardiovascular morbidity and mortality in the hypertensive population (*3*, *4*). Therefore, it could be hypothesized that in a higher risk population in which inflammatory mechanisms are frequently activated, such as hypertensives, the MMP-9/TIMP-1 system may be associated with elevated aortic stiffness and increased cardiovascular risk. Ishikawa *et al*. (*19*) reported that arterial stiffness in older, high-risk hypertensive patients involved extracellular matrix collagen metabolism. Thus, controlling vascular remodeling by modulating the MMP-9/TIMP-1 system may become a promising therapeutic modality. However, a large longitudinal study is warranted in order to elucidate the role of the MMP-9/TIMP-1 system as a marker for diagnosis and prognosis or as a potential target for treatment.

Acknowledgements

The authors would like to express their sincere thanks to the staffs at the Department of Cardiology of Xuanwu Hospital, Capital Medical University for their kind cooperation with this study.

References

1. Grundy SM: Inflammation, hypertension, and the metabolic syndrome. *JAMA* 2003; **290**: 3000–3002.
2. Amar J, Ruidavets JB, Chamontin B, Drouet L, Ferrieres J: Arterial stiffness and cardiovascular risk factors in a population-based study. *J Hypertens* 2001; **19**: 381–387.
3. Laurent S, Boutouyrie P, Asmar R, *et al*: Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension* 2001; **37**: 1236–1241.
4. Asmar R, Rudnichi A, Blacher J, London GM, Safar ME: Pulse pressure and aortic pulse wave are markers of cardiovascular risk in hypertensive populations. *Am J Hypertens* 2001; **14**: 91–97.
5. Asmar R, Benetos A, Topouchian J, *et al*: Assessment of arterial distensibility by automatic pulse wave velocity measurement: validation and clinical application studies. *Hypertension* 1995; **26**: 485–490.
6. Ni Y, Wang H, Hu D, Zhang W: The relationship between pulse wave velocity and pulse pressure in Chinese patients with essential hypertension. *Hypertens Res* 2003; **26**: 871–874.
7. Bland JM, Altman DG: Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; **1**: 307–310.
8. Visse R, Nagase H: Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res* 2003; **92**: 827–839.
9. Rodriguez-Pla A, Bosch-Gil JA, Rossello-Urgell J, Huguet-Redecilla P, Stone JH, Vilardell-Tarres M: Metalloproteinase-2 and -9 in giant cell arteritis: involvement in vascular remodeling. *Circulation* 2005; **112**: 264–269.
10. Yasmin, McEniery CM, Wallace S, *et al*: Matrix metalloproteinase-9 (MMP-9), MMP-2, and serum elastase activity are associated with systolic hypertension and arterial stiffness. *Arterioscler Thromb Vasc Biol* 2005; **25**: 372–378.
11. Karakaya O, Barutcu I, Esen AM, *et al*: Relationship between circulating plasma matrix metalloproteinase-9 (gelatinase-B) concentration and aortic root dilatation. *Am J Hypertens* 2006; **19**: 361–365.
12. Jacob MP: Extracellular matrix remodeling and matrix metalloproteinases in the vascular wall during aging and in pathological conditions. *Biomed Pharmacother* 2003; **57**: 195–202.
13. Tayebjee MH, Nadar SK, Macfadyen RJ, Lip GY: Tissue inhibitor of metalloproteinase-1 and matrix metalloproteinase-9 levels in patients with hypertension. Relationship to tissue Doppler indices of diastolic relaxation. *Am J Hypertens* 2004; **17**: 770–774.
14. Lindsay MM, Maxwell P, Dunn FG: TIMP-1: a marker of left ventricular diastolic dysfunction and fibrosis in hypertension. *Hypertension* 2002; **40**: 136–141.
15. Bouvet C, Gilbert LA, Girardot D, deBlois D, Moreau P: Different involvement of extracellular matrix components in small and large arteries during chronic NO synthase inhibition. *Hypertension* 2005; **45**: 432–437.
16. Boutouyrie P, Laurent S, Benetos A, Girerd XJ, Hoeks AP, Safar ME: Opposing effects of aging on distal and proximal large arteries in hypertensives. *J Hypertens Suppl* 1992; **10**: S87–S89.
17. Fichtlscherer S, Heeschen C, Zeiher AM: Inflammatory markers and coronary artery disease. *Curr Opin Pharmacol* 2004; **4**: 124–131.
18. Chua CC, Hamdy RC, Chua BH: Angiotensin II induces TIMP-1 production in rat heart endothelial cells. *Biochim Biophys Acta* 1996; **1311**: 175–180.
19. Ishikawa J, Kario K, Matsui Y, *et al*: Collagen metabolism in extracellular matrix may be involved in arterial stiffness in older hypertensive patients with left ventricular hypertrophy. *Hypertens Res* 2005; **28**: 995–1001.