Plasma Tissue Inhibitor of Metalloproteinase-1 Levels Are Elevated in Essential Hypertension and Related to Left Ventricular Hypertrophy

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**Background:** Essential hypertensive patients have an increased heart and arterial collagen concentration. Increased collagen synthesis can be assessed using pro-collagen III N peptide (PIIINP) and reduced collagen degradation measured using tissue inhibitor of metalloproteinase-1 (TIMP-1).

**Methods:** Plasma TIMP-1 and PIIINP levels were measured in 31 patients with essential hypertension and in 17 normotensive control subjects. The hypertensive patients were either treatment naive (n = 18) or had been without treatment for 1 month (n = 13). Both groups of patients were screened to exclude other fibrotic diseases.

**Results:** In the hypertensive patients, TIMP-1 levels were significantly (P < .0002) elevated (median 380 ng/mL, range 160 to 1560 ng/mL) compared with those of the normotensive control subjects (median 178 ng/mL, range 99 to 330 ng/mL). In hypertensive subjects who had never received antihypertensive therapy there were significant correlations between TIMP-1 and left ventricular posterior wall thickness in diastole (LVPWd) (r = 0.58) (P < .02) and left ventricular mass index (r = 0.58) (P < .02). There was no difference in PIIINP levels (mean ± 2 SD) between the hypertensive (0.56 U/mL ± 0.3) and normotensive groups (0.52 U/mL ± 0.2).

**Conclusions:** The increased tissue collagen III levels found in the heart and vessels of hypertensive patients is due to a reduction in collagen degradation because of high TIMP-1 levels, rather than an increase in synthesis of collagen type III. The tissue source of this TIMP-1 is unclear. Am J Hypertens 2002;15:269–272 © 2002 American Journal of Hypertension, Ltd.

**Key Words:** Essential hypertension, TIMP-1, PIIINP, LVH, angiotensin II.

Hypertensive patients have an increase in the amount of collagen in the heart and arteries.1 Left ventricular hypertrophy (LVH) is an established risk factor for all the sequelae of coronary artery disease with a three- to fivefold increase in cardiovascular mortality. The pathologic basis for LVH is a combination of myocyte hypertrophy and increased collagen deposition within the myocardium. Theoretically, elevated collagen levels may be due to an increase in collagen synthesis,2 or a decrease in collagen degradation, or both. The synthesis of collagen III can be monitored by measurement of plasma N terminal pro-collagen III peptide (PIIINP). A family of proteins called metalloproteinases (MMP) degrade extracellular matrix. One of the factors that inhibits the activity of MMP is the antiproteinase tissue inhibitor of metalloproteinase-1 (TIMP-1).3 Four members of the TIMP family (TIMP-1 to TIMP-4) have been described; all have six disulphide bonds and are capable of inhibiting matrix metalloproteinase activity. TIMP-1 has a molecular weight of 28,500 and is glycosylated on two sites.4 In the liver5 and heart,6 elevated levels of TIMP-1 cause an increase in tissue collagen III concentration by reducing the degradation of collagen.

We therefore measured left ventricular mass as well as plasma levels of TIMP-1 and PIIINP in a group of hypertensive patients, some of whom had LVH, and compared...
them with a control group to determine whether the elevated tissue levels of collagen in hypertension are associated with increased plasma TIMP-1 concentrations.

Methods

After obtaining informed consent, we enrolled 31 patients with essential hypertension and 17 normotensive control subjects. The hypertensive patients had either never been treated with antihypertensive medications (n = 18) or had been off treatment for 1 month (n = 13). Hypertension was confirmed by elevated supine blood pressure (BP) of >140/90 mm Hg on at least three separate clinic visits. Patients with any of the following conditions were excluded: those with secondary causes of hypertension; clinical history or biochemical evidence of renal disease; chronic liver disease or excessive alcohol use; lung or connective tissue disease; and previous major surgical procedures including injury resulting in scars in the previous year. Normotensive control subjects (n = 17) who satisfied the above inclusion criteria were age- and sex-matched to the hypertensive group.

A two-dimensional echocardiogram and Doppler study was performed on all patients by a trained echocardiographer using a Vingmed CFM 800 with Echopac analysis package. Two-dimensional guided M-mode measurements were made with left ventricular dimension being recorded at the level of the tips of the mitral valve leaflets. At least three measurements were made and the average of these measurements was used for analysis. Left ventricular mass was calculated by the formula of Devereux and Reichek.7

Heparinized blood samples, which were taken after the patient was supine for 15 min, were used in the analysis. The TIMP-1 was assayed8 using a sandwich ELISA kit modified by Amersham Biotrak (the company has recently changed the original antiserum, which can be purchased from Calbio (Cambridge, UK). The PIIINP was measured using a radioimmunoassay technique (CIS).9

The TIMP-1 data in the hypertensive group was non-Gaussian, and TIMP-1 results were normalized by loge transformation. The SPSS software program (SPSS, Cary, NC) was used to calculate significance between the normotensive and hypertensive groups. Other, normally distributed data were expressed as mean ± 2 SD, and the two-tailed unpaired Student t test was used to calculate significance.

Results

Table 1 shows the clinical and biochemical data for the normotensive and hypertensive groups of patients. As expected, the results for BP, left ventricular mass index (LVMI), IVSd, and LVPWd are significantly higher in the hypertensive group than in the normotensive control group. There was no significant difference in PIIINP levels between the hypertensive and normotensive groups, but the plasma TIMP-1 was significantly higher in the hypertensive patients than the normal control subjects. Plasma TIMP-1 levels were still elevated when the previously treated and previously untreated hypertensive patients were analyzed separately (both P < .002), but there was no significant difference between these two subgroups (Fig. 1).

There was no correlation between LVMI, IVSd, or LVPWd and TIMP-1 in the control subjects and the previously treated hypertensive patients. There was a significant correlation between lnTIMP-1 and LVPWd (r = 0.58, P < .02) and LVMI (r = 0.58, P < .02) (Fig. 2), but not IVSd in hypertensive patients who had never been treated.

Discussion

The present study shows a significantly higher level of TIMP-1 in a selected group of hypertensive patients in comparison with normotensive control subjects, which is in agreement with data reported by Laviades et al.10 However, our data show better discrimination between the hypertensive and normotensive groups than those of Laviades et al, who measured serum TIMP-1 (which, unlike plasma, is contaminated with platelet TIMP-1),11 and we demonstrated a correlation between plasma TIMP-1 and...
LVMI and LVPWd. Unlike Laviades et al, we could find no difference in PIIINP between our normotensive and hypertensive patients. During collagen synthesis and breakdown, PIIINP is released. However, in circumstances in which collagen degradation is reduced due to inhibition of MMP by TIMP-1, PIIINP released form collagen degradation will be minimal, and levels of plasma PIIINP will reflect collagen III synthesis.

We also showed a correlation between plasma TIMP-1 and LVMI and LVPWd in treatment-naive hypertensive subjects; however, there was no correlation in the previously treated hypertensive patients. This may be because withdrawal of antihypertensive treatment for 1 month is sufficient to change TIMP-1 to levels similar to those found in the treatment-naive group (Fig. 1). However, it will take more than 1 month to reach a new steady state associated with myocyte hypertrophy and matrix deposition.

If the source of the increased plasma TIMP-1 is only cardiac in origin and the left ventricle increased by 30% because of LVH, the highest predicted plasma TIMP-1 should be about 350 ng/mL. However, the plasma TIMP-1 concentration in hypertensive patients is at least two and up to five times the TIMP-1 levels present in the control subjects. Plasma TIMP-1 levels in subjects with chronic liver disease with severe fibrosis are similar to the TIMP-1 levels in hypertensive patients reported here. This suggests that cardiac tissue cannot be the only source of plasma TIMP-1 in these patients.

Angiotensin II is generally elevated in essential hypertension. Angiotensin II increases TIMP-1 levels in cultured rat endothelial heart cells, suggesting that the plasma TIMP-1 elevation may be from two sources: cardiac and, possibly, the vascular bed. Although the vascular bed is not a proven source of TIMP-1, other potential causes have been ruled out by clinical and biochemical assessments.

In agreement with Laviades et al, our unpublished data from a cross-sectional study show that angiotensin converting enzyme inhibitors reduce plasma TIMP-1 levels significantly. This observation emphasizes the important role of angiotensin II in increasing TIMP-1 levels in hypertensive patients, and provides support for the contention that the endothelium may produce TIMP-1.

From our data it appears that the increase in cardiac collagen type III is due to reduced degradation, rather than increased synthesis caused by elevations in TIMP-1. Elevations in other collagen types, however, may be due to an increase in synthesis as well as a reduction in degradation. This hypothesis is supported by the work of Funck et al, who showed that MMP-1 activity was low in angiotensin II stimulated cultured human cardiac fibroblasts.

Further work must be undertaken to determine the sources of TIMP-1, the measurement of which may prove important in biochemically assessing hypertensive patients as well as in highlighting an area of potential pharmacologic interest.

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References


