Circulating matrix metalloproteinase-2 is elevated in patients with congestive heart failure

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Abstract

Background and aims: It has been reported that matrix metalloproteinase (MMP) protein concentration and activity are upregulated in the failing human heart. However, there are few reports describing the role of elevated level of circulating MMPs in congestive heart failure (CHF) patients. This study examined whether circulating matrix metalloproteinases (MMPs) are also related to the pathogenesis of CHF.

Methods: We measured circulating levels of matrix metalloproteinase-2 (MMP-2) in 52 patients with CHF (left ventricular ejection fraction (LVEF) < 50%). The patients were also subdivided into two groups according to NYHA functional class; mild CHF (class II, n = 43) and severe CHF (class III, n = 9).

Results: The serum level of MMP-2 and MMP-2/ TIMP-2 ratio were significantly higher in CHF than in controls (P < 0.01). Among patient groups, serum levels of MMP-2 were significantly higher in patients with severe CHF than in patients with mild CHF (P < 0.01). Plasma levels of BNP had a significant positive correlation with circulating levels of MMP-2 (r = 0.78; P < 0.01) and MMP-2/ TIMP-2 ratio (r = 0.60; P < 0.01).

Conclusions: Our data showed that the circulating MMP-2 concentration was increased in CHF patients and that the levels were related to the plasma levels of BNP in CHF, suggesting that the elevated levels are related to developing heart failure syndrome.

Keywords: Matrix metalloproteinases; Congestive heart failure; Brain natriuretic peptide

1. Introduction

An important event in the progression to congestive heart failure (CHF) is left ventricular dilation and subsequent pump dysfunction [1,2]. Clinically, left ventricular remodeling has been reported to be an important initiating event in the transition to severe CHF, and an important predictor of morbidity and mortality in patients with CHF.

Matrix metalloproteinases (MMPs) are a family of enzymes that contribute to extracellular remodeling in several disease states [3]. Additionally, a family of inhibitors called tissue inhibitors of MMPs (TIMPs) have been shown to exist and to tightly regulate MMP activity [4]. MMP-2 is involved in the degradation of collagen type IV, a major component of the basement membrane.

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Previous studies have demonstrated that increased myocardial MMP activities are present in severe forms of CHF [5–8], suggesting that MMPs may contribute to myocardial remodeling in patients with CHF. However, there are no reports to describe the level of circulating MMP-2 in patients with CHF, although Kai et al. reported that circulating levels of MMP-2 were elevated in patients with acute coronary syndrome [9]. Thus the present study was undertaken to assess the level of circulating MMP-2, and their specific inhibitor TIMP-2 in patients with CHF.

2. Methods

2.1. Patients and study protocol

We studied 52 Japanese patients with chronic CHF [32 men and 20 women, aged 47 to 80 years (mean age 68 ± 9)] who were admitted to Fukui Medical University Hospital for heart failure between January 1999 and
December 2001, including 18 patients with dilated cardiomyopathy (DCM), 26 with old myocardial infarction (OMI, >6 months after onset), and eight with valvular disease. The diagnosis of DCM was established based on the finding of a normal coronary angiogram, the absence of valvular or pericardial heart disease, and the absence of a clinical history that would suggest myocarditis [10]. All patients experienced dyspnea or fatigue either on modest exertion or at rest [New York Heart Association (NYHA) functional class II (n = 43) and III (n = 9)]; left ventricular ejection fraction (LVEF) was <50% (as measured by left ventriculogram; average ejection fraction 38.7 ± 8.5%, range 14.0–49.8%). On entry into the study, 34 patients were being treated with angiotensin-converting enzyme inhibitors, 12 with beta-blockers, 34 with diuretics, and 11 with digitalis. The present study did not include patients with a history of neoplastic, hepatic, infectious, or autonomic disease, peripheral atherosclerotic disease, or any surgical procedure in the preceding 6 months.

All patients had stable symptoms for at least 3 months and none had inflammatory signs at the time of evaluation. The patients were also subdivided into two groups according to NYHA functional class; mild CHF (class II, n = 43) and severe CHF (class III, n = 9). We also selected 11 age-matched normal controls (mean age 56 ± 8, four men and seven women) who were admitted complaining of chest pain, but proved to be normal by coronary angiography and left ventriculography.

Left ventriculography was performed with contrast medium before or within 1 week after blood sampling. Left ventricular volume indices at end-diastole and end-systole were determined by a modification of Dodge’s formula from a single plane left ventriculogram, and left ventricular ejection fraction was calculated [11]. Plasma levels of brain natriuretic peptide (BNP) were measured using a commercially available radioimmunoassay kit (Shionogi, Osaka, Japan), and plasma levels of norepinephrine (NE) were measured by high-performance liquid chromatography [12,13] (Table 1).

The study was approved by the our hospital’s ethics committee and written informed consent was obtained from each subject.

### 2.2. Measurement of MMP-2 and TIMP-2

Whole blood was withdrawn from a forearm or femoral vein and kept on ice, then serum samples were separated by centrifugation within 30 min. After centrifugation, serum samples were frozen and stored at −80°C until use. Sandwich enzyme immunoassay was performed to measure concentrations of serum MMP-2 and TIMP-2 using commercial available kits with monoclonal antibodies against each substance according to the manufacturer’s instructions (Fuji Chemical Industries Ltd., Takaoka, Japan) [14–16].

### 2.3. Statistical analysis

Results are expressed as mean value ± S.D. Differences in clinical characteristics and some variables between patients and controls were determined by Mann–Whitney U-tests, as appropriate. One-way factorial analysis of variance followed by the Sheffe F-test was used for inter-group comparisons. Spearman rank test was used to detect relationships between circulating levels of MMP. Statistical analyses were performed with a commercial computer software package (Stat View-J5.0: Abacus Concepts, Inc., Berkeley, CA, USA). A P-value <0.05 was considered significant.

### 3. Results

#### 3.1. Hemodynamic and neurohumoral data

The left ventricular volume indices both at end-diastole and at end-systole were significantly higher, and the left ventricular ejection fraction was significantly lower in patients with CHF than in controls (Table 1). Plasma levels of BNP were significantly higher in CHF patients than in controls.

#### 3.2. Serum levels of MMP-2 and TIMP-2

Data are listed in Table 2. Serum levels of MMP-2 were significantly higher in patients with CHF than in:

### Table 1

Characteristics of subjects with and without congestive heart failure

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls (n=11)</th>
<th>CHF (n=52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58±10</td>
<td>67±8</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>6/5</td>
<td>32/20</td>
</tr>
<tr>
<td>Left ventriculography</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left ventricular volume indices</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At end-diastole (ml/m²)</td>
<td>79±20</td>
<td>126±47*</td>
</tr>
<tr>
<td>At end-systole (ml/m²)</td>
<td>23±6</td>
<td>79±40*</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (%)</td>
<td>71±5</td>
<td>39±8*</td>
</tr>
<tr>
<td>Plasma polypeptide hormones</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain natriuretic peptide (pg/ml)</td>
<td>25±29</td>
<td>383±404*</td>
</tr>
<tr>
<td>Norepinephrine (pg/ml)</td>
<td>235±142</td>
<td>308±151</td>
</tr>
</tbody>
</table>

*P < 0.01 vs. controls.

### Table 2

Serum levels of matrix metalloproteinases and tissue inhibitor of metalloproteinases

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=11)</th>
<th>CHF (n=52)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-2 (ng/ml)</td>
<td>533.5±158.3</td>
<td>861.4±277.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TIMP-2 (ng/ml)</td>
<td>36.0±17.6</td>
<td>37.9±25.3</td>
<td>0.69</td>
</tr>
<tr>
<td>MMP-2/TIMP-2 ratio</td>
<td>18.2±10.8</td>
<td>30.3±23.7</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Table 3

Correlation (Spearman correlation coefficient, rho) between serum levels of MMP-2 or MMP-2/TIMP-2 ratio and hemodynamic and neurohumoral variables in patients with CHF

<table>
<thead>
<tr>
<th>Variable</th>
<th>MMP-2</th>
<th>MMP-2/TIMP-2 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r )</td>
<td>( P )</td>
</tr>
<tr>
<td>LVEF</td>
<td>0.35</td>
<td>0.02</td>
</tr>
<tr>
<td>LVEDI</td>
<td>0.33</td>
<td>0.04</td>
</tr>
<tr>
<td>BNP</td>
<td>0.78</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NE</td>
<td>0.52</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

LVEF, left ventricular ejection fraction; LVEDI, left ventricular volume indices at end-diastole; BNP, brain natriuretic peptide; NE, norepinephrine.

In the patient group, serum levels of MMP-2 were significantly higher in patients with severe CHF than in those patients with mild CHF (1282 ± 414 vs. 827 ± 258 ng/ml; \( P < 0.01 \)). The levels of MMP-2 did not differ among patients with DCM (1028 ± 404 ng/ml), OMI (824 ± 250 ng/ml), and valvular disease (890 ± 365 ng/ml).

3.3. Relation between serum levels of MMP-2 and hemodynamic data

Coefficients of correlation are listed in Table 3. Circulating levels of MMP-2 had a significant positive correlation with left ventricular volume indices at end-diastole (LVEDI), and significant negative correlations with LVEF. Thus, these results suggested that circulating MMP-2 had been elevated along with the development of heart failure.

There were no significant correlations between serum MMP-2/TIMP-2 ratio and LVEDI or LVEF.

3.4. Relation between serum levels of MMP-2 and neurohumoral data

Plasma levels of BNP had a significant positive correlation with circulating levels of MMP-2 (Fig. 1) and MMP-2/TIMP-2 ratio. Moreover, there were also significant positive correlations between circulating levels of MMP-2 and plasma levels of NE. However, there were no significant correlations between plasma levels of NE and serum MMP-2/TIMP-2 ratio.

4. Discussion

In this study, serum MMP-2 levels were significantly increased in patients with CHF compared to controls. Among CHF patients, serum levels of MMP-2 were significantly higher in patients with severe CHF than in patients with mild CHF. To our knowledge, this is the first report demonstrating elevated peripheral blood levels of MMP-2 in patients with CHF. Moreover, serum MMP-2/TIMP-2 ratio was significantly increased in patients with CHF compared to controls. The actions of MMPs are known to depend on the balance between the enzymes and their inhibitors [17,18]. The altered balance between MMP-2 and TIMP-2 may contribute to the degradation of the extracellular matrix that leads to ventricular remodeling in the worsening process of heart failure. Several studies have identified changes in MMP expression and activity within left ventricular myocardium in both animals and patients with left ventricular dilation and the development of CHF. For example, Spinale et al. indicated that left ventricular end-diastolic dimension and the expression of MMP-2 were increased during the progression of CHF caused by pacing-induced supraventricular tachycardia in pigs [19]. They also reported that left ventricular myocardial zymographic MMP activity and MMP-2 abundance were increased in the myocardium of DCM patients [20].

We also demonstrated a significant correlation between the serum levels of MMP-2 and left ventricular volume indices at end-diastole and LVEF in these patients, although correlations between left ventricular volume indices at end-diastole or LVEF and serum MMP-2/TIMP-2 ratio were not significant. Since MMPs are known to play a significant role in extracellular remodeling, there has been a growing interest in the contribution of circulating MMP-2 in left ventricular remodeling in patients with CHF.

We also demonstrated a significant correlation between plasma levels of BNP and serum levels of MMP-2 or MMP-2/TIMP-2 ratio. Previous studies have
induced by profibrotic factors was a key factor in the stimulation of the secretion of BNP [22]. Tsuruda et al. reported that BNP induced protein expression of MMP-2 and increased MMP-2 release in cultured cardiac fibroblasts, suggesting that stimulation of MMPs by BNP may be a compensatory response to prevent excessive collagen deposition induced by profibrotic factors [23]. These findings may account for our results.

Plasma levels of NE were increased in patients with chronic CHF compared with those of age-matched controls in agreement with previous reports [24]. MMP-2 content was increased in the conditioned media of left ventricular myocytes incubated with isoproterenol [25], and these findings support our data that serum levels of MMP-2 correlated with plasma levels of NE. Therefore, the sympathetic nervous system may modulate MMP production in patients with CHF.

The circulating levels of MMP-2 were not related to the etiology of CHF in this study. However, Spinalet al. demonstrated that MMP-2 abundance in human myocardium was increased two-fold in non-ischemic DCM but was unchanged in ischemic DCM [20]. Because we could not define the main source of circulating MMP-2 in this study, it is difficult to explain the difference between these two studies. A number of cells, including fibroblasts, smooth muscle cells, endothelial cells, and myocytes are known to secret MMPs, myocardial MMP-2 is not necessarily the only factor regulating circulating MMP-2 [26,27]. Our data merely showed the elevated circulating MMP-2 levels in patients with CHF regardless of this etiology.

Serum levels of MMP-2 increased with the severity of CHF in this study, but its relationship to mortality in patients with CHF remains unknown. Moreover, it is unclear whether the effect of medications can be accurately evaluated by serum MMP-2 levels. Further studies are needed to clarify the role of medication, by repetitive measurement of MMP-2 before and after administration of drugs such as angiotensin-converting enzyme inhibitors or beta-blockers used to treat CHF patients.

Our data showed that the circulating MMP-2 concentration was increased in CHF patients and that the levels were related to the plasma levels of BNP in CHF, suggesting that the elevated levels are related to the developing heart failure syndrome.

References

References


