Association of Matrix Metalloproteinase Expression and Left Ventricular Function in Idiopathic Dilated Cardiomyopathy

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Myocardial remodeling is an important predictor for the development of dilated cardiomyopathy (DCM). Matrix metalloproteinases (MMPs) are the family of proteins responsible for extracellular remodeling, and tissue inhibitors of metalloproteinases (TIMPs) tightly control their activity. In the present study, the expression of MMP-2, MMP-9, TIMP-1 and TIMP-2 was determined by immunohistochemistry in right ventricular endomyocardial biopsy samples from 16 patients with idiopathic DCM, and its clinical significance was evaluated by comparison with parameters of cardiac function. To obtain a semi-quantitative assessment of MMP and TIMP expression, the average number of positive cells per high power field was counted. The left ventricular ejection fraction (LVEF) significantly correlated with the expression of both MMP-2 ($r=0.68$) and TIMP-2 ($r=-0.58$). Patients were classified into 2 groups according to the degree of MMP-2 expression: strongly positive and weakly positive. LVEF, left ventricular (LV) end-diastolic pressure, right ventricular end-diastolic pressure, pulmonary capillary wedge pressure and the plasma norepinephrine level were significantly greater in the strongly positive group ($p<0.05$). In conclusion, the expression of MMPs and TIMPs in the cardiac matrix of patients with idiopathic DCM is closely associated with myocardial remodeling and subsequent deterioration of LV performance. These findings suggest new therapeutic targets for patients with idiopathic DCM. (Jpn Circ J 2000; 64: 352–357)

Key Words: Dilated cardiomyopathy; Heart failure; Matrix metalloproteinases; Remodeling

The development of end-stage heart failure in humans is accompanied by left ventricular (LV) dilatation and pump dysfunction. Maladaptive myocardial remodeling contributes to diminished systolic performance as well as decreased compliance of the heart. Identification of the cascade of molecular and cellular events that contribute to myocardial remodeling is therefore likely to provide novel targets for the prevention of progressive heart failure.

Matrix metalloproteinases (MMPs) are the family of proteins responsible for extracellular collagen degradation and remodeling in a variety of tissues and their activity is tightly controlled by a family of closely related inhibitors known as tissue inhibitors of metalloproteinases (TIMPs). Recent clinical studies of the failed human heart have demonstrated that MMPs and TIMPs are expressed in the myocardium of end-stage dilated cardiomyopathy (DCM) explanted hearts. However, the significance of MMP and TIMP expression in the cardiac matrix of patients with mild or moderate DCM is unclear. In the failing heart, the activity and expression of MMPs can be influenced by a variety of soluble factors (neurohormones and cytokines) as well as by mechanical stress (pressure and volume overload). Therefore, the degree of MMP activity or expression may reflect the severity of the cardiac performance. In the present study, the expression of MMPs and TIMPs was evaluated in the myocardium of patients with mild or moderate idiopathic DCM using right ventricular (RV) endomyocardial biopsy samples, and the results were compared with parameters of cardiac function.

Methods

Patients

Sixteen patients diagnosed as idiopathic DCM comprised the study group (Table 1). The patients ranged in age from 22 to 76 years (mean, 53 years), and the male to female ratio was 10:6. Diagnosis was made based on history, 2-dimensional echocardiography, hemodynamics and coronary cineangiography. After giving informed consent, all patients underwent right heart catheterization, biplane left ventriculography, coronary angiography and right ventricular endomyocardial biopsy between May 1995 and September 1999 at the First Department of Internal Medicine, Shinsu University School of Medicine. All patients received a relatively standard therapeutic regimen including diuretics, digoxin and angiotensin-converting enzyme (ACE) inhibitors. One patient (patient 4) was treated with intravenous catecholamines in addition to the standard regimen. No patient was treated with $\beta$-blocker at the time cardiac biopsy was performed.

Sample Preparation and Immunohistochemistry

Two biopsy specimens, 1–3 mm in size, were obtained

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from each patient through the right femoral vein using a Cordis biortne. One sample was fixed in 10% neutral-buffered formalin and embedded in paraffin. The other was snap-frozen in Tissue-Tek optimum cutting temperature compound (Miles Inc, Elkhart, IN, USA) and stored at -70°C until use. Both the paraffin-embedded and frozen samples were cut at 6 μm and stained with hematoxylin and eosin (H&E). Immunohistochemistry was performed using frozen sections. Mouse monoclonal antibodies (mAbs) against human MMP-2 (42-SD2), MMP-9 (56-2A4), TIMP-1 (147-6D11) and TIMP-2 (67-4H11) (Fuji Pharmaceutical, Co., Toyama, Japan) were used. The antibodies were applied and allowed to incubate with the samples for 20 min. The sections were then incubated with biotinylated anti-mouse IgG (BA2000, Vector Labs Inc, CA, USA) for 20 min, and then avidin-biotin peroxidase complex solution (Vectastain ABC kit, Vector Labs Inc) for an additional 20 min. Antibody binding was visualized with 3-amino-9-ethyl-carbazole (Sigma Chemical Co, MO, USA).

Microscopic Examination
Light microscopic examination was performed after H&E staining. The degree of myocyte hypertrophy, myocardial degeneration and interstitial fibrosis were evaluated semiquantitatively according to a four-grade system: 0, normal or no remarkable change; 1, mild; 2, moderate; and 3, severe change.5 This score was compared with the degree of MMP and TIMP expressions.

Quantification of Expression
For quantitative assessment of MMP and TIMP expression, the number of positive cells in 5 high power fields (HPF, ×400) was counted. These data were obtained blindly by 2 independent observers and averaged for each patient. The patients were then classified into 2 groups according to the degree of MMP-2 expression: strongly positive (≥2.0 cells positive for MMP-2 per HPF) and weakly positive (<2.0 cells positive for MMP-2 per HPF).

Indices of Cardiac Function
The clinical data (New York Heart Association (NYHA) functional class, echocardiography, angiography, hemodynamics, and the levels of neurohormones) were evaluated at the time of endomyocardial biopsy. The LV end-diastolic (LVDD) and end-systolic (LVED) dimensions were measured from the M-mode echocardiograms using a Hewlett-Packard SONOS 1500 apparatus. Percent fractional shortening (%FS) was calculated as (LVDD-LVED)/LVDD. The LV ejection fraction (LVEF) was derived from left ventriculography data. Neurohormonal blood sampling was performed from venous blood (EDTA) at 08.00h after intravenous cannulation and the patient having been seated for 30 min. Assays for norepinephrine and atrial natriuretic peptide (ANP) were conducted according to established methods.6,17 The degree of MMP and TIMP expression was compared with these functional indices.

Statistical Analysis
The correlation between the degree of MMP and TIMP expression and the indices of cardiac function was examined by least squares linear regression analysis. The correlation coefficients are Pearson’s r values. Probability (p) values of less than 0.05 were considered statistically significant. Data are presented as the means ± standard deviation (SD) and were compared using the Mann-Whitney U test.
Results

Pathological Findings

Interstitial fibrosis was observed in almost all biopsy samples (Table 1). Fibrotic change was slight in most samples. Immunohistochemically, MMP-2 and TIMP-2 were expressed in spindle-shaped cells in the myocardial interstitium (Fig 1). MMP-9 and TIMP-1 were rarely observed in any of the samples. The mean number of positive cells expressing each enzyme is shown in Table 1. A significant correlation between MMP-2 and TIMP-2 expression was found (Fig 2). TIMP-2 expression predominated over that of MMP-2. There was no correlation between MMP or TIMP expression and the score of interstitial fibrosis. Fig 3 shows representative immunohistochemical findings of myocardium stained with anti-MMP-2. The score of interstitial fibrosis was slightly greater in the strongly positive group, but there was no significant difference in the score of interstitial fibrosis between the 2 groups (Table 2). Myocyte hypertrophy and myocardial degeneration was also observed in almost all biopsy samples, but there were no correlation between MMP or TIMP expression and these (data not shown).

Relation Between Clinical Findings and the Expression of MMP-2 and TIMP-2

The LVEF was negatively correlated with MMP-2 expression (Fig 4). A comparison of the clinical indices between the strongly positive and weakly positive groups is shown in Table 2. LVEF, LV end-diastolic pressure (LVEDP), RVEDP, pulmonary capillary wedge pressure (PCWP) and plasma noradrenaline levels were significantly greater in the strongly positive group. With respect to other indices, there were no significant correlations in either group. However, indices indicated slightly more severe conditions in the strongly positive group.
Discussion

To our knowledge, this is the first study to use right ventricular endomyocardial biopsy samples to determine the expression of MMPs and TIMPs in the cardiac matrix of patients with mild or moderate idiopathic DCM.

Expression of MMPs and TIMPs in the Failing Heart

MMP-2 and MMP-9 are known as gelatinase A and B, respectively. MMP-2 forms a complex with TIMP-2, and MMP-9 interacts with TIMP-1. In the present study, MMP-2 and TIMP-2 were expressed in the myocardial interstitium, whereas MMP-9 and TIMP-1 were rarely observed in the samples. These results indicate that the localization of MMP-2 and MMP-9 differs. Because limited information was available from the human biopsy samples in the present study, it was difficult to definitively determine the localization of MMP-9 and TIMP-1-positive cells. However, in our previous experimental study using cardiac allografts from Japanese monkeys, expression of MMPs and TIMPs was observed in rejected myocardium (unpublished data). In that, MMP-2 and TIMP-2 were strongly and diffusely expressed in spindle-shaped cells in the myocardial interstitium, considered to be activated fibroblasts or myofibroblasts. MMP-9 and TIMP-1-positive cells were observed in perivascular lesions or thickened intima and media. It is true that the mechanism of myocardial damage is different, but it is possible that MMP- and TIMP-positive cells are similarly distributed in human DCM myocardium and non-human rejected myocardium.
Table 2: Comparison of the Clinical Indices Between the Strongly Positive Group and Weakly Positive Group

<table>
<thead>
<tr>
<th></th>
<th>Strongly positive group (n=7)</th>
<th>Weakly positive group (n=9)</th>
<th>p value</th>
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</thead>
<tbody>
<tr>
<td>LVEDd (mm)</td>
<td>68±9.9</td>
<td>67±6.2</td>
<td>NS</td>
</tr>
<tr>
<td>LVDS (mm)</td>
<td>60±11.3</td>
<td>56±4.4</td>
<td>NS</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>24±2.2</td>
<td>36±5.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>FS (%)</td>
<td>12±5.2</td>
<td>16±5.3</td>
<td>NS</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>20±8.7</td>
<td>10±4.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CI (L·min⁻¹·m⁻²)</td>
<td>2,250±20.0</td>
<td>2,750±90.0</td>
<td>NS</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>21±6.5</td>
<td>8±6.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BP (mmHg)</td>
<td>41±15.1</td>
<td>39±5.1</td>
<td>NS</td>
</tr>
<tr>
<td>RVDEP (mmHg)</td>
<td>128±6.6</td>
<td>124±3.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NE (pg/ml)</td>
<td>0.62±0.33</td>
<td>0.27±0.08</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ANP (pg/ml)</td>
<td>127±94</td>
<td>75±56.6</td>
<td>NS</td>
</tr>
<tr>
<td>Intermittent fibrosis</td>
<td>1.3±0.76</td>
<td>0.8±0.33</td>
<td>NS</td>
</tr>
</tbody>
</table>

*LVEF, LVEDP and the plasma norepinephrine level were significantly greater in the strongly positive group. ANP, atrial natriuretic peptide; CI, cardiac index; FS, fractional shortening; LVEDd, left ventricular end-diastolic diameter; LVDS, left ventricular end-systolic diameter; LVEDP, left ventricular end-diastolic pressure; LVEF, left ventricular ejection fraction; NE, norepinephrine; PCWP, pulmonary capillary wedge pressure; SD, standard deviation.

Myocardial Remodeling and Matrix Metalloproteinases

Features of DCM are ventricular dilatation and pump dysfunction. Remodeling of the extracellular matrix is an important contributory event in the progression of end-stage DCM. A recent clinical study demonstrated that MMP activity was increased in the endomyocardium of both the LV and RV in DCM. In the present study, we demonstrated that the MMP-2 expression in RV biopsy samples was negatively correlated with LVEF. It has been reported that the histological findings in a RV biopsy are in good agreement with those in LV biopsy obtained from the same heart and reflect the LV function. The present results suggested that MMP-2 expression in the RV reflects cardiac functional deterioration. The degree of interstitial fibrosis was slightly more severe in the strongly positive group, but there was no statistical relation between the degree of MMP expression and the score of the microscopic findings. These results suggest that the expression of MMPs in the RV cardiac matrix could reflect pathophysiological changes of DCM that can not be observed by light microscopic examination.

Myocardial remodeling is influenced by mechanical overload as well as by various chemical agents. In the failing heart, mechanical stress accelerates the production of vasoactive agents, angiotensin II, endothelin and catecholamines. These agents cause a receptor-mediated increase in protein kinase C in the myocardium, and protein kinase C is involved in the induction of MMP transcription. In the present study, indices of mechanical overload, such as LVEDP, tended to be greater in the strongly positive group. The plasma norepinephrine level was also significantly greater in the strongly positive group. Elevated plasma norepinephrine levels activate the renin-angiotensin system in the myocardium, and angiotensin II increases the level of several species of myocardial MMPs. Therefore, it is suggested that the expression of MMPs and TIMPs reflects the responses of the myocardium and interstitium to physical stress and soluble factors. However, experimental data that support this speculation are required.

Clinical Implications

The expression of MMPs in the RV cardiac matrix may reflect pathophysiological changes of DCM that can not be observed by light microscopy. Although the present the follow-up period was too short to be confirmatory, it is interesting to speculate whether the MMP expression in the cardiac matrix will also reflect the patient’s response to medication. Further long-term follow-up needs to be done.
Several recent studies have examined the use of synthetic MMP inhibitors.33-35 Tyagi et al described the prospect of gene therapy in improving cardiac function by overexpression or downregulation of extracellular matrix components.36 We demonstrated in the present study that MMPs and TIMPs are expressed in the myocardium of patients with mild or moderate (relatively early stage) idiopathic DCM. On this basis, we suggest that the use of synthetic MMP inhibitors in early stage idiopathic DCM could be significantly therapeutic.

Study Limitations

The endomyocardial biopsy specimen was often so small that it was difficult to quantify MMPs and TIMPs at the mRNA level or the gelatinolytic activity. As described, the possibility of differential localization of MMP-2 and MMP-9 remains. Samples were obtained from patients who had received medical treatment and ACE inhibitors (all patients), as well as catecholamines (15 patients), possibly influence MMP expression in cardiac matrix.37 In addition, the number of patients was relatively small and a further sampling error should be considered because the biopsy samples obtained from one patient were so small.

Acknowledgments

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