Matrix Metalloproteinase Inhibition Attenuates Left Ventricular Remodeling and Dysfunction in a Rat Model of Progressive Heart Failure

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Background—Matrix metalloproteinase (MMP) activation contributes to tissue remodeling in several disease states, and increased MMP activity has been observed in left ventricular (LV) failure. The present study tested the hypothesis that MMP inhibition would influence LV remodeling and function in developing LV failure.

Methods and Results—LV size and function were measured in 5 groups of rats: (1) obese male spontaneously hypertensive heart failure rats (SHHF) at 9 months (n=10), (2) SHHF at 13 months (n=12), (3) SHHF rats treated with an MMP inhibitor during months 9 to 13 (PD166793 5 mg · kg⁻¹ · d⁻¹ PO; n=14), (4) normotensive Wistar-Furth rats (WF) at 9 months (n=12), and (5) WF at 13 months (n=12). Plasma concentrations of the MMP inhibitor (116±11 μmol/L) reduced in vitro LV myocardial MMP-2 activity by ∼100%. LV function and geometry were similar in LV rats at 9 and 13 months. LV peak +dP/dt was unchanged at 9 months in SHHF but by 13 months was reduced in the SHHF group compared with WF (3578±477 versus 5983±109 mm Hg/s, P<0.05). LV volume measured at an equivalent ex vivo pressure (10 mm Hg) was increased in SHHF at 9 months compared with WF (443±12 versus 563±33 mL, P<0.05) and increased further by 13 months (899±64 mL, P<0.05). LV myocardial MMP-2 activity was increased by ∼2-fold in SHHF at 9 and 13 months. With MMP inhibition, LV peak +dP/dt was similar to WF values and LV volume was reduced compared with untreated SHHF values (678±28 mL, P<0.05).

Conclusions—MMP activity contributes to LV dilation and progression to LV dysfunction in a rodent HF model, and direct MMP inhibition can attenuate this process. (Circulation. 2001;103:2303-2309.)

Key Words: ventricles • remodeling • hypertrophy • hypertension • systole

Left ventricular (LV) remodeling is an important determinant in the progression to heart failure (HF).¹ The matrix metalloproteinases (MMPs) are a family of zinc-dependent enzymes demonstrated to contribute to tissue remodeling in a number of disease states.² Moreover, increased MMP zymographic activity and species expression have been identified in end-stage human HF.³-⁵ Thus, increased MMP activity may directly participate in the progressive LV remodeling process in HF. This study tested the hypothesis that increased MMP activity directly contributes to the LV remodeling process by evaluating whether MMP-inhibitor treatment preserves LV geometry and function in a rodent HF model, the spontaneously hypertensive HF (SHHF) rat.

Methods

MMP Inhibitor PD166793: Specificity and Pharmacology

The broad-spectrum MMP inhibitor PD166793, or (S)-2-[(4'-bromobiphenyl-4-sulfonylamino)-3-methyl-butyric acid, was used in this study. The MMP-inhibitory profile of PD166793 is shown in Table 1. Unlike first-generation MMP inhibitors, the peptide hydroxymates (eg, batimastat, marimastat),⁶ PD166793 is a selective MMP inhibitor and does not inhibit other metalloproteases like tumor necrosis factor-α convertase.

Experimental Design of Efficacy Experiment

Male normotensive Wistar-Furth (WF) rats (Harlan, San Diego, Calif) and obese SHHF rats (Genetic Models, Indianapolis, Ind) were divided into 5 groups: (1) SHHF at 9 months (n=10), (2) SHHF at 13 months (n=15), (3) SHHF treated with an MMP inhibitor during months 9 to 13 (PD166793 5 mg · kg⁻¹ · d⁻¹ PO in chow) (n=14), (4) normotensive WF at 9 months (n=12), and (5) WF at 13 months (n=12). Drug administration was initiated in 9-month-old rats and continued for 4 months. The 5-mg · kg⁻¹ · d⁻¹ dose of PD166793 was selected to produce a plasma drug level of 100 μmol/L, which, on the basis of the in vitro inhibition for both rat and human MMPs, was expected to abolish MMP activity (Figure 1). Three rats in the SHHF-13 vehicle control group died during the last month of testing. The cause of death was unknown, and these rats are not included in the analysis. Death did not occur in any other group.
LV Function and Geometry

Rats were anesthetized with sodium pentobarbital (25 mg/kg IP for SHHF-13, 50 mg/kg IP for all other groups). Rats were ventilated with 50% oxygen, and LV pressure was measured in closed-chest rats with a Millar pressure transducer inserted via the right carotid artery. Data were recorded (500 Hz) on a digital data acquisition system (Gould Po-Ne-Mah HD-4). Baseline hemodynamic measurements were made over a 30- to 40-second interval. LV dP/dt at 40 mm Hg of LV pressure was computed by use of digitized data, thus providing an index of the rate of LV pressure development at an equivalent LV pressure. Blood was withdrawn to determine plasma drug levels by high-performance liquid chromatography.

The heart was arrested by intravenous KCl injection and rapidly excised. The LV was cannulated, and dilation was measured by ex vivo LV pressure-volume (PV) curves as described elsewhere. From the LVPV data, LV peak circumferential global average wall stress was computed from a spherical model of reference:

\[
s = \frac{P(1+\frac{h}{D})}{4(1-\frac{h}{D})} \times 1.36, \]

where \(P\) is LV systolic pressure, \(D\) is minor-axis dimension determined at 10 mm Hg filling pressure, and \(h\) is wall thickness at 10 mm Hg filling pressure. LV minor axis dimension and wall thickness were computed on the basis of a spherical model using LV mass and volume.8 LV circumferential sections were stained with hematoxylin-eosin for myocyte cross-sectional measurements and picrosirius red for fibrillar collagen measurements by use of techniques described elsewhere.9,10

LV Myocardial MMP Activity and Abundance

Specific MMP-2 activity in LV myocardial extracts (25 μg) was measured by an antibody capture method described elsewhere.6 These studies were also performed with WF myocardial extracts in the presence of increasing concentrations of PD166793 (0 to 50 μmol/L), and activity measured (ng · h⁻¹ · g⁻¹). LV myocardial samples were also subjected to immunoblotting for the gelatinases (MMP-9 and MMP-2) and the predominant rodent form of interstitial collagenase (MMP-13).11 LV myocardial extracts were prepared in the presence of protease inhibitors and subjected to SDS gel electrophoresis under denaturing conditions as described previously.10 The fractionated proteins were then transferred to nitrocellulose membranes (Amersham) and incubated with anti–MMP-2 (1:200 dilution, MS-806-P0, NeoMarkers, Inc), anti–MMP-9 (1:2000 dilution, TP221, Torrey Pines Biolabs), or anti–MMP-13 (1.0 μg/mL, MAB3321, Chemicon International). The membranes were incubated overnight at 4°C and washed. The positive immunoreactive signal was detected with a conjugated secondary antibody and chemiluminescence (ECL, Amersham). Luminescent signal intensity at the molecular weight corresponding to the zymogen form was subjected to densitometric analysis (ImageQuant v 5.0, Molecular Dynamics). The densitometric signal was normalized to WF-9 values and expressed as a percentage.

Statistical Analysis

All data are expressed as group mean ± SEM. A 1-way ANOVA was used to test for treatment effects, and between-group differences were assessed with a post hoc t test (Tukey’s test, SigmaStat Version 2.0, Jandel Scientific). Statistical significance was based on a value of \(P \leq 0.05\).

Results

Drug Levels and LV Function and Geometry

The plasma concentration of PD166793 measured after 4 months of dosing was 116 ± 11 μmol/L. In vivo hemodynamic and PV measurements are summarized in Table 2. No differences in mean arterial pressure or LV function and geometry were detected between 9- and 13-month-old WF rats, and these data were combined. Mean arterial pressure and LV peak pressure were higher in the 9-month-old SHHF than in WF rats. At 13 months of age, LV peak pressure, dP/dt, and arterial pressure were reduced in untreated SHHF rats compared with 9-month values. In the 13-month-old SHHF rats treated with PD166793, LV peak pressure and pressure development were similar to 9-month SHHF values.

LVPV measurements were performed ex vivo (Figure 2). LV volumes were highest in the untreated 13-month-old SHHF group at all filling pressures and were reduced in the...
13-month-old SHHF group treated with PD166793. LV peak pressure obtained in vivo and autopsy LV mass, LV wall thickness, and peak stress were calculated by use of the LV volumes obtained at 10 mm Hg (Table 2). LV wall thickness was greater in 9-month-old SHHF than in normotensive WF rats, and wall thickness decreased in untreated 13-month-old versus 9-month-old SHHFs. LV peak stress was higher in the SHHF rats at 9 months, but this did not reach statistical significance ($P > 0.05$) and was unchanged in the SHHF rats at 13 months. In the 13-month-old SHHF group treated with PD166793, LV wall thickness was similar to that of SHHF rats at 9 months. Because LV systolic pressure and volumes were higher in the treated SHHF group than WF values, this was translated into a higher LV peak wall stress.

LV mass/volume ratio increased in the SHHF group at 9 months, consistent with LV hypertrophy. The LV mass/volume ratio fell, however, in 13-month-old non–drug-treated SHHFs, indicating inadequate LV hypertrophy and development of ventricular dilatation. In the treated SHHF group, LV mass/volume ratio was similar to SHHF 9-month values. Figure 3 shows representative LV circumferential sections demonstrating the changes in LV geometry in all groups.

**LV Myocardial Morphometry**

LV myocyte cross-sectional area was increased in the SHHF group at 9 and 13 months compared with age-matched normotensive values. Endocardial fibrillar collagen percent area was significantly increased in 9-month-old SHHF, compared with age-matched normotensive rats (Table 3). Percent collagen area was significantly greater across all 3 regions of the LV wall in 13-month-old untreated SHHFs than in age-matched WF rats. Endomyocardial and midmyocardial collagen percent area increased with age in untreated SHHF rats. MMP inhibitor treatment did not alter percent fibrillar collagen versus the untreated 13-month-old SHHF control.

**LV Myocardial MMP Activity and Abundance**

With an MMP-2 capture assay, LV MMP-2 activity was increased in all SHHF groups compared with both WF groups.

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### TABLE 2. Systemic Hemodynamics and LV Geometry

<table>
<thead>
<tr>
<th></th>
<th>WF</th>
<th>SHHF-9</th>
<th>SHHF-13</th>
<th>SHHF-793</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean blood pressure, mm Hg</td>
<td>121±3</td>
<td>184±8*</td>
<td>120±12†</td>
<td>184±8‡</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>402±7</td>
<td>331±19*</td>
<td>295±19*</td>
<td>379±13‡</td>
</tr>
<tr>
<td>LV peak pressure, mm Hg</td>
<td>141±2</td>
<td>209±8*</td>
<td>126±11†</td>
<td>213±9†</td>
</tr>
<tr>
<td>Peak positive $dP/dt$, mm Hg/s</td>
<td>5983±109</td>
<td>6714±376</td>
<td>3578±477‡</td>
<td>7228±356‡</td>
</tr>
<tr>
<td>LV peak pressure at 40 mm Hg, mm Hg/s</td>
<td>3418±73</td>
<td>3842±235</td>
<td>1865±293†</td>
<td>3842±235‡</td>
</tr>
<tr>
<td>LV volume at pressure of 10 mm Hg, μL</td>
<td>443±12</td>
<td>563±33*</td>
<td>899±64¶</td>
<td>678±28†‡</td>
</tr>
<tr>
<td>LV wall thickness, mm</td>
<td>11.3±0.2</td>
<td>15.6±0.4*</td>
<td>13.5±0.8®</td>
<td>15.9±0.3‡</td>
</tr>
<tr>
<td>LV peak stress, g/cm²</td>
<td>362±11</td>
<td>409±27</td>
<td>338±26†</td>
<td>429±20‡</td>
</tr>
<tr>
<td>LV mass, mg</td>
<td>779±16</td>
<td>1326±30*</td>
<td>1436±65*</td>
<td>1515±30¶†</td>
</tr>
<tr>
<td>LV mass/volume ratio, g/mL</td>
<td>1.8±0.1</td>
<td>2.4±0.1*</td>
<td>1.7±0.1†</td>
<td>2.3±0.1¶†</td>
</tr>
<tr>
<td>Sample size, n</td>
<td>24</td>
<td>10</td>
<td>12</td>
<td>14</td>
</tr>
</tbody>
</table>

SHHF 793 indicates SHHF rats treated with PD166793 (see text). LV volumes, wall thickness, and stress were computed with a common end-diastolic filling pressure of 10 mm Hg. Values are mean±SEM.

* $P<0.05$ vs WF, † $P<0.05$ vs SHHF-9, ‡ $P<0.05$ vs SHHF-13.
These in vitro measurements require extraction techniques resulting in removal of the MMP inhibitor, which may have been operative in vivo. Therefore, MMP-2 activity was examined in the presence of increasing concentrations of the MMP inhibitor PD166793 (Figure 4). The effective concentration of PD166793 resulting in 50% inhibition of LV myocardial MMP-2 activity (EC50) was computed to be 3 μmol/L. At 50 μmol/L of PD166793, MMP-2 activity was nearly extinguished, and higher concentrations completely eliminated any MMP-2 activity. Therefore, the plasma levels of PD166793 achieved in the present study were higher than that necessary to inhibit the panel of MMPs listed in Table 1.

Relative LV myocardial levels of MMP-9 and MMP-2 were increased in the SHHF compared with the WF group (Figure 5). In the 13-month-old SHHF groups, myocardial MMP-2 levels were increased from 9-month SHHF values, irrespective of treatment (P<0.05). MMP-9 levels were lower in the MMP-inhibition group at 13 months than untreated values. MMP-13 was observed in LV extracts taken from all groups, and it increased with age (Figure 6). Myocardial MMP-13 levels were increased in all 13-month-old groups versus 9-month normotensive values; relative myocardial MMP-13 levels were higher in the 13-month-old untreated SHHF group; however, this did not reach statistical significance (P=0.09).

**Discussion**

A milestone in the progression of HF is myocardial remodeling. Recent clinical and experimental studies implicate MMPs in the myocardial remodeling characteristic of developing HF in humans and animal models. Whether and to what degree myocardial MMP activity directly contributes to the transition to HF, however, remains to be established. Accordingly, this study tested the hypothesis that MMP inhibition instituted early during the progressive development of HF in the SHHF rat would attenuate the degree of LV remodeling that invariably occurs as a function of age in this animal model. The important findings of this study were...
Moreover, past studies have instituted MMP inhibition at the onset of the HF stimulus, whereas the present study initiated MMP inhibition during the progressive development of HF. The results from the present study demonstrate for the first time that MMP inhibition can delay and/or attenuate the time-dependent progression of LV remodeling in a model of HF.

**MMP Inhibition and LV Function and Geometry**

Previous studies have demonstrated that MMP inhibition reduced the degree of LV dilation in a mouse MI model and in the pacing HF model. In the pacing HF model, concomitant MMP inhibition during the pacing period reduced the degree of LV peak systolic wall stress. The reduction in LV wall stress achieved with MMP inhibition in the pacing HF model was associated with improved LV fractional shortening. Improvement in LV fractional shortening with pacing HF was primarily due to preserved LV geometry and reduced LV afterload, rather than an intrinsic improvement in myocyte contractile function. In the present study, MMP inhibition maintained computed LV peak wall stress in hypertensive rats with developing HF. This maintenance of peak LV wall stress with MMP inhibition was due to the preservation of LV chamber dimensions and myocardial wall thickness, which was accomplished by the continuation of elevated LV peak pressure. Therefore, MMP-inhibitor treatment preserved LV geometry in hypertensive HF rats despite elevated peak LV wall stress, a potent stimulus of myocyte remodeling. The present study did not evaluate LV pump function in vivo. Therefore, the effects of MMP inhibition on LV pump function under ambient systemic loading conditions and neurohormonal influences in this rodent HF model remain to be established. In both the present and pacing HF studies, MMP-inhibitor treatment was associated with improved fractional shortening compared with the untreated HF group. In light of these findings, future studies that serially measure the effects of long term MMP inhibition on indices of LV pump function in this rodent model of progressive HF are warranted.

Diastolic dysfunction is an important factor contributing to the signs and symptoms associated with the development of HF. One of the more common causes for diastolic HF is LV hypertrophy and an attendant reduction in chamber compliance. In the present study, the hypertensive rat model resulted in significant LV hypertrophy by 9 months of age that persisted with the progression of HF. MMP inhibition instituted in the pacing HF model reduced LV chamber size and preserved myocardial wall thickness but was associated with increased chamber and myocardial stiffness properties. Several factors influence LV chamber compliance, including myocardial tissue composition and active relaxation processes. The changes in LV chamber compliance that occurred, and whether and to what degree MMP inhibition influenced this index of LV diastolic function, remain to be established. The observation in this study that MMP inhibition limited the degree of LV chamber dilation without a concomitant reduction in the degree of LV hypertrophy raises an issue requiring additional study.

**LV Myocardial MMPs and Remodeling**

Past experimental studies have demonstrated a relationship between myocardial MMP activity and LV remodeling in models of myocardial infarction (MI) and HF. For example, Rohde and colleagues demonstrated that MMP inhibition attenuated the degree of LV dilation in a mouse MI model. In a pacing HF model, progressive LV dilation and myocardial wall thinning were temporally related to myocardial MMP expression. Moreover, MMP inhibition in this pacing HF model reduced the degree of LV dilation and was associated with improved LV ejection performance. These past studies, however, examined the effects of MMP inhibition over a fairly short time period and with rapidly progressive LV remodeling. The present study builds on these past reports by demonstrating that MMP inhibition instituted over a 4-month period attenuated the degree of LV dilation and wall thinning that occurred in this rodent model of HF.
Myocardial MMPs and Myocardial Structure

The MMPs constitute a family of ~20 zinc-dependent neutral endopeptidases categorized on the basis of substrate specificity and structure. MMPs are expressed and synthesized by all primary cell types within the myocardium, including myocytes. With the development of HF, increased expression of certain MMP species has been identified. For example, increased myocardial levels of the gelatinases (ie, MMP-2 and MMP-9) have been identified in patients with end-stage HF as well as in several animal models of HF. The present study demonstrated increased myocardial levels of MMP-2 and MMP-9 in rats with HF. These findings, coupled with past reports, suggest that increased myocardial levels of these MMP species is a fairly uniform event in HF development. Recent studies using transgenic mouse models of MMP deletion have provided important additional insight into particular MMP species that are likely to be involved in LV remodeling. For example, in MMP-9 gene–knockout mice, the degree of LV remodeling after surgically induced MI was reduced. In this past report, the degree of macrophage and collagen accumulation was reduced in the MMP-9–deficient mice after MI. These results suggest that MMP-9 may contribute to the LV remodeling process in the post-MI period by direct proteolysis of myocardial matrix components as well as through facilitating an inflammatory response. In the present study, long-term treatment with the MMP inhibitor PD166793 resulted in myocardial drug levels significantly higher than that necessary to inhibit all the MMPs profiled in Table 1, including MMP-9. Moreover, an ex vivo activity assay demonstrated that these myocardial levels of the MMP inhibitor completely inhibited MMP-2 activity. Substrates for MMP-2 and MMP-9 include the basement membrane components collagen IV and laminin. Thus, increased MMP-2 and MMP-9 activity within the myocardium can contribute to a discontinuity of the basement membrane, thereby disrupting the normal myocyte-matrix interface. The findings from the present study contribute to the body of evidence suggesting that MMP-2 and MMP-9 are involved in LV myocardial remodeling.

The MMP species primarily responsible for fibrillar collagen degradation are the collagenases: MMP-1, MMP-13, and MMP-8. Rodents do not express MMP-1, and MMP-8 is synthesized and released primarily by inflammatory cells. Thus, the predominant collagenase within the rodent myocardium is MMP-13. In the present study, myocardial MMP-13 levels were increased in the hypertensive 13-month-old rats. The levels of MMP inhibition achieved in the present study should have resulted in complete inhibition of myocardial MMP-13 activity. Whether and to what degree these in vitro measurements of myocardial MMP-13 and MMP-inhibitor levels were translated into inhibition of in vivo collagenolytic activity, however, remains to be established. An issue surrounding the assessment of myocardial MMP activity is that MMPs are synthesized and released in an inactive (zymogen) form requiring proteolytic processing for full activation. A number of MMP species contribute to proteolytic degradation of the extracellular matrix as well as participating in the activation cascade of other MMP species. For example, Stromelysin (MMP-3) and membrane type 1 MMP (MMP-14) have been reported to possess proteolytic activity against a wide complement of extracellular proteins and can contribute to full activation of several MMP species. Because the present study, along with past reports, demonstrated that MMP activity can contribute to the LV remodeling process in the setting of HF, future studies that begin to identify the specific complement of myocardial MMPs that contribute to this process are warranted.

In the present study, long-term treatment with a broad-spectrum MMP inhibitor did not appear to cause an upregulation of myocardial MMPs. In fact, myocardial MMP-9 levels were reduced from untreated HF values, perhaps because ventricular remodeling was largely prevented. In certain enzyme cascade systems, interruption of proteolytic activity can cause a feedback effect that ultimately increases protein expression. For example, long-term ACE inhibition induces an increase in plasma renin activity as a result of a substrate feedback effect. In MMP-9–knockout mice, increased levels of MMP-2 and MMP-13 were observed compared with wild-type littermates, suggesting a compensatory induction of MMP expression. The present study provides the first evidence to suggest that inhibition of MMP activity may not be associated with a feedback induction of MMP expression. This issue, however, requires further study through the use of long-term MMP inhibition in other species and models of HF.

MMP activity is regulated by an endogenous family of at least 4 proteins called the tissue inhibitors of MMPs (TIMPs). Past clinical studies demonstrate that TIMP levels and TIMP-1/MMP stoichiometry change in patients with end-stage HF. TIMP-1–deficient mice exhibit significant LV remodeling, suggesting that constitutive control of myocardial MMP activity is important in maintaining a normal LV phenotype. The present study did not evaluate TIMP levels in the myocardium from HF rodents and how these levels might have been altered with long-term MMP inhibition. TIMP expression is a physiologically relevant counterbalance, modulating MMP activity in LV remodeling, which warrants further study.

Age is another aspect of MMPs that may be relevant to HF progression. LV collagenase expression increases with age in SHR rats. The effect of age on MMP expression may account for why MMP-13 levels were higher in both 13-month-old normotensive and HF rats in this study. Because HF is primarily a disease of the elderly, the observation that MMP species expression changes in mature rodent myocardium and can increase further with hypertension and HF may be of particular relevance.

Summary

LV MMP content and activity was higher in HF rats than in normotensive rats. MMP upregulation was progressive between 9 and 13 months of age and preceded the development of HF. MMP-inhibitor treatment significantly reduced LV dilation and preserved systolic function but did not prevent further hypertrophy, exacerbate fibrosis, or induce an increase in MMP expression. These results suggest that LV dilation mediated by MMP activity is an important event in the transition to HF.
Acknowledgments

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