Effects of Matrix Metalloproteinase Inhibition on Ventricular Remodeling Due to Volume Overload

Amanda L. Chancey, BS; Gregory L. Brower, DVM, PhD; J. Thomas Peterson, PhD; Joseph S. Janicki, PhD

Background—Left ventricular (LV) hypertrophy and dilatation are important compensatory responses to chronic volume overload. Although LV function is initially preserved by these responses, the continued structural remodeling of the myocardium ultimately becomes maladaptive, leading to the development of heart failure. We have shown previously that increased myocardial matrix metalloproteinase (MMP) activity precedes LV dilatation induced by a chronic volume overload. Accordingly, this study focused on the effects of MMP inhibition therapy (PD 166793, 1 mg · kg⁻¹ · d⁻¹) on LV size and function in a rat model of volume overload–induced heart failure.

Methods and Results—Rats were divided into the following groups: treated and untreated infrarenal abdominal aortocaval fistula and treated and untreated sham-operated (control). LV weights of both fistula groups were increased above that of the control group (868±79 mg; P≤0.001); LV weights in the treated fistula group, however, were lower than in the untreated fistula group at 8 weeks (1447±186 versus 1715±279 mg, respectively; P≤0.012). The marked ventricular dilatation seen in the untreated fistula group was significantly diminished in the treated fistula group, although the increase in LV compliance was similar in both treated and untreated fistula hearts.

Conclusions—MMP inhibition significantly attenuates the myocardial remodeling associated with chronic volume overload, as evidenced by prevention of dilatation, a marked reduction in LV hypertrophy, and preservation of ventricular function. (Circulation. 2002;105:1983-1988.)

Key Words: hypertrophy ■ dilatation ■ ventricles ■ fistula

Chronic ventricular volume overload induces predictable and reproducible changes in ventricular weight, end-diastolic volume, and function.¹⁻⁵ For this characteristic myocardial remodeling to occur, however, there must be alterations in the interstitial collagen matrix.⁶⁻⁷ We and others have previously shown that an increase in matrix metalloproteinase (MMP) activity resulting in a partial degradation of the extracellular matrix precedes ventricular remodeling induced by a chronic volume overload or rapid pacing.⁸⁻¹⁰ MMPs are a group of zinc-dependent enzymes that function in the turnover of the extracellular matrix¹¹ and have been implicated in contributing to myocardial remodeling in animal models and humans with congestive heart failure (CHF) and cardiomyopathy.⁵,¹⁰,¹²,¹³ Although humans and rodents possess different portfolios of MMPs, it is agreed that the collagenases (MMP-1, MMP-8, and MMP-13), the stromelysins (MMP-3 and MMP-10), and the gelatinases (MMP-2 and MMP-9) are particularly relevant in myocardial remodeling.¹⁴ Consequently, MMP inhibitors have been advocated as a potential therapeutic approach for the prevention of cardiovascular diseases.¹⁵⁻⁾ Twenty Although various MMP inhibitors have been studied in animal models of rapid pacing–induced heart failure,¹⁶ hypertension,¹⁵ and myocardial infarction,¹⁸ the effectiveness of MMP inhibition in models of chronic volume overload has not been adequately addressed. Therefore, the goal of this study was to determine the effects of MMP inhibition therapy on myocardial remodeling and function in normal rats subjected to an infrarenal aortocaval (AV) fistula. The results demonstrate a marked reduction in hypertrophy, prevention of ventricular dilatation, and preservation of ventricular function by MMP inhibition.

Methods

All experiments were performed on 6-week-old male Sprague-Dawley rats housed under standard environmental conditions and maintained on commercial rat chow and tap water ad libitum. All studies conformed with the principles of the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals and were approved by our Institution’s Animal Care and Use Committee. Anesthesia for the surgical procedure and at the experimental end point was effected with sodium pentobarbital (50 mg/kg IP).

Surgical Preparation and Experimental Protocol

Chronic biventricular volume overload was induced using an infrarenal AV fistula model previously characterized by this laboratory.¹,⁵ Significant changes in myocardial size (ie, ventricular dilatation) and function consistently occur in this model within 8 weeks after fistula surgery.
Rats were randomly divided into groups as follows: controls, consisting of sham-operated rats that received either vehicle only (n = 10) or 1 mg · kg⁻¹ · d⁻¹ PD 166793 (n = 5); untreated fistula rats, which received vehicle only (n = 8); and treated fistula rats, dosed at 1 mg · kg⁻¹ · d⁻¹ with PD 166793 (n = 13). PD 166793 is a broad-spectrum inhibitor of MMP-1, -2, -3, -7, -9, and -13, as described elsewhere. This dosage produced plasma levels of 37,000 ± 9400 ng/mL, which have been shown to be adequate to achieve MMP inhibition. The inhibitor was dissolved in a vehicle of sterile 0.5% methylcellulose and administered by daily gavage. Rats were weighed before initial dosing and weekly thereafter during treatment to ensure constant dosing. Each group received drug and/or vehicle via gavage beginning 2 weeks before fistula or sham surgery and continued to receive the assigned treatment until the completion of the functional studies, the atria and great vessels were separated and weighed. Lung wet weight was obtained after the esophagus and trachea were trimmed away and the pleural (RV) were separated and weighed.

**TABLE 1. Body, Lung, and Heart Weights**

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight, g</th>
<th>Lung Weight, mg</th>
<th>LV Weight, mg</th>
<th>RV Weight, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>394±26</td>
<td>1456±122</td>
<td>868±79</td>
<td>214±33</td>
</tr>
<tr>
<td>Treated sham</td>
<td>393±39</td>
<td>1555±181</td>
<td>988±81</td>
<td>265±20</td>
</tr>
<tr>
<td>Treated fistula</td>
<td>405±41</td>
<td>2009±384*</td>
<td>1447±186*</td>
<td>484±76*</td>
</tr>
<tr>
<td>Untreated fistula</td>
<td>421±63</td>
<td>2477±579†</td>
<td>1715±279†</td>
<td>562±117*</td>
</tr>
</tbody>
</table>

Values are mean±SD.

*P<0.05 vs control; †P<0.05 vs treated fistula.

Assessment of Ventricular Size and Function

LV volume and function were evaluated in vitro by use of a blood-perfused, isolated heart preparation as previously described. Briefly, the descending thoracic aorta is cannulated for continuous perfusion of the heart during the functional study, and the heart is then attached to the perfusion apparatus, which consists of a pressurized reservoir (95 to 105 mm Hg) containing arterial blood from a support rat. Once the heart is hung, the pulmonary artery is cut to allow for unimpeded drainage of coronary venous flow, which is collected in a reservoir and returned to the support rat. The left atrium is removed to allow insertion of a balloon into the LV, and the pressure developed within the ventricle is measured via an attached pressure transducer. By filling the balloon with saline in 10- to 20-μL increments and measuring the pressure developed by the ventricle, we could obtain pressure-volume curves and use them to analyze LV diastolic and systolic function.

Data and Statistical Analysis

Statistical analyses were performed with Systat 9.0 software (SPSS Inc). All grouped data are expressed as mean±SD. Grouped data comparisons were made by 1-way ANOVA. Intergroup comparisons were analyzed by Bonferroni post hoc testing, with statistical significance taken to be P≤0.05.

Results

Average body, lung, and ventricular weights are presented in Table 1. There were no statistically significant differences between the sham-operated groups receiving either vehicle or drug among the parameters listed, nor were there differences between these 2 groups in other measured parameters (ie, myocyte size, pressure-volume relationships, contractility, and mass-to-volume ratio). Therefore, data from the sham-treated group are not presented elsewhere in the text, and the untreated sham-operated group is subsequently referred to as control.

No significant differences in body weight were observed between the control and fistula groups. Lung weights in both treated and untreated fistula groups were significantly elevated above control values (38% and 70%, respectively), but were also significantly different from each other. LV weights also displayed significant differences among all 3 groups, with treated and untreated fistula groups being 67% and 98% greater than controls, respectively. RV weights of treated and untreated fistula animals were not significantly different from each other but were increased significantly above controls, by 126% and 163% above controls, respectively. LV myocyte size showed significant differences among the groups, with both treated and untreated fistula groups displaying myocyte hypertrophy consisting of significant increases in length (178.7±8.4 and 188.9±21.4 μm, respectively; P=0.006) and modest increases in width (73.9±10.2 and 75.9±8.3 μm, respectively) compared with control values for length and width of 151.9±25.2 and 64.5±14.2 μm, respectively.

The extent of LV dilatation can be determined by close examination of the end-diastolic pressure—end-diastolic volume (EDP-EDV) relationship. The average LV EDP-EDV curves for each group and the average LV EDP-EDV curves normalized for the LV volume that produced an LVEDP of 0 mm Hg (V₀) are shown in Figure 1, A and B, respectively. As can be seen, there are 2 components that contribute to the marked ventricular dilatation seen after fistula. The first is related to a structural remodeling of the ventricle, producing a larger ventricular chamber. The contribution this enlargement of the ventricular chamber makes to dilatation can be assessed by the change in the volume of the unstressed ventricle (ie, V₀). Changes in this component of ventricular dilatation produce a parallel shift to the right in the EDP-EDV relation, provided that there is no change in myocardial compliance. Thus, the marked increase in V₀ in the untreated fistula hearts (519±148 μL), which was significantly greater than the corresponding V₀ of either the control or treated fistula hearts (269±60 and 313±80 μL, respectively, P=0.001), indicates significant structural remodeling. This component of myocardial remodeling was prevented by MMP inhibition, because there was no difference in V₀ between control and treated fistula hearts (P=0.80). The second element contributing to LV dilatation derives from changes in the material properties of the myocardium. The contribution of this functional (ie, dependent on alteration of in vivo LVEDP) component to LV dilatation is best illus-
trated in Figure 1B, in which the EDP-EDV relationship has been normalized to remove the dilatation contributed by the structural component. The volume required to increase EDP from 0 to 25 mm Hg ($\Delta V_{25}$) was determined so as to assess the change in LV compliance. As can be seen from Table 2 and Figure 1B, myocardial compliance was increased in both groups after fistula, although this increase was attenuated by MMP inhibition.

The slope of the peak isovolumetric pressure–EDV ($P_{\text{max}} - V$) relationship presented in Figure 2 has previously been demonstrated to be an accurate index of LV contractility and was calculated as previously described. The relationship between peak isovolumetric pressure and EDV was highly linear, as evidenced by correlation coefficients that were >0.9 in all groups. The slopes of the $P_{\text{max}} - V$ relationships of the treated and untreated fistula groups were both decreased below that of the control group (0.386 ± 0.200 and 0.190 ± 0.062 versus 0.937 ± 0.410, respectively; $P$ < 0.001), indicating a decrease in contractility in both fistula groups. Because MMP inhibition produced significant reductions in hypertrophy and dilatation after fistula, the LV mass/EDV (M/V) ratio presented in Figure 3 was calculated as a measure of ventricular compensation in response to chronic volume overload.

### Table 2. Values for $V_0$ and $\Delta V_{25}$

<table>
<thead>
<tr>
<th>Group</th>
<th>$V_0$ (μl)</th>
<th>$\Delta V_{25}$ (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>269 ± 60</td>
<td>98 ± 36</td>
</tr>
<tr>
<td>Treated fistula</td>
<td>313 ± 80</td>
<td>247 ± 128*</td>
</tr>
<tr>
<td>Untreated fistula</td>
<td>519 ± 148*</td>
<td>330 ± 89*</td>
</tr>
</tbody>
</table>

Values are mean ± SD. $V_0$ indicates volume required to reach an EDP of 0 mm Hg; $\Delta V_{25}$, volume required to change EDP from 0 to 25 mm Hg.

* $P$ < 0.05 vs control.

### Discussion

Although alterations in LV size induced by chronic volume overload may represent an important compensatory mechanism for maintaining cardiac function, it is crucial that continued “remodeling” of the heart be prevented. This is
evidenced by the findings of Lee et al., indicating that marked LV dilatation in patients with heart failure was associated with increased mortality. Although several recent studies have evaluated the effects of MMP inhibition in cardiovascular disease, the studies to date have had variable outcomes with regard to the ability of MMP inhibition to prevent adverse remodeling. Furthermore, nothing is known with regard to the efficacy of MMP inhibition in preventing myocardial remodeling in normal hearts subjected to a chronic volume overload.

Accordingly, we sought to determine whether MMP inhibition would be efficacious in preventing myocardial remodeling in the rat infrarenal AV fistula model of chronic volume overload. It has been established that this model produces biventricular volume overload in which heart rate and blood pressure are relatively normal, and we have previously characterized the progressive myocardial remodeling in this model of heart failure. In addition to consistently developing CHF, hearts subjected to this biventricular volume overload invariably develop extensive myocardial hypertrophy and marked ventricular dilatation with no obvious myocardial necrosis and minimal fibrosis. LV dilatation accompanied by a significant increase in LV compliance is seen consistently by 8 weeks after fistula, and it is this progressive dilatation that leads to the eventual decompensation of the ventricle. We have previously shown that a rapid activation of MMP-2 after a fistula has been created is associated with subsequent degradation of the fibrillar collagen matrix in the heart. Significant increases in MMP activity also accompany the marked ventricular dilatation associated with the development of CHF.

In this study, treatment with the MMP inhibitor PD 166793 largely prevented the adverse remodeling characteristically seen in the AV fistula model. LV dilatation was significantly diminished, although the increase in LV compliance was only slightly attenuated. The slope of the Pmax-V relationship is an index of ventricular contractility, and although this value was significantly lower in both groups after fistula relative to control, contractility in the treated fistula group was better than or comparable to this assessment of systolic function previously reported in compensated fistula hearts.

Conversely, the decrease in the slope of the Pmax-V relationship in the untreated fistula group was much closer to values for hearts from rats with CHF. These results indicate that treated fistula hearts remain compensated and are better able to maintain contractility, thereby ejecting more blood per cardiac cycle. Thus, although the contractile dysfunction secondary to AV fistula was not completely prevented by MMP inhibition, ventricular function was maintained.

Similarly, although myocardial hypertrophy was not prevented, the increase in LV weight after fistula was significantly reduced by MMP inhibition. Superficially, this seems at odds with a physiological impetus to normalize the forces responsible for inducing adverse myocardial remodeling. The implication of these differences in LV hypertrophy and dilatation among the groups, however, is better understood by examining the M/V ratio or the ratio of wall thickness to radius (h/R ratio). A report by Gaasch found that hearts of patients with chronic compensated LV volume overload maintain a normal LV h/R ratio (ie, developing a corresponding increase in LV hypertrophy comparable to ventricular dilatation). Grossman et al., however, found that end-diastolic wall stress was consistently and significantly increased in patients with chronic volume overload even when a normal h/R ratio was maintained. Thus, although there might be sufficient hypertrophy to maintain the M/V ratio, the increased end-diastolic wall stress induced by volume overload would not be normalized. The pertinence of this can be appreciated from the findings in Figure 3, in that the M/V ratio for the untreated fistula group was similar to or below control, whereas the LV M/V ratio in the treated group was significantly greater than normal, reflecting a relative increase in LV mass without a corresponding increase in chamber volume (ie, dilatation). Considering this in the context of Grossman’s finding that end-diastolic wall stress is increased in volume overload, it appears that by preventing the marked ventricular dilatation, MMP inhibition allows for development of a sufficient increase in mass to normalize diastolic wall stress and compensate for the additional hemodynamic workload imposed on the heart by the AV fistula.
increased by 40% above controls in overt heart failure, even though ventricular stiffness was significantly decreased.5 This is consistent with the hypothesis that prevention of adverse myocardial remodeling by MMP inhibition is beneficial in preventing the development of CHF.

In a study by Peterson et al., treatment of spontaneously hypertensive heart failure (SHHF) rats with PD 166793 prevented cardiac dilatation, preserved contractility, and reduced myocardial fibrosis compared with untreated SHHF controls. This suggested that the beneficial effects of MMP inhibition are mediated by limiting cardiac remodeling, thereby slowing the progression to heart failure. In contrast, Rhode et al.10 reported that MMP inhibition with CP-471,474 produced a variable attenuation of early ventricular dilatation in mice with experimental myocardial infarction. One factor that may be responsible for the conflicting results in these studies is that different levels of myocardial damage and dysfunction inherent to the animal models used. Taken together, these studies suggest that MMP inhibition has great potential to limit adverse myocardial remodeling. Even so, a more thorough understanding of the underlying mechanisms of these drugs modulate is clearly needed.

Further investigation is also needed to ascertain the effects of MMP inhibition on collagen structure, function, and cross-linking and how these factors contribute to the maintenance of ventricular function. Similar MMP inhibition studies by Spinale et al.15,30 found that concomitant treatment with PD 166793 in pigs undergoing rapid pacing attenuated the degree of LV dilatation, but was associated with a qualitative increase in interstitial collagen and an abnormal increase in myocardial stiffness. They concluded that the increase in ventricular stiffness was due to a greater amount of fibrillar collagen in the hearts of treated animals, suggesting that MMP inhibition might also have negative effects by inhibiting normal collagen turnover. Peterson et al.19 however, showed a reduction of myocardial fibrosis in SHHF rats treated with the same MMP inhibitor. In both of the aforementioned studies, collagen volume fraction (CVF) was used to measure fibrillar collagen. Although CVF is often used as an index of collagen structure, in our experience the composition of the extracellular matrix is dynamic and CVF does not consistently correlate with ventricular stiffness. This is highlighted by a recent study in which myocardial CVF was increased by 40% above controls in overt heart failure, even though ventricular stiffness was significantly decreased.5 Similarly, a recent study by Norton et al.31 found that increased myocardial stiffness was not associated with changes in CVF in rats with streptozotocin-induced diabetes mellitus.

In summary, MMP inhibition with PD 166793 prevents ventricular dilatation and attenuates the hypertrophy typically induced by chronic volume overload. Prevention of this adverse ventricular remodeling by MMP inhibition also preserved ventricular function compared with the untreated fistula group. The efficacy of MMP inhibition in slowing the progression to heart failure in this and other studies exemplifies the significant role of MMPs in the remodeling of diseased hearts and stresses the need to inhibit their expression, activation, and/or activity.

Acknowledgments
This work was supported in part by NIH grants HL-62228 and HL-59981 and a research grant from Pfizer Pharmaceuticals. The authors would like to thank James A. Stewart, Jr, for his technical assistance.

References

Increased myocyte length is another factor that is thought to correlate with ventricular dilatation and dysfunction in heart failure.29,32 Accordingly, myocyte size was measured to assess possible differences in remodeling at the cellular level between the treated and untreated fistula groups. Although both treated and untreated fistula hearts had similar increases in myocyte length relative to controls, the increases in length were proportional to the extent of ventricular hypertrophy and increased compliance. In addition, these subtle differences in myocyte size could account for the observed changes in LV weight when the total number of myocytes in the heart is taken into account. One limitation of these studies is that myocyte length was obtained from unstressed hearts and may not precisely reflect in vivo lengths. Accordingly, additional studies examining the relationship between changes at the cellular level and ventricular remodeling are needed.


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_Circulation_. 2002;105:1983-1988; originally published online April 1, 2002;
doi: 10.1161/01.CIR.0000014686.73212.DA
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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