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Matrix Metalloproteinases in the Progression of Heart Failure

Potential Therapeutic Implications

Yun You Li and *Arthur M. Feldman*

Cardiovascular Institute, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

Abstract

Matrix metalloproteinases (MMPs) are a family of functionally related zinc-containing enzymes that denature and degrade fibrillar collagens and other components of the extracellular matrix. Myocardial extracellular matrix remodelling and fibrosis regulated by MMPs are believed to be important contributors to the progression of heart failure. The role of MMPs in cardiac fibrosis and the progression of heart failure, along with the possibility of halting the progression of heart failure by modulating extracellular matrix remodelling are important issues under intense study.

MMPs are increased in the failing hearts of both animal models and patients with heart failure. MMP inhibition may therefore modulate extracellular matrix remodelling and the progression of heart failure. It is a great advantage that various MMP inhibitors have been developed initially for the treatment of cancer, arthritis and other diseases believed to be associated with increased MMP activity. Several preclinical studies have shown that treatment of heart failure in animal models with MMP inhibitors results in less collagen matrix damage, favourable extracellular matrix remodelling, and improved cardiac structure and function. The results suggest that modulation of MMP activity can prevent myocardial dysfunction and the progression of heart failure through alterations in the remodelling process of extracellular matrix and the left ventricle.

Although these promising results suggest potential benefits of MMP inhibition for human heart failure, no clinical data evaluating MMP inhibitors in heart failure have been reported. As the preclinical evidence continues to grow and the potential of MMP inhibition for the treatment of heart failure continues to unfold, MMP inhibition may prove to be an effective treatment for heart failure.

Each year nearly half a million patients are diagnosed with heart failure in the US. Left ventricular (LV) remodelling and dilation contribute to the progression of chronic heart failure and have been associated with increased morbidity and mortality.[1] Myocardial extracellular matrix (ECM) remodelling accompanied by LV remodelling may play a permissive role in LV dilation. Changes in myocardial ECM structure and properties, such as collagen type shifting,[2,3] collagen cross-linking,[4,5] and collagen denaturation,[6,7] are important features of ECM remodelling and one of the pathognomonic findings in the failing heart.

Matrix metalloproteinases (MMPs) in the myo-

cardium are capable of degrading all the ECM components including fibrillar collagens. It has recently been shown that MMPs are increased in the failing heart of both animal models and humans.[6,8-10] However, despite increased activities of MMPs, the failing heart usually has increased collagen content which may be ascribed to matrix fragments (matrikines)-induced collagen expression. Therefore, modulation of MMP activity may alter myocardial ECM remodelling processes, cardiac dilation and eventually myocardial function. The activity of MMPs can be regulated through gene expression, proenzyme activation,[11] endogenous physiological inhibitors such as tissue inhibitors of metalloproteinases (TIMP) and α_2-macroglobulin,[12] and by pharmacological agents such as batimastat (BB-94), PD-166793 and CP-471474. In this review, we discuss new insights into the role of MMPs in the progression of heart failure and therapeutic implications of new pharmacological agents which modulate MMP activity.

1. Matrix Metalloproteinases (MMPs) and Extracellular Matrix (ECM) in the Myocardium

MMPs are a family of functionally related zinc containing enzymes that cleave ECM components resulting in fibrillar collagen denaturation and degradation, and the synthesis of new fibrous tissue.[10,13-20] MMPs are secreted by fibroblasts, smooth muscle cells, endothelial cells and adult mammalian myocytes[21] as a proenzyme, or zymogen, and are activated by proteolytic cleavage to yield the active MMPs. In the rat myocardium, indirect immunofluorescence localised proMMPs/MMPs to the endothelium and subendothelial space of the endocardium and throughout the interstitial space found between groups of muscle fibres.[22]

The various MMPs are differentiated by their structure and specificity for substrates with the collagenase (MMP-1) degrading fibrillar collagens, the stromelysin (MMP-3) degrading proteoglycans and glycoproteins, and the gelatinases (MMP-2 and MMP-9) degrading denatured collagens and basement membrane collagens. Currently there are more than 20 MMPs in this family and there are undoubtedly still more to be identified in the future.

Common molecular structural features of MMPs have been identified and used for structure-based inhibitor design.[23] The conserved isostructural sequence (HEXXHXXGXXH) creates a common locus for active site zinc atom and an exceptionally large and deep S1′ pocket as part of the extended binding site for collagen-like substrates.[24,25]

The activity of MMPs can be regulated at multiple levels. The expression of MMPs can be regulated at levels including transcription, translation and post-translational modification by a number of chemical agents, neurohormones and cytokines.[14,18,20,21,26,27] After secretion, the proMMPs bind to various ECM components, which may serve as a means of extracellular storage for rapid activation and mobilisation upon stimulation. The activation processing of proMMPs by proteases, organomercurials and plasmin plays an important role in regulating their activity. In most cases, the activation of proMMPs involves the 'cysteine-switch' mechanism.[28] The final control of MMP activity is by physiological inhibitors, the TIMPs and α_2-macroglobulin. TIMPs not only inhibit MMPs directly, but also form complexes with proMMPs to control MMP activation and stability.[29,30] However, this tight regulation seems to get lost during the development of several diseases including cancer,[31,32] chronic liver disease,[33] arthritis,[34] atherosclerosis[35] and heart failure.[9,36-38]

MMPs not only play a role in the degradation of matrix components but may also modulate collagen synthesis, which may be accomplished by regulating the formation of matrikines,[19,39] such as Glycyl-Histidyl-Lysine, and releasing biologically active factors from the ECM (including transforming growth factor-β_1, insulin-like growth factor and fibroblast growth factor).[40] The end result is often an increase in MMPs accompanied by increased fibrosis, such as that seen in the failing heart, and decreased MMP activity accompanied by reduced fibrosis.[6,41-43]

The ECM is a fibrillar network composed of collagens, basement membrane, fibronectin, proteoglycans, laminin, MMPs and growth factors. The most important components of the ECM are fibrillar collagens which are also the major components of fibrous tissue. Type I and III are the major collagens in the myocardium with type I being predominant in the adult heart.[44] The collagen matrix provides the support essential for maintaining alignment of myofibrils within the myocyte as well as for maintaining myocyte alignment within the myocardium.[45,46] Under normal conditions the ECM is an important determinant of cardiac mechanics: (i) it maintains the structural alignment of myocytes and prevents myocyte slippage during contraction; (ii) during systole it transduces force that facilitates re-lengthening; and (iii) it is the main determinant of diastolic stiffness.[7]

That remodelling of the ECM is of critical importance to cardiac remodelling and to the development of heart failure is supported by a large body of evidence in both animal models and patients with heart failure. Individual myocyte function is preserved during aging; however, myocyte loss and replacement by ECM contributes substantially to ventricular dilation, diminished LV function and reduced ejection fraction.[47,48] The direct digestion of collagens is an essential part of matrix remodelling. MMP-1 initiates the digestion of collagens by hydrolysing the peptide bond following a Gly residue located at a distance of three-fourths of the collagen molecule length from the amino terminus.[15] The resulting ¾ and ¼ fragments are completely degraded by MMP-2 and MMP-9 in addition to MMP-1 and MMP-3.

Using scanning electron microscopy, Rossi et al.[49] demonstrated that the myocardial collagen matrix is a 3-dimensional structure that supports individual myocytes. Breaks in the overall structure of the ECM would therefore result in a loss of continuity and integrity of this 3-dimensional support and in so doing alter myocardial function.[50]

2. MMPs and ECM in Heart Failure

2.1. Ventricular Remodelling in the Progression of Heart Failure

The myocardium of most animal models of heart failure as well as patients with heart failure commonly demonstrates increased content and activity of MMPs.[9,10,51] The activated MMPs have been suggested to contribute to ECM remodelling, progressive LV remodelling and dilation, and the progression of heart failure.[6,50-55] In ischaemic cardiomyopathy, latent myocardial MMPs are activated which in the absence of inhibitors degrade ECM and lead to ventricular dilation and dysfunction.[56] In coronary artery ligation-induced myocardial infarction, collagen degradation exceeds synthesis during the early phase of repair at the infarct site which coincides with increases in MMP gelatinolytic activity.[57] Although various mechanisms may account for the regulation of MMP activity, one potential mechanism for the increased myocardial MMP activity in the failing heart is a loss of endogenous inhibition by TIMPs or other physiological inhibitors.[9]

In the rapid pacing-induced pig heart failure model, MMP-1, MMP-2 and MMP-3 are increased after 7 days of rapid pacing.[36] MMP-1 appears to increase in a time-dependent manner, increasing by 150% after 7 days and 360% after 21 days. MMP-2 and MMP-3 levels increase by 200% after 7 days of rapid pacing and plateau with longer durations of rapid pacing. Consistent with these findings, the gelatinolytic activity of MMPs also increases after 7 days and remains increased at 14 and 21 days of rapid pacing.[36] This change in MMPs was assumed to contribute to the decreased myocardial collagen confluence and content, which may occur as early as within 24 hours of pacing,[58] in animals with rapid pacing-induced heart failure. The change in myocardial collagen is similar to that found in acute myocardial ischaemia,[59] but differs from that seen in other forms of heart failure,[3,47,60,61] in which an increase in MMP activity is associated with normal or increased collagen content. Thus, the regulation of collagen expression

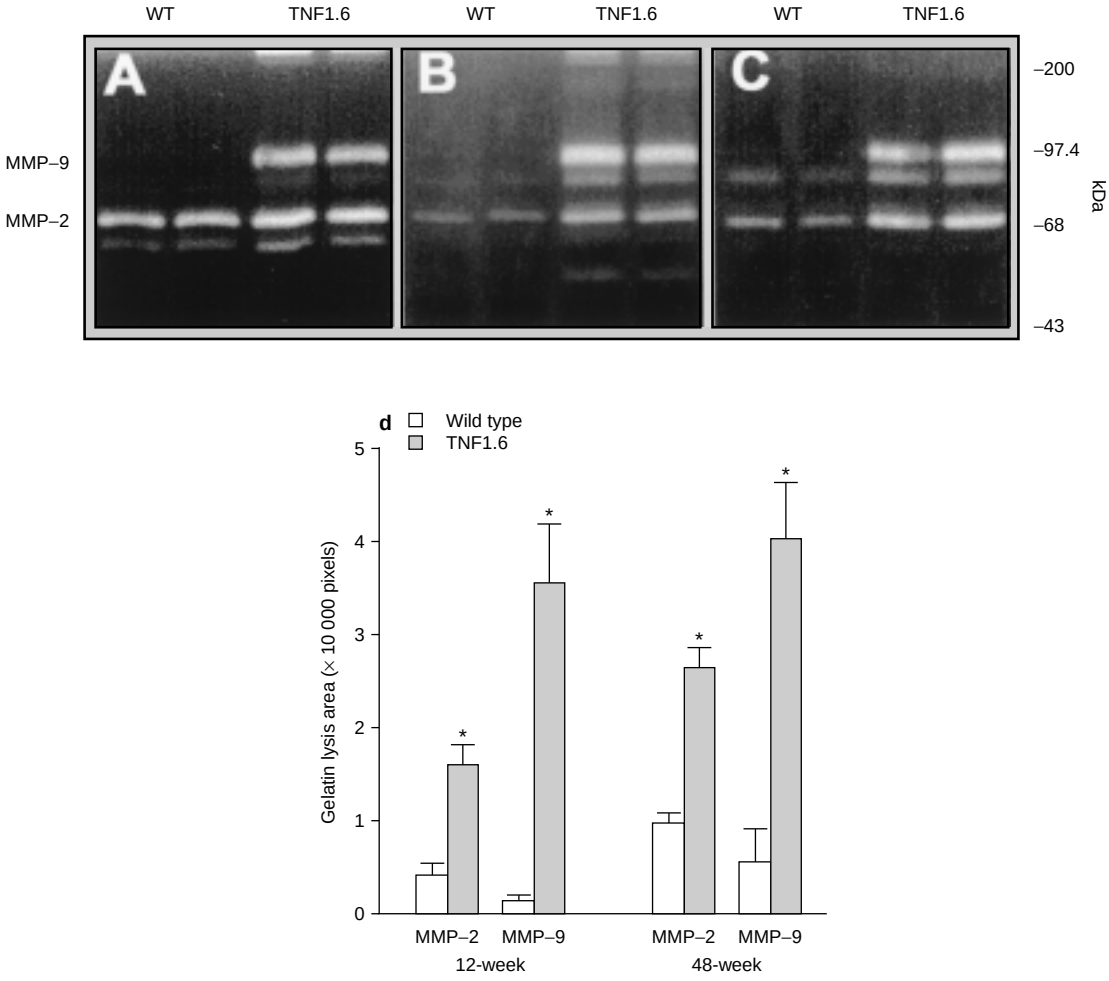

Fig. 1. Increased matrix metalloproteinase (MMP)-2 and MMP-9 in the TNF1.6 mouse heart. Gelatin zymography of ventricular extracts of (**A**) neonates, (**B**) 12-week-old and (**C**) 48-week-old wild-type (WT) and TNF1.6 mice; (**d**) summary of the quantitative data of MMP-2 and MMP-9 in 12- and 48-week-old mouse heart (n = 4 each). * $p < 0.01$ compared with respective wild-type. After Li et al.,[6] with permission. **TNF1.6** = transgenic mice overexpressing tumour necrosis factor-α.

is highly complex and may involve multiple enzymes, matrix proteins and regulatory pathways.

In mice with heart failure induced by cardiac-specific overexpression of tumour necrosis factor-α (TNFα; TNF1.6 mice),[6,62] progressive ventricular hypertrophy and dilation are accompanied by a significant increase in MMP-2 and MMP-9, an increase in collagen synthesis, deposition and denaturation, and a decrease in undenatured soluble collagens (figs 1, 2).[6] The increase in MMP-2 and MMP-9 could be demonstrated from the initial postnatal period (fig. 1). In young TNF1.6 mice, the changes in the ECM are associated with marked diastolic dysfunction as demonstrated by significantly reduced transmitral Doppler echocardiographic E/A wave ratio.[6]

During LV remodelling after myocardial infarction in rats, MMP up-regulation evolves over time.

MMP-2, MMP-9 and MMP-13 were significantly elevated during the first week post-infarction and MMP-14 was elevated at approximately 16 weeks post-infarction.[38] In spontaneously hypertensive rats (SHR), MMP-2 and MMP-9 gelatinolytic activity was increased in both 9-month-old rats at the stage of compensatory hypertrophy and 13-month-old rats at the stage of decompensated failure.[63] There was an age-related progressive increase in the active forms of MMP-2 and MMP-9 compared with age-matched controls. Similarly, MMP-13 protein levels were also increased in the failing heart. Concomitant changes in collagen synthesis and deposition were also observed in this heart failure model. Both collagen transcripts (type I and III) and collagen volume fraction were elevated in 9- and 13-month-old rats.[63]

The potential importance of MMPs in ECM remodelling and in the progression of heart failure has been further demonstrated by transgenic myocardial overexpression of MMP-1 in mice.[41,64] Myocardial overexpression of MMP-1 produces LV hypertrophy and hypercontractility in young mice, and ventricular dilation and failure in old mice. The 6-month-old MMP-1 transgenic mice had increased cardiac type III collagen concentration in the myocardium. Prolonged overexpression of MMP-1 produced loss of cardiac collagens coincident with deterioration of systolic and diastolic function at 12-months of age.[41] By contrast, targeted deletion of the MMP-9 gene in mice has been shown to decrease collagen accumulation in the myocardium and attenuate LV enlargement after experimental myocardial infarction.[65] However, knockout of MMP-3, MMP-9 or MMP-12 genes had no effect on collagen accumulation in the infarct area,[42] suggesting an essential role of MMPs in collagen deposition and a different mechanism in collagen deposition in the infarct area from areas remote to the infarction.

Cardiac fibrosis is defined not only as an increase in the concentration of matrix collagens in the interstitium, but also changes in collagen type,[2,3] organisation,[6,66] cross-linking[4,5] and denaturation.[6,66] Myocardial ECM is not a pas-

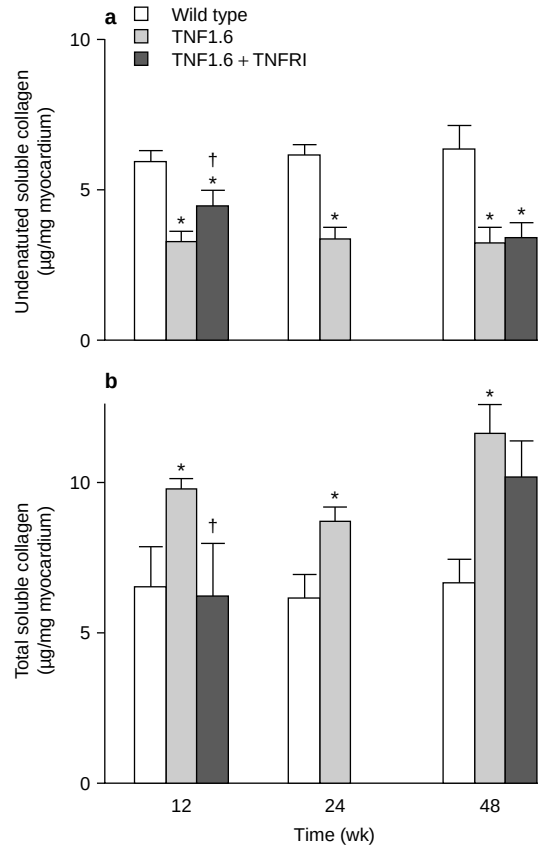

Fig. 2. Changes in collagens in the TNF1.6 mouse heart. Collagens excessively exposed to active metalloproteinases (MMPs) may denature and lose their normal functions. After solubilisation, undenatured soluble collagens were measured with the Sircol collagen assay based on their property of quantitatively binding picrosirius red, and the denatured collagens and newly synthesised collagens not quantitatively binding picrosirius red. Total soluble collagens were measured with hydroxyproline assay. (**a**) Reduction in undenatured soluble collagen in TNF1.6 mice was partially prevented by AdTNFRI treatment; (**b**) Total soluble collagen content was increased in TNF1.6 mouse heart, and the increase was prevented by AdTNFRI treatment in 12-week-old TNF1.6 mice (n = 8 in each group). * $p < 0.05$ compared to wild-type; † $p < 0.05$ compared to TNF1.6. After Li et al.,[6] with permission. **AdTNFRI** = an adenoviral vector allowing systemic overexpression of a similar human tumour necrosis factor receptor-α type I-murine IgG fusion protein; **Ig** = immunoglobulin; **TNF1.6** = transgenic mice overexpressing tumour necrosis factor-α.

sive structural support but a metabolically reactive entity.[67] Various changes in the composition of collagen types and cross-linking during the development of cardiac fibrosis in different animal models as well as in patients with heart failure have been reported.[3,68-70] In the ischaemic failing human heart, multiple foci of reactive fibrosis account for more than two thirds of the fibrous tissue, whereas the infarct scar constitutes only one third.[71] In fibrotic myocardium, the normal collagens are denatured and degraded by increased MMPs, and the collagen chains are fractured and replaced by poorly structured bands and sheets of collagen or more soluble matrix components, which leads to ventricular dilation.[48,51] Because myocardial compliance ($\Delta V/\Delta P$) is directly affected by the concentration as well as the ratio of different types of collagens in the heart,[3,72] these changes may lead to alterations in the mechanical properties of the tissue. Therefore, active MMPs play an essential role in cardiac ECM remodelling, cardiac dilation and the progression of heart failure.

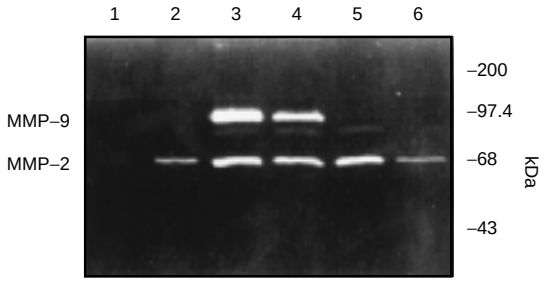

Fig. 3. Modulation of metalloproteinases (MMPs) in the TNF1.6 mouse heart by anti-TNFα gene therapy. Gelatin zymography demonstrates that AdTNFRI treatment abolished MMP-9 after 2 weeks (lane 5), and reduced MMP-2 to wild-type level after 6 weeks (lane 6). Controls are negative without known MMPs (lane 1), FVB wild-type (lane 2), TNF1.6 (lane 3), and TNF1.6 treated with AdLacZ control vector (lane 4). After Li et al.,[6] with permission. **AdTNFRI** = an adenoviral vector allowing systemic overexpression of a similar human TNFα receptor type I-murine IgG fusion protein; **FVB** = an inbred mouse strain commonly used for transgenic studies; **TNF**α = tumour necrosis factor-α; **TNF1.6** = transgenic mice overexpressing TNF-α.

2.2. Rationale for MMP Inhibition in the Treatment of Heart Failure

Currently, there is increasing interest in attenuating maladaptive cardiac remodelling and dilation through the modulation of ECM remodelling. Modulation of the renin-angiotensin-aldosterone system by ACE inhibitors, angiotensin II receptor antagonists or aldosterone antagonists, alters the progression of myocardial fibrosis, although the mechanisms involved are poorly defined.[73,74] As discussed in section 2.1, MMP-mediated myocardial ECM remodelling contributes to the dilation and dysfunction of the failing heart, and so MMPs may also be regarded as viable targets in heart failure therapy.[48,51,75] Because the constant remodelling of the ECM is regulated by MMPs,[67] which in turn are regulated by growth factors, cytokines, activators and inhibitors,[76,77] modulation of either of these regulators may change the course of ECM remodelling and therefore the outcome of heart failure.

We have shown previously that LV assist device support reduces MMP levels and brings about favourable changes in collagens in the failing human heart,[43] which may be mediated by alterations in TNFα expression.[78] Therefore, modulation of the myocardial remodelling process and ultimately myocardial function might be achieved by changing the activity of MMPs either through anti-cytokine treatment or direct MMP inhibition.

Indeed, anti-TNFα treatment with etanercept (p75 TNF receptor Fc fusion protein) has been reported to cause regression of LV remodelling and dilation in humans with heart failure.[79] To examine the mechanisms of the beneficial effects of anti-TNFα therapy, we studied the expression of MMPs, extent of collagen deposition and denaturation, and cardiac function after inoculation with an adenovirus allowing systemic overexpression of a similar human TNF receptor type I-murine immunoglobulin (Ig)G fusion protein (AdTNFRI) in the TNF1.6 heart failure model. Not surprisingly, we have demonstrated that anti-TNFα therapy with AdTNFRI reduced both MMP-2 and MMP-9 (fig. 3), prevented further collagen synthesis, deposition

and denaturation (fig. 2), and preserved myocardial diastolic function in young TNF1.6 mice. The results suggest a critical role of TNFα and MMPs in myocardial matrix remodelling and functional regulation, and support the hypothesis that both TNFα and MMPs may serve as potential therapeutic targets at an appropriate time point in the treatment of heart failure.[6] However, it is important to point out that these salutary effects were not seen in older animals with well-established disease.

Because MMPs are significantly reduced after anti-TNFα therapy (fig. 3),[6] we hypothesised that these beneficial effects might come from reduced levels of MMPs. Indeed, we have demonstrated in the same heart failure model that MMP inhibition using batimastat (BB-94) recapitulated the molecular, histochemical and functional changes in Ad-TNFRI treated TNF1.6 mice.[80] In addition, direct modulation of physiological regulatory system has also been shown to change collagen deposition in the heart. Either uPA knockout or adenoviral mediated TIMP-1 or plasminogen activator inhibitor (PAI) overexpression reduces collagen deposition in infarcted heart.[42]

The above results suggest that MMP activation plays a key role in modulating myocardial function through the regulation of ECM remodelling. Thus, the MMPs represent an attractive therapeutic target for heart failure.

3. Preclinical Studies

MMP inhibition has been studied for the treatment of cancer,[31,32] arthritis,[34] chronic liver diseases,[33] cardiovascular diseases including abdominal aortic aneurysm,[81,82] coronary artery vein graft failure[83] and atherosclerosis.[83-87] There are many potential ways to inhibit MMPs,[12] including suppressing MMP gene expression, blocking the MMP activation pathways, inhibiting MMPs by enhancing TIMP gene expression or by administering synthetic MMP inhibitors (MMPIs).

Although etanercept anti-TNFα therapy in heart failure is aimed at TNFα, it may act through the down-regulation of MMP expression. This strategy may eventually prove to be a viable alternative for heart failure treatment. Currently, there are no data on the modulation of the activation pathways of MMPs *in vivo*.

Enhancing TIMP gene expression may be achievable in the future when gene therapy techniques mature.[83] However, administration of TIMPs has not proven suitable for pharmacological applications, although TIMP-1 has been shown to completely protect mice against cardiac rupture after experimental myocardial infarction.[42]

A series of low molecular weight MMPIs (table I), with varying efficacy and specificity of MMP inhibition, have been developed and evaluated in animal models as well as in humans for the treatment of cancer or other conditions such as abdominal aortic aneurysm with promising results.[32,88-90]

Chemically modified tetracyclines were the first to obtain approval for clinical use in anti-MMP therapy of periodontal diseases.[91]

Recently, several of the synthetic MMPIs have been evaluated in the treatment of experimental heart failure. The initial results in modulating ECM remodelling are promising. MMPIs improve cardiac pump function and block the progression of heart failure in different models of cardiac dysfunction.[55,63,80]

Table I. Matrix metalloproteinase (MMP) 50% inhibitory concentrations (IC_{50}) as nmol/L of MMP inhibitors

	Batimastat	Marimastat	CP-471474	PD-166793	Prinomastat	Tanomastat	Doxycycline
MMP-1	3	5	1170	6100	8.3	>5000	15000
MMP-2	4	6	0.7	47	0.05	11	
MMP-3	20	230	16	12	0.03	134	
MMP-7	6	16	-	8100	54	-	
MMP-9	10	3	13	9900	0.26	301	<50000
MMP-13			0.9				

3.1. PD-166739

PD-166739 is a broad-spectrum MMPI but is not active against endothelin- or angiotensin converting enzymes, neutral endopeptidase or TNFα convertase.[55] It appears to be most potent in inhibiting MMP-2 and MMP-3 (table I).

Administration of PD-166739 during the development of rapid pacing heart failure resulted in increased and thicker collagen weaves.[55] The treatment also increased endocardial shortening and LV myocardial stiffness, reduced end-diastolic dimension, LV wall stress and myocyte length. The reduced LV dilation and increased myocardial stiffness observed with concomitant MMP inhibition during rapid pacing were assumed to be the result of increased collagen content and improved ECM support.[55,92] This study confirms the concept that the use of MMPI during the development of heart failure is beneficial to the preservation of ECM integrity and cardiac function.

PD-166739 has also been evaluated in SHR.[63] In this model, PD-166739 treatment of 9-month-old rats for 4 months reduced myocardial MMP-2, MMP-9 and MMP-13. MMP-2 mRNA expression was also reduced by PD-166739. In addition, PD-166739 reduced LV dilation and preserved systolic function. In contrast to pacing-induced heart failure, these beneficial effects of MMPI were associated with reduced collagen volume fraction, although PD-166739 had no effect on collagen mRNA levels.[63]

It is well established that inhibition of ACE is effective in preventing fibrosis and improving survival of patients with heart failure.[93,94] The beneficial effects of ACE inhibition and MMP inhibition have been compared in both the rapid pacing heart failure and SHR models.[63,92] Both ACE inhibition and MMP inhibition blocked ventricular dilation and improved cardiac function in both models of heart failure. Both ACE inhibition and MMP inhibition reduced myocardial fibrosis in SHR heart. However, in rapid pacing heart failure, concomitant administration of ACE inhibitor did not prevent collagen damage, whereas PD-166739 did.[92] The discrepancies in collagen matrix changes in these two heart failure models may be due to different pathophysiology during the development of heart failure.

3.2. CP-471474

CP-471474 selectively inhibits MMP-2 and MMP-13, but less potently inhibits other MMPs (table I). Administration of CP-471474 attenuated early LV dilation after experimental myocardial infarction in mice.[95] The treatment also prevented worsening of fractional shortening in the mice. Furthermore, the effects of CP-471474 on end-systolic and end-diastolic areas are most prominent in animals that had greater initial LV dilation.

However, because of the dissimilarity of acute ischaemia- or pacing-induced heart failure in terms of alterations in ECM to the clinical forms of heart failure of other aetiology, such as chronic ischaemia and hypertension, the results derived from those models may not be generalised without extensive testing of MMPIs in other animal models of heart failure that mimic heart failure in humans.

3.3. PNU-171829

PNU-171829, a broad-spectrum MMPI, was tested in rats with aorto-caval fistula induced heart failure.[96] Treatment with PNU-171829 for 4 weeks increased ejection fraction and reduced cardiac hypertrophy (heart weight/body weight ratio). However, the treatment had no effect on end-systolic and end-diastolic volume.

3.4. Batimastat and Marimastat

Batimastat (BB-94) was the first synthetic MMP inhibitor with a collagen-mimicking hydroxamate structure that worked by competitive, potent but reversible inhibition, and was the first MMPI in its class evaluated to treat human diseases.[97-99] Batimastat inhibits MMPs with 50% inhibitory concentration (IC_{50}) values in the low nanomolar range and has broad specificity for members of the MMP family but little activity against ACE or enkephalinase (table I).[100]

We have evaluated the effect of batimastat on the development of heart failure in TNF1.6 mice.[62,80] Four- and 40-week-old TNF1.6 mice were injected intraperitoneally with batimastat or the vehicle 3 times a week for 8 weeks. The treatment significantly reduced myocardial expression of type I and type III collagens and total collagen content, increased the ratio of undenatured collagen to total collagen, prevented myocardial hypertrophy and dilation, and improved diastolic function in 12-week-old TNF1.6 mice. Furthermore, the treatment significantly improved cumulative survival of TNF1.6 mice.[80] The results suggest that MMP inhibition therapy with batimastat significantly ameliorates the heart failure phenotype and improves survival of TNF1.6 mice.

The usefulness of batimastat has been limited by extremely poor water solubility, which requires intraperitoneal administration of a detergent emulsion of the drug. Phase III trials in cancer treatment were initiated, but closed soon afterward because of slow accrual, local tissue reaction (peritonitis) and the development of water soluble marimastat (BB-2516).

Marimastat is similar to batimastat in potency and spectrum in MMP inhibition. In contrast to batimastat, marimastat is orally available and currently in Phase III trials for the treatment of various cancers.[101] However, it has not been tested in the treatment of heart failure. The successful use of marimastat in cancer treatment may imply that it could be the most promising MMPI for clinical testing in heart failure treatment.

3.5. Other MMPIs

Various other MMPIs have been synthesised and many have been tested in the treatment of cancer, arthritis and aortic aneurysm. By defining their efficacy and adverse effects from those studies, one may infer their potential usefulness in future tests in heart failure treatment. The following are several potential candidates.

RS-113456 is a competitive wide spectrum MMP inhibitor that limits flow-mediated arterial enlargement in a dose-dependent fashion.[102,103] Prinomastat (AG-3340) has a molecular structure similar to CP-471474 that selectively inhibits MMP-2, MMP-3, MMP-9 and MMP-14.[90,104] It has a short half-life of 3 hours and musculoskeletal adverse effects such as arthralgia and body aches presumably due to the inhibition of MMP-1.[105,106] Those adverse effects may also present in patients receiving other wide spectrum MMPIs including batimastat. More selective MMPIs that spare MMP-1 may have fewer adverse effects.[106-108] For example, tanomastat (BAY-129566) selectively inhibits MMP-2, MMP-3, and MMP-9. It is orally available, has a long half-life of 5 to 7 days and reportedly showed no signs of musculoskeletal adverse effects in phase 2 clinical trials.[109] Nevertheless, a clinical trial of tanomastat for cancer treatment has been discontinued because of concerns about efficacy.[32] The newly discovered selective MMP-2 inhibitor ABT-770 spares MMP-1. It is orally bioavailable and effective in an *in vivo* model of tumour growth.[110] It may also hold great promse in the treatment of heart failure.

Doxycycline noncompetitively inhibits MMPs with much lower potency compared to other MMPIs. Doxycycline 50 μmol/L completely inhibits the phorbol myristate acetate-mediated induction of MMP-8 and MMP-9.[111] It inhibits not only the activity of MMP-8 and MMP-9 but also the synthesis of MMPs in human endothelial cells and abdominal aortic aneurysm tissues.[112]

4. Potential Therapeutic Implications

As discussed above MMPIs have been used in various experimental animal models of heart failure with promising results.[55,63,80,92] Although the results vary possibly because of the different selectivity in MMP inhibition and use of different animal models, a common finding is the preservation of collagen matrix integrity and myocardial function and, to different degrees, cardiac structure and geometry. In situations in which MMP inhibition did not alter collagen content, the beneficial effect may lie in the improvement of the quality of collagens and the integrity of ECM.[113] The relative abundance of different types of collagens, collagen

cross-linking, alignment, maturation and denaturation all contribute to the strength and integrity of ECM. However, these findings must be evaluated with great care. For example, MMPs are critical in the healing process associated with myocardial infarction, and the strutting afforded by the ECM may require a certain fluidity during cardiac stress.

In general, synthetic MMPIs can be administered at very high doses without significant toxicity or lethality in animal studies. It should be noted although that the specificity of MMPIs may be dose-dependent, at extreme doses synthetic MMPIs will inhibit other proteinases and induce untoward side effects. The mechanism of high dose MMPI-induced tendonitis may be caused by the inhibition of MMP-1 or TNFα convertase, but no evidence is available to address this. Most of the hydroxamate-based MMPIs are nonspecific and may induce muscle and joint pain attributable to tendonitis which can be alleviated by discontinuing the drug for a period of time and re-starting the treatment at reduced dosages.[114,115] More selective MMPIs usually do not have such adverse effects. Thus, the development of more selective MMPIs will be of most significance in the long term treatment of heart failure. We can expect improved inhibitors with increased specificity based on radiographic structures.

One should also consider vascular remodelling in heart failure and during treatment with MMPIs. A recent study has shown that rats treated with either doxycycline or adenoviral vector carrying human TIMP-1 had higher pulmonary artery pressure and a greater level of right ventricular hypertrophy, which were associated with increased muscularisation and periadventitial collagen accumulation in distal arteries.[116] In contrast, oral marimastat induced favourable changes by inhibiting constrictive arterial remodelling in favour of both neutral and expansive remodelling in pigs who had undergone balloon dilation of peripheral arteries.[117]

5. Conclusion

Myocardial ECM remodelling regulated by MMPs is implicated in LV remodelling and the progression of heart failure. The increased levels of MMPs in the failing heart, and their potential role in ECM remodelling and the progression of heart failure make MMP inhibition a reasonable strategy in heart failure treatment. It has been shown that inhibition of MMPs in the failing heart, either directly or through factors that affect MMP activity, alters the ECM remodelling process and slows the progression of heart failure. While there is great potential for MMP inhibition in the treatment of heart failure, there is still much to be learned about the exact role of MMPs in the development of the heart failure phenotype. Because the field is new and emerging, and there is a significant overlap in substrate specificity among MMPs, further study will be needed to test more promising MMPIs, including more selective ones, for the treatment of heart failure.

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Correspondence and offprints: Dr *Yun You Li,* Cardiovascular Institute, University of Pittsburgh School of Medicine, Room 1750, Biomedical Science Tower, 200 Lothrop Street, Pittsburgh, PA 15213, USA.
E-mail: liyuny@pitt.edu