Myocardial matrix degradation and metalloproteinase activation in the failing heart: a potential therapeutic target

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Abstract
A fundamental structural event in the progression of heart failure due to dilated cardiomyopathy is left ventricular (LV) myocardial remodeling. The matrix metalloproteinases (MMPs) are an endogenous family of enzymes which contribute to matrix remodeling in several disease states. The goal of this report is to summarize recent findings regarding the myocardial MMP system and the relation to matrix remodeling in the failing heart. In both experimental and clinical forms of dilated cardiomyopathy (DCM), increased expression of certain species of myocardial MMPs have been demonstrated. Specifically, increased myocardial levels of the gelatinase, MMP-9 has been identified in both ischemic and non-ischemic forms of human DCM. In addition, stromelysin or MMP-3 increased by over four-fold in DCM. The increased levels of MMP-3 in DCM may have particular importance since this MMP degrades a wide range of extracellular proteins and can activate other MMPs. In normal human LV myocardium, the membrane type 1 MMP (MT1-MMP) was detected. These MT-MMPs may provide important sites for local MMP activation within the myocardium. In a pacing model of LV failure, MMP expression and activity increased early and were temporally associated with LV myocardial matrix remodeling. Using a broad-spectrum pharmacological MMP inhibitor in this pacing model, the degree of LV dilation was attenuated and associated with an improvement in LV pump function. Thus, increased LV myocardial MMP expression and activity are contributory factors in the LV remodeling process in cardiomyopathic disease states. Regulation of myocardial MMP expression and activity may be an important therapeutic target for controlling myocardial matrix remodeling in the setting of developing heart failure. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Cardiomyopathy; Extracellular matrix; Remodeling; Heart failure

1. Introduction
A central component in the development and progression of congestive heart failure (CHF) is left ventricular (LV) pump dysfunction. The LV pump dysfunction observed with CHF is paralleled by changes in LV geometry, which is referred to as myocardial remodeling. LV myocardial remodeling and subsequent chamber dilation have been associated with increased morbidity and mortality in patients with CHF; irrespective of the etiology [1,2]. Moreover, clinical as well as experimental studies suggest that LV remodeling is an important contributory event in the progression to end-stage CHF [3–15]. However, the structural basis and contributory mechanisms for the changes in LV geometry which occur during the progression of CHF are not fully understood. The LV myocardial fibrillar collagen matrix contributes to the maintenance of LV geometry and the structural alignment of adjoining myocytes. Alterations in collagen structure and composition have been reported to occur within the LV myocardium in several cardiac disease states, which in turn may influence LV geometry [3–16]. An endogenous family of enzymes responsible for extracellular collagen degradation...
are the matrix metalloproteinases (MMPs) [16–18]. The purpose of this report is to examine the biology of MMPs and to place this proteolytic system in the context of LV remodeling which occurs in end-stage CHF, with particular focus on the dilated cardiomyopathies.

2. The myocardial collagen matrix

The myocardial fibrillar collagens such as collagen types I and III, ensure structural integrity of adjoining myocytes, provide the means by which myocyte shortening is translated into overall LV pump function and are essential for maintaining alignment of myofibrils within the myocyte through a collagen–integrin–cytoskeletal–myofibril relation. The development of LV dilation in patients and animals has been shown to result in discontinuity of the fibrillar collagen network [4,5,8–16]. Furthermore, increased collagen degradation products have been measured in the plasma of CHF patients and prognostic relationships have been described [19,20]. The results from these past studies have provided the working hypothesis that changes in fibrillar collagen structure and support, which primarily occur through degradative pathways, is a structural mechanism by which progressive LV dilation and remodeling occurs with developing CHF [11,13,21–28].

3. Collagen degradation and the matrix metalloproteinases

The matrix metalloproteinases (MMPs) are a family of zinc-dependent enzymes which have a high affinity for extracellular matrix components and therefore play a fundamental role in tissue remodeling processes [16–18]. More recently, changes in MMP expression profiles and activational states have been identified in a number of evolving cardiovascular disease states. For example, increased MMP activity has been identified to occur in aortic aneurysms and has been postulated to significantly contribute to dilation and progression of the aneurysm [29,30]. In coronary artery disease, atherosclerotic plaque rupture has been shown to be temporally associated with heightened MMP activity within the lesion [31–33]. Finally, increased MMP expression and activation accompany LV remodeling in CHF. Thus, the identification of MMP species which are involved in cardiac disease states and the mechanisms which control MMP expression and activation has become an area of active research.

4. The structure and classification of the matrix metalloproteinases

A generic structure of the MMP enzyme is shown in Fig. 1 with respect to three common domains: pro-peptide, catalytic and C-terminal domain. The MMP catalytic domain contains a highly conserved zinc binding region which may be essential for maintaining enzymatic activity [18]. This region of the catalytic domain is a common target for the pharmacological development of MMP inhibitors [34–38]. MMPs can be broadly categorized into sub-groups based upon specificity and structure. These sub-groups include the interstitial collagenases, gelatinases, stromelysins, and membrane type MMPs (MT-MMPs). The MMP species which may have particular relevance to the LV matrix remodeling process are summarized in Table 1.

Interstitial collagenases include MMP-1, MMP-8 and MMP-13. MMP-1 cleaves the fibrillar collagens such as collagen type-I and -III at specific sites of the collagen molecule [18]. MMP-8 has similar specificity to that of MMP-1, but the key feature of MMP-8 is that it is the predominant form of interstitial collagenase found in neutrophils. Increased MMP-8 expression and activity are commonly found in inflammatory and wound healing processes [18]. Interestingly, it has been reported that MMP-8 was detected in samples taken from end-stage cardiomyopathic disease [5]. MMP-13 is the predominant form of interstitial collagenase found in rodents [18]. MMP-13 has been identified to be expressed in human breast carcinoma and in osteoarthritic chondrocytes [39]. Using immunoblotting techniques [11,21,23,26], we observed a strong signal for MMP-13 in normal human myocardial samples (Fig. 2). Thus, all of the interstitial collagenases have been detected in human LV myocardium. MMP-2 and MMP-9 possess the capacity to degrade a number of interstitial proteins [16–18]. Both MMP-2 and MMP-9 have been demonstrated previously to be highly expressed in LV myocardium [11,21,23,26]. Moreover, a
Table 1
The matrix metalloproteinases with potential relevance to myocardial remodeling

<table>
<thead>
<tr>
<th>Name</th>
<th>Number</th>
<th>Substrate/Function</th>
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<tbody>
<tr>
<td>Collagenases</td>
<td></td>
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<tr>
<td>Interstitial collagenase</td>
<td>MMP-1</td>
<td>Collagens I, II, III, VII and basement membrane components</td>
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<tr>
<td>Collagenase 3</td>
<td>MMP-13</td>
<td>Collagens I, II, III</td>
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<tr>
<td>Gelatinases</td>
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<tr>
<td>Gelatinase A</td>
<td>MMP-2</td>
<td>Gelatins, collagens I, II, III, VII and basement membrane components</td>
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<tr>
<td>Gelatinase B</td>
<td>MMP-9</td>
<td>Gelatins, collagens I, II, III, VII and basement membrane components</td>
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<tr>
<td>Stromelysins</td>
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<tr>
<td>Stromelysin 1</td>
<td>MMP-3</td>
<td>Fibronectin, laminin, collagens III, IV, IX, and MMP activation</td>
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<td>Membrane-type MMPs</td>
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<tr>
<td>MT1-MMP</td>
<td>MMP-14</td>
<td>Collagens I, II, III, fibronectin, laminin, and activates proMMP-2 and proMMP-13</td>
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recent study from this laboratory has demonstrated that these MMPs exist within LV myocytes (Fig. 3) [25]. Thus, it is likely that LV myocytes are an important source of MMPs within the myocardium and therefore have the capacity to directly participate in the LV matrix remodeling process through the expression, synthesis and release of MMPs.

The stromelysins, such as MMP-3, degrade a large number of extracellular substrates such as collagen and basement membrane components [18,40,41]. MMP-3 is ubiquitously expressed in a number of tissues and by a large number of cell types. One of the unique features of this particular MMP is the capacity to activate other MMPs [40,41]. Another important class of MMPs with respect to MMP activation are the MT-MMPs. While there are now four species of MT-MMPs which have been identified, MT1-MMP has been clearly shown to participate in the MMP activational process in a number of cell systems [42–44]. Using immunoblotting techniques, we were able to identify MT1-MMP in normal human LV myocardial preparations (Fig. 2). The relevance of MMP-3 and MT1-MMP expression within the LV myocardium with respect to MMP activation and matrix degradation is discussed in a subsequent section.

Fig. 2. Normal human LV myocardial extracts were subjected to immunoblotting as described previously [21]. A monoclonal antibody solution (1.0 µg/ml) for MMP-13 (MAB3321, Chemicon), MT1-MMP (MAB3317, Chemicon), or EMMPRIN (MAB1354, Chemicon) was used. A positive control was included in all immunoblots (MMP-13: CC068, MT1-MMP: CC1042, Chemicon; Human Breast Cancer Preparation for EMMPRIN). A robust signal was observed for the MMP-13 and for MT1-MMP indicating that these MMP species exist in human LV myocardium. A strong positive signal for the MMP inducer protein EMMPRIN was also observed.
5. Control of matrix metalloproteinase expression

In tumor cell lines, MMP mRNA has been shown to be upregulated by bioactive peptides and cytokines [45,46]. Protein kinase C (PKC) induces MMP transcription in several cell systems [45,47–49]. CHF is accompanied by increased circulating levels of catecholamines, angiotensin-II and endothelin, which in turn can cause a receptor mediated increase in PKC. MMP genes contain response elements such as TRE, which binds proto-oncogene products of the fos and jun family [45,48]. Bioactive peptides and cytokines, such as tumor necrosis factor-α (TNF-α) which stimulate the production of proto-oncogenes, have been demonstrated to increase MMP transcription in several cell systems [46,50–52]. Increased levels of TNF-α have been reported in patients with LV failure and TNF-α levels are associated with disease progression [53–57]. A past study demonstrated that increased circulating TNF-α levels in normal rats caused LV remodeling and subsequent pump dysfunction [24]. Moreover, these pathophysiological relevant concentrations of TNF-α caused alterations in the extracellular matrix such as diminished collagen con-
fluence and continuity of the fibrillar matrix between myocytes [24]. Thus, one intriguing hypothesis is increased levels of TNF-α can induce MMP expression in LV myocytes which in turn cause changes in extracellular matrix structure thereby facilitating the LV remodeling process in CHF.

While extracellular stimuli induce MMP expression, endogenous control systems which regulate MMP transcription remain poorly understood. However, more recent studies have identified a novel 58-kDa protein, called extracellular matrix metallocproteinase inducer (EMMPRIN) on the cell surface of specific tumor cell lines which caused the induction of MMP expression [58,59]. Moreover, we have identified that EMMPRIN exists in normal human LV myocardium (Fig. 2). Thus, an important local induction system for MMPs exists within the human LV myocardium which may be an important regulatory process for MMP expression. The potential role of this local MMP induction system in the progression of CHF warrants further study.

6. Activational control of MMPs

MMP activation begins with proteolytic cleavage ahead of the cysteine residue which results in a partially active intermediate form and release of the propeptide sequence [16–18,40,41]. Following the initial proteolytic cleavage by serine proteases, conversion to the fully active MMP can be achieved through a final common enzymatic pathway requiring stromelysin (MMP-3) [40,41]. Thus, an important regulatory step in overall MMP activation involves the expression and activational state of MMP-3. The critical role that MMP-3 plays in the activation of key MMP species is indicated in Table 2.

While a proteolytic cascade can result in fully active MMPs, the MT-MMPs have been shown to be an important site for MMP activation in tumor cell systems [41–44]. MT1-MMP has been clearly shown to participate in the MMP activational process in a number of cell systems [44]. Specifically, pro-MMP-2 will bind to a specific extracellular domain of MT1-MMP and result in full activation of MMP-2 [43,44]. Moreover, MMP-13 is directly activated by MT1-MMP [41]. The MT-MMPs provide for a local MMP activation system which will result in focalization of MMP activity and therefore extracellular remodeling. Our preliminary results have demonstrated that MT1-MMP exists in normal human LV myocardium (Fig. 2) and may be an important system for the activation of MMPs within the myocardium. In certain cell systems, it has been demonstrated that cytokines such as TNF-α upregulate MT1-MMP levels [44,52]. Whether increased cytokine levels and other bioactive peptides which occur in developing CHF influence myocardial MT-MMP expression and activity remains unknown.

The tissue inhibitors of MMPs (TIMPs) bind to MMPs in a stoichiometric 1:1 molar ratio and therefore form an important endogenous system for regulating MMP activity in vivo [18,41,60–62]. TIMPs bind to the active site of the MMPs by blocking access to the collagen substrate. TIMP-1 forms a complex with several MMPs which include MMP-1 and MMP-9. TIMP-4 has been recently characterized as having a relatively unique expression pattern [61]. Specifically, the only organ which was observed to have a high expression pattern for TIMP-4 was the myocardium [61]. However whether TIMP-4 possesses different MMP inhibitory activity within the myocardium remains unclear. In light of the potential importance of TIMPs to modulate MMP activity, the TIMPs are an area of active interest in cardiovascular biology [22,29].

7. Pharmacological development of matrix metalloproteinase inhibitors

Studies have clearly shown a positive correlation between MMP activity and metastatic tumor behavior and inflammation [16–18]. Accordingly, a number of synthetic MMP inhibitors have been developed with a broad range of specificity as well as compounds which have very select specificity for MMP species [34–38]. Current targets for MMP inhibition include corneal ulcerations, malignant ascites, tumor metastases and arthritis [63–66]. In initial efforts, a Parke-Davis compound with global MMP inhibitory activity (PD166793) was evaluated in a model of CHF and the results are summarized in the following section [27]. This MMP inhibitor demonstrated inhibitory activity against a number of MMP species potentially

<table>
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<th>Zymogen</th>
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<th>Activation by final step</th>
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<td>ProMMP-1</td>
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<td>MMP-3, MT1-MMP</td>
<td>MMP-3, MMP-3, MMP-2</td>
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<td>ProMT1-MMP</td>
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<td>Intracellular activation</td>
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*Adapted from Nagase [62].
relevant to myocardial matrix remodeling but was not active against other proteolytic systems [27]. Thus, orally active MMP inhibitors can be successfully constructed which do not posses activity against other proteolytic systems relevant to the CHF process. Recent evidence has accumulated that global MMP inhibition which results in direct MMP-1 inhibition, caused undesirable systemic effects [63,64]. Specifically, non-selective MMP inhibition using hydroxymate constructs such as the British Bio-Tech compounds [64–66], can induce muscle and joint pain [63,64]. An alternative approach would be to inhibit the activational process of MMPs through selective inhibition of MMP-3, but to actually ‘spare’ direct inhibitory actions on the MMP-1 enzyme. The development of more selective MMP inhibitors will be of significant importance if these are to be considered for use in cardiovascular disease states where chronic treatment will likely be required.

8. LV myocardial matrix metalloproteinase expression in CHF

Several studies have demonstrated increased MMP expression and abundance in experimental models of CHF and with end-stage cardiomyopathic disease in humans [5,21,22,26,67]. Initial studies by this laboratory were focused upon determining whether and to what degree MMP species expression and activation were altered with the progression of CHF. Since LV dilation and remodeling are important sequelae and can actually precede the development of LV pump dysfunction, the goal was to relate potential changes in MMP expression and activity to the LV remodeling process in CHF. For these studies, we used a model of rapid pacing induced CHF in pigs which produces the clinical phenotype of CHF [9–11,14,15,27,68]. We have demonstrated previously that this model of chronic pacing produces well defined, predictable, and progressive LV dilation and matrix remodeling [10,11,14,15]. The pacing model of CHF causes biventricular dilation and dysfunction similar to that of dilated cardiomyopathy. However, this review will focus on the LV with respect to matrix remodeling and MMP activity. The relative abundance of MMPs was examined in LV myocardial extracts during the development of pacing CHF by immunoblotting for MMP-1, 2, and 3 [11,23]. A strong immunoreactive signal corresponding to MMP-1, 2 and 3 was detected in LV myocardial extracts (Fig. 4). Den-sitometry revealed a time dependent increase in MMP abundance with the progression of pacing CHF. At day 7 of pacing, a robust increase in MMP-3 and MMP-2 was observed. The early increase in MMP-3 expression would potentially contribute to the activational cascade for a number of MMPs. Thus, an early event in the progression of pacing CHF was increased LV myocardial MMP abundance. These findings demonstrated that changes in collagen degradative pathways occur within the LV myocardial interstitium during the progression of CHF.

In order to determine whether MMP expression is induced in human CHF, LV myocardial extracts from normal and DCM samples were subjected to immunoblotting for MMP-1, 2, 3 and 9 as described previously [21,26]. Increased levels of MMP-9 were observed in these DCM samples. Interestingly, MMP-1 levels were reduced in the end-stage cardiomyopathy samples when compared to normals. However, a significant and robust signal for MMP-3 was observed with DCM (Fig. 5). The increased expression of MMP-3 with DCM would potentially contribute to a cooperative, step-wise process necessary in order to achieve fully active MMPs within the myocardium [40,41].

9. LV myocardial matrix metalloproteinase activity in CHF

The most common approach for the assessment of MMP activity in tissue is through the extraction of the samples and performing in vitro assays [5,11,17,21–23,25–27]. The tissue extracts are often first electrophoretically separated and then exposed to MMP substrates in order to assess proteolytic activity. For example, MMP zymography consists of electrophoretic gels which are impregnated with an MMP substrate and the tissue samples run into the gel [11,21]. Proteolytic activity within the tissue extracts can then be identified at different molecular weights. LV myocardial MMP gelatinase activity was examined in pacing CHF samples using digital zymographic methods (Fig. 4) [11,21]. These studies demonstrated a time-dependent increase in MMP zymographic activity during the progression of pacing CHF. More importantly, MMP zymographic activity was increased early in the time course of pacing CHF, which preceded significant changes in LV myocyte contractile dysfunction [11].

Studies from this laboratory and others have clearly demonstrated that increased MMP zymographic activity occurs in end-stage DCM [21,22,26]. Using LV myocardial extracts from non-ischemic and ischemic origin, significantly greater LV myocardial zymographic MMP activity was observed (Fig. 6). Thus, in both forms of cardiomyopathy, LV myocardial MMP activity is increased and potentially contributes to the remodeling process which invariably occurs in these cardiac disease processes. However, it remains unclear whether different MMP species expression occurs in these two different etiologies of cardiomyopathic disease. It is very likely that different extracellular stimuli will induce different portfolios of MMP expression within the myocardium. For example, this laboratory has recently reported that differential expression and activation occurs following the induction of either an acute pressure or volume overload stimulus [69].
Fig. 4. (A) Immunoblotting was performed on LV myocardial extracts for interstitial collagenase (MMP-1), 72-kDa gelatinase (MMP-2), and stromelysin (MMP-3) taken from control (time 0) and with each week of chronic rapid pacing. A time-dependent increase in MMP abundance was observed following 7 and 14 days of pacing with a strong signal observed after 3 weeks of pacing. A positive control (+) for the MMPs was included as described previously [11,27]. (B) MMP zymographic activity was examined in LV myocardial extracts using gelatin as a proteolytic substrate in control samples and with each week of chronic rapid pacing. A positive control from an HT1080 cell line (+) was used as described previously [11,27,31]. In the zymogram, proteolytic activity was observed in LV myocardial extracts in the 50–90-kDa region. Under non-activated and activated conditions, MMP zymographic activity was increased in the rapid pacing samples. Moreover, a robust increase in MMP zymographic activity occurred by 7 days of pacing indicating that an early increase in MMP myocardial activity occurred during the development of pacing CHF. Reproduced from Ref. [11] with permission from the American Heart Association.

Specifically, the induction of a volume overload due to mitral regurgitation was associated with increased myocardial expression of MMP-3 [69]. In contrast, the induction of a pressure overload due to aortic stenosis was not accompanied by an induction of MMP-3. Thus, different wall stress patterns may invoke distinct patterns of MMP expression, which in turn would contribute to the type of LV matrix remodeling which occurs during adaptation to a prolonged volume or pressure overload stimulus.

An important consideration regarding the LV myocardial zymographic measurements is that these are in vitro studies which cannot completely represent the degree of in vivo MMP activity. For example, using in vitro assay systems, local inhibitors such as TIMPs may dissociate from MMP binding sites during myocardial sample preparation. Furthermore, in vitro assay systems are performed under optimal conditions with purified substrates. More recently, we have described an MMP antibody capture assay which allowed for quantitation of myocardial MMP activity without electrophoretic separation [27]. With the development and application of MMP inhibitors, improved methods for measuring indices of MMP tissue activity will be necessary if the local effects of these MMP inhibitors are to be rigorously examined. Nevertheless, measurements of zymographic activity provide evidence that the increased potential for heightened MMP activity occurs in failing myocardium.

One potential mechanism for the increased myocardial MMP activity with end-stage CHF is a loss of endogenous inhibitory control. A study by Li et al. [22] provided evidence to suggest that changes in the MMP/TIMP stoichiometric ratio occurred with end-stage cardiomyopathic disease. Specifically, TIMP-1 and TIMP-3 levels were reduced in cardiomyopathic samples whereas TIMP-2 levels were unchanged when compared to controls. It has been demonstrated previously that TIMP-1 and TIMP-2 expression are differentially regulated by a number of external stimuli [62]. For example, cytokines such
Fig. 5. Immunoblotting for stromelysin (MMP-3) was performed in normal human LV myocardial samples and in dilated cardiomyopathy (DCM) samples as described previously [21]. Each lane represents an individual normal (N) or DCM (D). A robust increase in MMP-3 was observed in all samples. Using densitometry, over a four-fold increase in myocardial MMP-3 was observed with DCM (* P<0.05 vs. normal). Reproduced from Ref. [21] with permission from the American Heart Association.

as TNF-α can modify the expression of TIMP-1 through the induction of nuclear transcription factors, but TIMP-2 expression appears to be unaffected by cytokine stimulation [46,48,62]. Thus, differences in TIMP-1 and TIMP-2 levels may occur in the setting of increased myocardial neurohormone and cytokine production. The different TIMPs exhibit species selectivity with respect to forming complexes with pro-MMPs. For example, TIMP-1 binds with the pro-peptide sequence of MMP-9 and TIMP-2 binds to pro-MMP-2 [18,44,60]. The TIMP binding domain for the pro-MMPs is located at the C-terminal of the TIMPs and is distinct from the domain which inhibits activated MMPs [18,41]. It has been demonstrated that pro-MMP-2/TIMP-2 complexes can be activated in vitro by organomercurial agents in the same fashion as free pro-MMP-2 [18,41]. Thus, formation of pro-MMP/TIMP complexes does not necessary prevent activation of the complexed MMP. These pro-MMP/TIMP complexes may reflect additional functions of TIMPs in controlling MMP activational state and stability.

In a past study, Li et al. [22] reported a reduction in TIMP-1 abundance with in end-stage cardiomyopathy. In order to begin to address whether this reduction in TIMP-1 levels may result in alterations in inhibitory control, we measured MMP-1/TIMP-1 complex formation in normal and DCM LV myocardial samples (Fig. 7) [21,26]. In both non-ischemic and ischemic cardiomyopathy, an absolute reduction in MMP-1/TIMP-1 complex formation occurred. One contributory factor for the reduction in the levels of this specific MMP/TIMP complex was the absolute reduction in MMP-1 levels in the DCM samples [21]. These results suggest that reduced MMP/TIMP complex formation may occur in cardiomyopathic disease states which in turn would contribute to increased myocardial MMP activity.

10. Matrix metalloproteinase inhibition and developing CHF

The studies outlined above identified increased myocardial MMP expression and activity with the development of severe CHF. However, whether heightened MMP activity contributes to the LV matrix remodeling which occurs during the evolution of the CHF process remained to be established. Accordingly, the goal of a recently completed study was to determine whether chronic MMP inhibition would influence LV geometry and function in the setting of developing CHF [27]. This study was designed in order to test the hypothesis that interruption of myocardial MMP activity will reduce the LV dilation which invariably occurs in the pacing model of CHF. The global MMP inhibitor PD166793, as discussed in the previous section was used in the pig model of pacing CHF [27]. Representative serial LV echocardiographic recordings are shown in Fig. 8. The degree of LV dilation and posterior wall thinning which occurs in a time dependent fashion with chronic rapid pacing was attenuated with MMP inhibition. After 21 days of pacing, the degree of LV dilation had significantly progressed without treatment and was accompanied by
Fig. 7. A quantitative ELISA system was performed for the MMP-1/TIMP-1 complex (RPN 2610-2614, BIOTRAK, Amersham) in normal human LV myocardial extracts (NORM; \( n = 13 \)), and in samples taken from patients with end-stage DCM due to non-ischemic (NONISC; \( n = 21 \)) or ischemic (ISCH; \( n = 16 \)) origin. The absolute MMP-1/TIMP-1 complex abundance was reduced in DCM samples compared to normal (* \( P < 0.05 \)) and was further reduced from non-ischemic values in ischemic DCM samples (+ \( P < 0.05 \)). Thus, alterations in MMP/TIMP complex formation occurred with end-stage human DCM.

Fig. 6. LV myocardial extracts from normal human LV myocardium (NORM; \( n = 13 \)), and patients with end-stage DCM due to non-ischemic (NONISC; \( n = 21 \)) or of ischemic (ISCH; \( n = 16 \)) origin were subjected to gelatin zymography. LV myocardial MMP zymographic activity was detected in all LV myocardial samples in the 50–90-kDa range, consistent with the molecular weights of several MMP species. Conditioned media from an HT1080 cell line was used as a positive control (+). MMP zymographic activity was increased in the DCM samples when compared to normal (* \( P < 0.05 \) vs. normal).

Reduced pump function and posterior wall thickness. With concomitant MMP inhibition, the degree of LV dilation was attenuated and LV pump function improved. The reduction in LV volumes and the attenuation of wall thinning with MMP inhibition resulted in a reduction in LV peak wall stress [27]. In isolated myocyte studies, contractility was reduced to an equivalent degree in both pacing groups irrespective of MMP inhibition treatment. These observations suggested that the basis for the improvement in LV pump function with MMP inhibition was primarily due to beneficial effects on LV geometry.

LV myocardial specimens from the study outlined above were prepared for light and scanning electron microscopy. Representative micrographs are shown in Fig. 9 for control, pacing CHF and CHF with MMP inhibition. In control sections, the fibrillar collagen weave could be readily appreciated between adjoining myocytes. With pacing CHF, the collagen weave surrounding individual myocytes appeared reduced and disrupted. In the rapid pacing and MMP inhibition group, the fibrillar collagen weave appeared increased between adjoining myocytes. The attenuation in the degree of LV dilation which was observed with concomitant MMP inhibition during rapid pacing may have been due, at least in part, to increased myocardial collagen content and improved extracellular support. MMP inhibition was associated with a relative increase in collagen content from both untreated CHF and control values [27]. Changes in the relative composition of the myocardial fibrillar collagen matrix have been implicated to influence LV myocardial stiffness characteristics. In this past study, indices of LV chamber and myocardial stiffness were increased when compared to untreated CHF values [27]. Since LV myocardial stiffness reflects intrinsic material properties of the myocardium itself, then this increase in myocardial stiffness with MMP inhibition was likely due changes in myocardial collagen content and structure. Thus, while MMP inhibition reduced LV chamber dimensions during the development of pacing CHF, this was accompanied by potentially negative effects on LV chamber compliance. However, it must be recognized that this study used a high dose of a non-selective MMP inhibitor. More recent studies from this laboratory have demonstrated that combined angiotensin converting enzyme and MMP inhibition reduced LV chamber dimensions in pacing CHF without an associated increase in...
myocardial stiffness properties [70]. Since LV diastolic dysfunction forms an important component of CHF, then modulation of MMP activity will need to be carefully evaluated with respect to alterations in myocardial stiffness properties.

The results detailed above demonstrated that a contributory mechanism for the LV remodeling which occurs during the progression of the CHF process is heightened MMP activity within the LV myocardium. Moreover, these results provide proof of concept that the use of pharmacological MMP inhibitors will alter the course of a developing CHF process. In a myocardial infarction model in mice, Rohde et al. [28] demonstrated that MMP inhibition attenuated the LV dilation which occurred in the early post-infarction period. Thus, it is likely that increased MMP expression and activation directly contribute to the LV matrix re-
modeling process in a number of cardiac disease states which can potentially accelerate the development of LV pump dysfunction.

11. Summary

Most current therapies for CHF do not retard or reverse the underlying cause for the LV dysfunction, and therefore the LV failure continues to progress with the ultimate demise of the patient. The statistics regarding the development of CHF in the US patient population are unsettling. Specifically, approximately one-half million patients develop CHF each year and the incidence has been predicted to increase significantly in the next 10 years worldwide. With the beginning of the new millennium, it may be appropriate to review the development and treatment of CHF (Fig. 10). A transition in the philosophy and treatment of CHF has occurred in which recognition that modulating the physical and biochemical environment which surrounds the failing LV does not completely retard the progression of the disease [56]. Instead, strategies which directly influence the adaptive and perhaps maladaptive myocardial growth and matrix remodeling response may alter the inevitable course to end-stage CHF. Regulation of myocardial MMP expression and activity may be an important therapeutic approach in controlling matrix remodeling in the setting of developing CHF. With the development of high throughput pharmacological screening, the development of potent and selective pharmacological MMP inhibitors has accelerated and eclipsed our knowledge of MMP biology within the myocardium. Mouse models have been created with alterations in the
MMP/TIMP genotype [71–73] and will likely serve as an important tool in future studies to examine and understand MMP biology and matrix remodeling. An integrative approach using genetically engineered mice, large animal models and clinical studies of CHF will be necessary in order to address mechanistically based questions regarding the role of MMPs in the pathogenesis of heart failure. The questions which will need to be answered from these studies include how matrix remodeling/degradation directly affects LV geometry and function, which MMP species contribute to this process, and how MMP inhibition will be integrated into the pharmacological armamentarium of CHF.

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