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Targeting Matrix Metalloproteinases in Heart Disease: Lessons from Endogenous Inhibitors

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Abstract

Basic pharmacological/transgenic studies have clearly demonstrated a cause-effect relationship between the induction and activation of matrix metalloproteinases (MMPs) and adverse changes in the structure and function of the left ventricle (LV). Thus, regulation of MMP induction and/or activation would appear to be a potential therapeutic target in the context of cardiovascular disease, such as following myocardial infarction (MI). However, pharmacological approaches to inhibit MMPs have yet to be realized for clinical applications. The endogenous inhibitors of the MMPs (TIMPs) constitute a set of 4 small molecules with unique functionality and specificity. Thus, improved understanding on the function and roles of individual TIMPs may provide important insight into the design and targets for pharmacological applications in LV remodeling processes, such as MI. Therefore, the purpose of this review will be to briefly examine biological functions and relevance of the individual TIMPs in terms of adverse LV remodeling post-MI. Second is to examine the past outcomes and issues surrounding clinical trials targeting MMPs in the post MI context and how new insights into TIMP biology may provide new pharmacological targets. This review will put forward the case that initial pharmacological attempts at MMP inhibition were over-simplistic and that future strategies must recognize the diversity of this matrix proteolytic system and that lessons from TIMP biology may lead to future therapeutic strategies.

Keywords

myocardial remodeling; tissue inhibitors of matrix metalloproteinases; fibroblasts; myocardial infarction

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Introduction

Heart failure (HF) remains a major cause of morbidity, mortality, and constitutes a significant portion of medical care costs. In general clinical terms, HF is manifested by defects in cardiac pump function (ejection, filling, or a combination of both), which in turn will cause clinical signs and symptoms that are often progressive and result in emergent presentation and hospitalization. While the current standard of care for HF is appropriately focused upon the reduction in symptomatology, therapeutic strategies which specifically target the fundamental underpinnings of the HF process remains an unmet medical need and hence an important area for research and development. The term HF is not defined by a specific pathological stimulus but rather the downstream consequence of multifactorial events with the underlying causes being quite diverse, and as such, classification schemes can be problematic. Nevertheless, a generalized dual classification system has been developed that encompasses the HF presentation, and key underlying physiological manifestations have emerged. [1] Specifically, patients with a HF presentation and primarily left ventricular (LV) systolic dysfunction such as that which can occur following a myocardial infarction (MI), or that of primarily LV diastolic dysfunction, which can occur with a sustained pressure overload such as hypertension. The changes in LV geometry and myocardial structure, often referred to as LV remodeling, can also be different in these two classifications, and as such the therapeutic targets and pathways may also be distinctly different. For the purposes of this review and to maintain focus, the prototypical example of LV remodeling and progressive HF, as it applies to myocardial infarction (MI), will be utilized.

Despite significant improvements in the management of acute coronary syndromes and myocardial ischemic events, residual injury to the affected region of the myocardium (MI) can occur. This myocardial injury sets in motion a number of cellular and extracellular matrix (ECM) events. The death of cardiac myocytes in the context of ischemic injury and MI first occurs through the classical cell death pathway, necrosis. This region of necrotic myocytes then causes a cascade of biological events, which include the expression of inflammatory molecules and egress of inflammatory cells, proliferation and transdifferentiation of fibroblasts, and the induction of ECM degradation/synthetic pathways.[2,3] While this process is initially considered to be an appropriate and adaptive wound healing response, the persistence of these biological events, particularly that of continued ECM turnover, is considered to be maladaptive and contribute to the pathophysiology of LV remodeling and progression to HF.[3–5] Most specifically, the changes in ECM structure can contribute to a structural milestone in adverse post-MI remodeling - infarct expansion.[5,6] The affected region following the MI that contributes to infarct expansion, progressive LV remodeling, and systolic dysfunction not only is composed of the MI region itself but can also affect the viable myocardium surrounding the MI. Significant changes within the ECM occur during all time points post-MI and likely contribute to the overall adverse LV remodeling process. Firstly, the inflammatory response causes the release of matrix metalloproteinases (MMPs) as well as other proteases to degrade the ECM and allow for margination of inflammatory cells.[8–10] However, with a persistent inflammatory state, MMP induction will also destabilize the newly formed ECM

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and the nascent scar. Secondly, the transformed fibroblast population within the MI region as well as the surrounding viable myocardium causes a shift in the relative balance of MMPs and endogenous tissue inhibitors of MMPs (TIMPs),[6,8,9,11–14] favoring accelerated ECM turnover and a failure of mature scar formation. These observations led to significant initial exuberance by both industry and medical academia for the development of pharmacological reagents, which would inhibit MMP activity for the purposes of interrupting adverse LV remodeling post-MI.[15–24] However, this initial enthusiasm has been tempered by the recognition that MMPs constitute a diverse family of enzymes, all with unique functionality, and that only a subset of these MMPs may hold therapeutic relevance in terms of post-MI remodeling.[6,21–23,25,26] At the same time, there is growing awareness that the TIMPs may also be multifaceted in terms of biological roles relevant to the post-MI remodeling process well beyond that of inhibition of active MMPs.[27–36] Specifically, there are 4 known TIMPs, which appear to be differentially regulated in terms of temporal expression following tissue injury, may differentially affect MMP activity, and regulate fibroblast growth and viability.[27–32,35–43] Therefore, the purpose of this review is to place into context the functionality, expression profiles, and potential therapeutic application of these individual TIMPs in terms of post-MI remodeling.

TIMPs – small molecules with diversity of function

The TIMPs constitute 4 unique, small molecular weight proteins (~20 kDa) that are distinctly different gene products with 50% or less homology.[27–30] The first TIMP, TIMP-1, was identified in the late 1970s and TIMP-4 was first described in the late 1990s,[44,45] and while initially considered to simply bind to active MMPs in a 1:1 stoichiometric ratio, these molecules have unique and differential effects upon key aspects of ECM biology relevant to the post-MI remodeling process (Table 1). Using primary fibroblast cultures taken predominantly from either myocardial samples or from cancer related regions, [27–37] divergent effects of specific TIMPs have been identified with respect to cell growth and viability. For example, TIMP-1 induces a robust effect on fibroblast growth and proliferation, whereas TIMP-2 and TIMP-4 appear to have a more modest or negative effect.[29–37,41] On the other hand, TIMP-1 has been shown to reduce relative apoptosis rates, whereas other TIMPs can accelerate cell death.[31,32,37,41] With respect to TIMP-2, a robust effect on fibroblast transdifferentiation, as defined as a transition to a more contractile phenotype, has been reported.[37] In contradistinction to other TIMPs, TIMP-3 modulates cytokine processing through an inhibition/interruption of a disintegrin-metalloproteases (ADAMs), including both ADAM-10 and ADAM-17. [27–30,39,40]

Other unique differences in TIMPs can be found in the relative affinity for MMP inhibition as well as activation. Specifically, TIMP-1 has been shown to have a very low affinity for the transmembrane MMPs, such as MT1-MMP.[27,28,46] Since MT1-MMP has been shown to play a significant role in ECM remodeling, including post-MI remodeling,[47,48] then the weak inhibitory capacity of TIMP-1 on MT1-MMP likely holds relevance when considering TIMPs as a therapeutic. With respect to TIMP-2, it has now been well established that an activation complex is formed between proMMP-2, TIMP-2, and MT1-MMP, which will facilitate activation of MMP-2.[27,35,47,49] Moreover, while other TIMPs, such as TIMP-4, can bind to pro-MMP-2, these pro-MMP-2/TIMP-4 complexes do

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not appear to facilitate MMP-2 activation.[27,28,35,36] In fact, *in-vitro* kinetic studies have identified TIMP-4 will inhibit the interaction and activation of pro-MMP-2 via the TIMP-2/MT1-MMP cascade and is also a potent inhibitor of MT1-MMP.[27–29,35,36,42,50] Thus, a duality of function exists for TIMP-2 whereby both MMP activation and inhibition can occur simultaneously and provide for a very precise localization of ECM turnover. With respect to TIMP-4, the direct and indirect effects on MMP activation and activity, along with the relatively restricted expression pattern to that of hollow muscular organs, such as the heart and uterus, [42,51,52] underscore the unique functionality of each TIMP and potential relevance to the post-MI remodeling process.

While TIMP binding to specific MMP sequences results in both inhibition and activation, there is growing evidence that TIMPs can directly influence cell growth and function through a ligand-receptor mediated pathway.[29–34] In cancer associated fibroblasts, it has been demonstrated that TIMP-1 binds to the membrane receptor CD63 and can cause extracellular signal-regulated kinase activation.[31] In transformed fibroblasts, TIMP-1 induced activation of protein kinase B (Akt) pathway, whereby this cellular transduction event was demonstrated to be MMP independent.[32] In cancer associated fibroblasts, TIMP-2 has been demonstrated to reduce mitogen activated signaling through a cyclic AMP mediated pathway, which in turn reduced fibroblast proliferation.[33] Moreover, TIMP-2 binds to transformed fibroblasts in a ligand-receptor mediated fashion that is saturable, whereby the binding kinetics were unaffected by co-incubation with an MMP inhibitor.[33] In other *in-vitro* binding studies, it has been demonstrated that the likely cognate receptor for TIMP-2 is an alpha-3/beta-1 integrin.[34] While the majority of these studies have been performed in transformed fibroblast cell lines in the context of cancer, there is likely great relevance to the post-MI remodeling process. Specifically, there are significant parallelisms in the expression profiles in the transdifferentiation process of fibroblasts associated with cancer and those which occur in fibroblasts post-MI.[13,14] Thus, the identification of specific TIMP receptors in cardiac cells, such as the myocardial fibroblast, may afford a specific mechanism by which to regulate proliferation and function of this critical ECM cell type. Indeed, differential effects of individual TIMP types on myocardial fibroblast growth and transdifferentiation have been demonstrated [37] and were likely due to specific receptor mediated interactions.

Through sequencing analysis and examination of TIMPs in invertebrate species, [27,28] it is clear that TIMPs are ancient molecules, which therefore underscore the overall biological relevance of these molecules. What is now becoming clear is past canonical thought that TIMPs simply bind and inhibit active MMPs must be revised. The diversity in biological effects of each TIMP upon cell growth, viability, and signaling as well as those that can actually facilitate MMP activation hold important considerations when developing these molecules as pharmacological agents, such as with post-MI remodeling.

TIMP profiles and expression post-MI

A number of animal and clinical studies have profiled changes in TIMP expression in the post-MI period. [see reviews 6,7,25,47] Overall, these studies uniformly demonstrated a divergence in MMP and TIMP expression and induction in the early post-MI period.

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Specifically, in animal and patient studies of post-MI remodeling, a robust induction of MMPs, particularly those associated with inflammation, occur early (~3–7 days post-MI) but are not necessarily accompanied by a concomitant increase in TIMPs. For example, in a rat MI model, Peterson and colleagues demonstrated temporal changes in TIMP induction over an approximate 2 week post-MI period.[53] Specifically, at early post-MI time points (<3 days) an increase in relative TIMP-1 and TIMP-2 mRNA levels occurred, which was then followed by a time-dependent decline over longer post-MI time periods. This study also reported that relative TIMP-4 concentrations actually fell from referent control values during this post-MI time period. In contrast, this past study demonstrated a robust and persistent induction of a number of MMP types, which included MMP-13, MMP-2, MMP-9, and MT1-MMP. The changes in TIMP levels post-MI also appear to be region specific within the LV, whereby TIMP levels are substantially extinguished within the MI region, and within normal limits or elevated in the remote viable regions of the myocardium.[12] Clinical studies have utilized plasma samples to profile TIMP levels,[55–56] and while arguably the sources for these TIMPs can be of concern, they can serve as a reasonable surrogate for changes in TIMP levels which are likely occurring within the myocardium of patients post-MI.[57] A summary of clinical studies that comprise over 5,000 subjects, which have measured TIMPs following MI, is shown in Table 2. Overall, these studies identified an increase in relative plasma TIMP-1 levels in patients post-MI, but relative TIMP-2 and TIMP-4 levels either remained unchanged or were reduced from referent normal values. However, these TIMP levels were also accompanied by robust increases in plasma MMP levels in the post-MI period, and the elevated MMP levels persist for weeks to months. Moreover, the relative magnitude of the increase in MMP levels is associated with the rate and extent of adverse post-MI remodeling in patients.[6,54,56] The lack of concordance between increased MMP levels and TIMP levels is further exemplified when the stoichiometric ratio of MMP/TIMP is considered. For example, a 3–4 fold increase in the MMP-9/TIMP-4 ratio has been reported in post-MI patients, which illustrates the imbalance of this ECM proteolytic system in the post-MI context.[54]

Despite the relative imbalance between MMP and TIMP levels in the post-MI period, relative TIMP-1 levels increase from normal values in both animal models and patients, particularly at later post-MI time points.[6,8,53,54] This raises the question as to why elevated TIMP-1 levels in the post-MI period do not appear to favorably alter the post-MI remodeling process. Moreover, elevated plasma TIMP-1 levels have actually been demonstrated to be associated with a worsening of clinical outcomes and cardiovascular events.[57] The underlying reasons for this apparent conundrum regarding TIMP-1 may be due to several biological and structural reasons. First, the increased induction of TIMP-1 may be insufficient to effectively prevent the exuberant ECM proteolysis that persists in the post-MI period. Specifically, the relative MMP/TIMP-1 levels remain elevated throughout the post-MI period, thus favoring continued ECM degradation. Furthermore, TIMP-1 is a poor inhibitor of MT1-MMP, a likely critical MMP type in the post-MI remodeling process. Second, TIMP-1 causes fibroblast growth and transdifferentiation (Table 1), which may actually exacerbate post-MI remodeling. Third, in myocardial samples from patients with end-stage ischemic HF, TIMP-1 levels are increased, but MMP/TIMP-1 complexes are actually reduced.[70,71] This observation suggests that defects in TIMP-1 binding to active

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MMPs may be impaired. It has been demonstrated that post-translational modification of TIMPs, can directly affect the affinity and inhibition of active MMPs.[28–30] For example, carbamylation of the N-terminal amino group of TIMP-1 greatly reduces MMP inhibitory capacity.[66] These past binding/structural studies suggest the intriguing possibility that post-translational modification of TIMPs may occur in the context of post-MI remodeling.

TIMP modulation by transgenics and relevance to post-MI remodeling

In order to examine the potential consequence and roles of individual TIMPs in terms of post-MI remodeling, transgenic constructs through deletion of genomic fragments of the targeted TIMP gene were utilized, which in general will prevent initiation of transcription.[17,38,40,43] None of the individual TIMP transgenic knockout lines were embryonically lethal, and stable colonies could be developed. This however does not imply that the global loss of a specific TIMP yielded a phenotypically normal organism. For example, TIMP-3 deletion resulted in abnormal lung development and function as well as enhanced inflammation.[67,68] While there are a number of inherent limitations with global gene deletion in transgenic mice, MI induction in these murine constructs through coronary ligation have provided a unique insight into post-MI remodeling and TIMPs.[17,38,40,43]

In general terms, TIMP deletion accelerated the adverse LV remodeling process post-MI, but there were differences in the changes in myocardial structure (Table 1). In the TIMP-1–/– mouse, induction of an MI caused an acceleration in global LV dilation compared to wild-type littermates, presumably due to increased infarct expansion and a loss of ECM structural integrity.[17] Notably, these effects of TIMP-1 gene deletion post-MI in terms of increased acceleration of the adverse post-MI remodeling process could be mitigated somewhat by concomitant pharmacological MMP inhibition.[17] The most significant work regarding the effects of targeted TIMP deletion and the consequences upon post-MI remodeling is from the Kassiri laboratory.[38,40,43] Using a TIMP-2–/– mouse line, these investigators demonstrated that MI induction caused increased loss of ECM structural integrity by second harmonic generation and multi-photon imaging, which was in turn associated with increased infarct expansion and LV dilation.[38] Moreover, these authors demonstrated that in these TIMP-2 null mice, which effectively abolished the pro-MMP-2/TIMP-2/MT1-MMP activation cascade, accelerated adverse LV remodeling was observed. These findings underscored several important points regarding MMP-TIMP interactions. First, this study demonstrated the requirement of TIMP-2 in an *in-vivo* context or MMP-2 activation. Second, these investigators identified enhanced MT1-MMP activity in the TIMP-2 null mice, underscoring the tight interaction of MMP types to specific TIMPs. Perhaps the most profound effects on post-MI remodeling was observed in the TIMP-3–/– mouse.[39] In these studies, the Kassiri laboratory demonstrated that MI induction in the TIMP-3 null mouse was associated with increased and persistent myocardial inflammation and increased MI rupture.[39] While myocardial rupture is a rather rare event post-MI in humans, this occurs with enough frequency in murine MI models to be a quantifiable response variable.[8–10,19] The increased MI rupture in the post-MI period resulted in worse survival in the TIMP-3–/– mouse when compared to strain matched wild type mice and clear evidence of LV dilation in the surviving mice (Figure 2). Finally, these investigators demonstrated differential effects of TIMP-4 gene deletion with respect to LV remodeling and survival

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secondary to MI or a pressure overload.[43] While the effects of TIMP-4 gene deletion in the context of a sustained pressure overload appeared modest, adverse LV remodeling and poor survival was observed post-MI (Figure 2).

Clinical studies of pharmacological MMP inhibition post-MI: Lessons learned

The initial pharmacological compounds advanced to clinical application for MMP inhibition contained structures which would bind to the common sequence of the catalytic domain of active MMPs. These initial MMP inhibitors were non-peptides and contained a hydroxymate structure.[6,21–23] Continued improvements in the structural design yielded MMP inhibitors with nanomolar potency and were successfully used in several animal models.[15–17,19,24] One of the MMP inhibitors that was successfully advanced to clinical trials for the indication of post-MI remodeling was the pharmacological MMP inhibitor (PG116800).[16,20,69] This specific MMP inhibitor had been utilized in pre-clinical large animal models in order to develop pharmacokinetic data and initial proof of concept.[16,70] Following which, a clinical study was undertaken entitled Selective Matrix Metalloproteinase Inhibitor to Prevent Ventricular Remodeling After Myocardial Infarction (Prevention of Myocardial Infarction Early Remodeling-PREMIER).[20,69] The PREMIER study recruited 253 patients, primarily from international study centers that were with an identified MI, and then randomized to an active treatment arm consisting MMP inhibition or placebo. Treatment began within 48 hours of presentation with an MI, and initially the study utilized a 200 mg dose to be given orally twice daily for the entire study interval of 180 days.[69] However, due to historical concerns regarding the potential risk of systemic side effects, the dosing regimen was reduced to 200 mg dose once/day.[20,69] Changes in LV end-diastolic volume from baseline compared to that obtained at 90 days post-MI was the primary response variable. Surprisingly and contrary to other major clinical studies,[1–7] LV volumes only increased slightly (~10%), suggesting that infarct expansion had not occurred in these post-MI patients, irrespective of treatment. As a function of baseline values, the change in LV volumes was 8.4% in the PG11680 group and 10.3% in the placebo group (p=0.31). The neutral findings from the PREMIER trial were likely multifactorial and included an inadequate dosing regimen and a minimal change in the primary response variable. For example, based upon pharmacokinetic computations as well as the known volume of distribution for humans of PG11680,[16,69] it is unlikely that this study achieved significant therapeutic efficacy, that is MMP inhibition, in a large number of patients [for further computations and discussion, see reference 6]. Inexplicably, plasma profiles for PG11680 were not reported in the PREMIER trial, and thus this issue remains of concern. In the PREMIER study, the relative magnitude of post-MI remodeling as a function of change in LV end-diastolic volume were unusually modest (5 mL/m^2). Based upon the initial power estimates,[69] it would be necessary for PG11680 to reduce the change in LV end-diastolic volume during the post-MI period by 80% when compared to placebo values. These dramatic reductions in LV end-diastolic volumes have never been achieved in pre-clinical animal studies of MMP inhibition despite optimal dosing conditions and experimental designs. Despite the significant shortcomings in design and limitations in the primary response variable, the PREMIER trial did have one effective outcome: the closure

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of a number of research and development programs focusing upon MMP inhibition for adverse LV remodeling.

An entirely different and initially rather surprising MMP inhibitor that was advanced to clinical trials was that of doxycycline.[18,71] In a preclinical large animal study, Villarreal and colleagues demonstrated that specific doxycycline doses would reduce adverse LV remodeling post-MI and that this effect was independent of any anti-microbial action.[18] Tetracyclines were developed as a result of the screening of soil samples for antibiotic organisms (an extensive review on the pleiotropy of tetracyclines can be found in [72]). This family of antibiotics was found to be highly effective against various pathogens including rickettsiae, Gram-positive, and Gram-negative bacteria, thus becoming a class of broad-spectrum antibiotics. The antibiotic mechanism of action of tetracyclines is thought to be related to the inhibition of protein synthesis. Over time, many other "protective" actions have been reported for tetracyclines. Minocycline, which can readily cross cell membranes, is known to be a potent anti-apoptotic agent. Its mechanism of action appears to relate to specific effects exerted on apoptosis signaling pathways. Another tetracycline, doxycycline, is known to exert antiprotease activities. Doxycycline can inhibit MMPs, which can contribute to tissue destruction activities as seen with gingivitis, thus their FDA approval for this use under the category of MMP inhibition. A large body of literature has provided evidence for additional "beneficial" actions of tetracyclines, including their ability to act as oxygen radical scavengers and anti-inflammatory agents. Their unique capacity to accumulate in injured tissues (such as infarcted myocardium) also makes them appear to act as smart drugs. The recognition by scientists and clinicians of these collections of properties and of the safety profile of this class of drugs led to the implementation of pre-clinical and clinical trials to explore their possible beneficial effects in the setting of a wide variety of diseases including cardiovascular pathologies.

Villarreal and colleagues demonstrated that early, short term treatment of rats subjected to MI with doxycycline significantly decreased cardiac hypertrophy, myocyte cross-sectional area, and internal LV diameter while preserving infarcted wall thickness.[18] Doxycycline yielded parallel left shifts in LV pressure-volume relationships and epicardial scar area strain patterns similar to normal myocardium. The assessment of LV global MMP and MMP-2 and -9 activities 1 hour after MI also evidenced significant differences with doxycycline. Subsequent studies from the same research group further substantiated these observations in large animals [73] and also expanded on the anti-remodeling properties of doxycycline when given later post-MI (from 2–7 days post-infarction). [74] Interestingly, the anti-protease properties of doxycycline also appear to be exerted on other enzymes known to potentially play adverse roles in the setting of ongoing remodeling, such as plasmin. [75] As a result of these efforts, a clinical trial was recently implemented.[76] The "TIPTOP" trial evaluated the efficacy of submicrobial doses of doxycycline (100 mg for 7 days BID started immediately post-percutaneous intervention, n=110) on post-MI remodeling. Results yielded significant decreases in LV end-diastolic volumes, infarct size, and severity in the doxycycline treated group. Thus, emerging evidence support a possible role for tetracyclines in ameliorating adverse cardiac remodeling. However, larger clinical studies will be implemented to validate the use of this class of compounds as a means to limit adverse cardiac remodeling and may include non-antimicrobial variants (known as

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chemically modified tetracyclines or CMTs), which have demonstrated an apparent safe clinical profile. [77]

TIMP delivery to the myocardium as a therapeutic - Proof of concept

In light of the fact that systemic delivery of broad spectrum MMP inhibitors (with the possible exception of doxycycline or CMTs) is unlikely to gain a clinical foothold in terms of post-MI remodeling, then more specifically targeted approaches must be developed. One possible therapeutic direction is to augment local TIMP levels on a regional basis following MI. As briefly outlined in a previous section and detailed previously,[27–30,39,40] TIMP-3 demonstrates unique biological functions which include a high affinity to bind to the ECM through interactions with glycosaminoglycans, an influence on cytokine processing, and the ability to alter fibroblast phenotype *in-vitro*. Thus, localized augmentation of TIMP-3 in the context of post-MI remodeling constitutes a novel and translationally relevant therapeutic approach. Accordingly, a study was recently completed whereby the effects of regional delivery of exogenous TIMP-3 within the MI region upon infarct expansion and the course of post-MI remodeling was examined.[78] Specifically, a recombinant, full length human TIMP-3 (rTIMP-3) was synthesized and delivered to the MI region in adult pigs. In order to maintain a continuously high level of this low molecular weight construct, and to avoid systemic, off target delivery, the rTIMP-3 was encapsulated in a hyaluronic acid (HA) based hydrogel. As such, targeted injections of a HA hydrogel formulation that achieved sustained release of rTIMP-3 into the MI region was attained (Figure 3). Using this localized approach for rTIMP-3 delivery and post-MI large animal model, significant changes in the post-MI remodeling process were observed. Specifically, the rate of infarct expansion was reduced as was the degree of LV dilation - both key indices of post-MI remodeling. This translational study provides the proof of concept that regional, sustained delivery of a recombinant TIMP effectively interrupts the adverse post-MI remodeling process. This puts forth a new therapeutic paradigm in terms of modulating local biology of the MI and the infarct expansion process. Finally, this proof of concept study demonstrates that systemic or for that matter global interference of matrix proteolytic pathways is not necessary to favorably affect post-MI remodeling.

Developing TIMPs for therapeutic interventions on post-MI remodeling - Future directions

Since there are unique peptide sequences of each TIMP, then identifying the specific structure-function relationship of the regions within each TIMP with respect to MMP binding and other biological effects would allow for improved specificity in drug design. Indeed, past studies have already demonstrated that the N-terminus sequence and that of the C-terminus sequence of each TIMP may hold unique functionality in terms of MMP inhibition and non-MMP inhibitory roles.[28–30,79,80] For example, it has been shown that the C-domain of TIMP-2 is essential for formation of the proMMP-2 activation cascade but is not required for MMP inhibition.[70] On the other hand, a single point mutation in the N-terminal domain of TIMP-3 did not alter ADAM-17 inhibition but significantly impaired MMP inhibition.[80] Thus, truncated forms of TIMPs, which contain either the C or N-terminus regions, may provide enhanced specificity relevant to the post-MI remodeling

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process. In addition, engineering recombinant TIMPs with additional functional side chains may also provide for more localized specificity. For example, Diafarzadeh et al. added a glycosylphosphatidylinositol anchor to a recombinant form of human TIMP-1 which resulted in increased binding to the cell surface, and more localized effects on MMP activity.[81] Moreover, these investigators demonstrated that this membrane anchored form of TIMP-1 increased the rate of experimental wound closure. It has been demonstrated that specific point mutations, such as within the TIMP-1 sequence, can result in specific discrimination between MMP types.[26–29] For example, double and triple mutations at position Val4 and Ser68 within the TIMP-1 molecule altered inhibitory profiles for MMP-1, -2, and -3 when compared to the native form.[82]. Higashi et al reported that a fusion protein, whereby a 10 amino acid sequence was added to the N terminus of TIMP-2, resulted in significant inhibitory selectivity for active MMP-2.[83] Thus, future directions would include the design of recombinant TIMPs with specific amino acid substitutions that may confer MMP selectivity and hence target MMP types that are considered to play pathological roles in post-MI remodeling. It may also be possible to engineer a TIMP structure to prevent rapid proteolytic degradation as well as improve tissue penetration and specificity. Indeed, regions of the nascent TIMPs have been identified to be glycosylated and form cross-linked regions, which in turn affect 3-dimensional structure and biological activity.[28] Moreover, localized delivery of recombinant TIMPs, such as through the use of eluting biomaterials as exemplified in the previous paragraph or through induction of TIMP designs through gene transfer techniques, would also enhance the specificity as well as local concentrations of these molecules.

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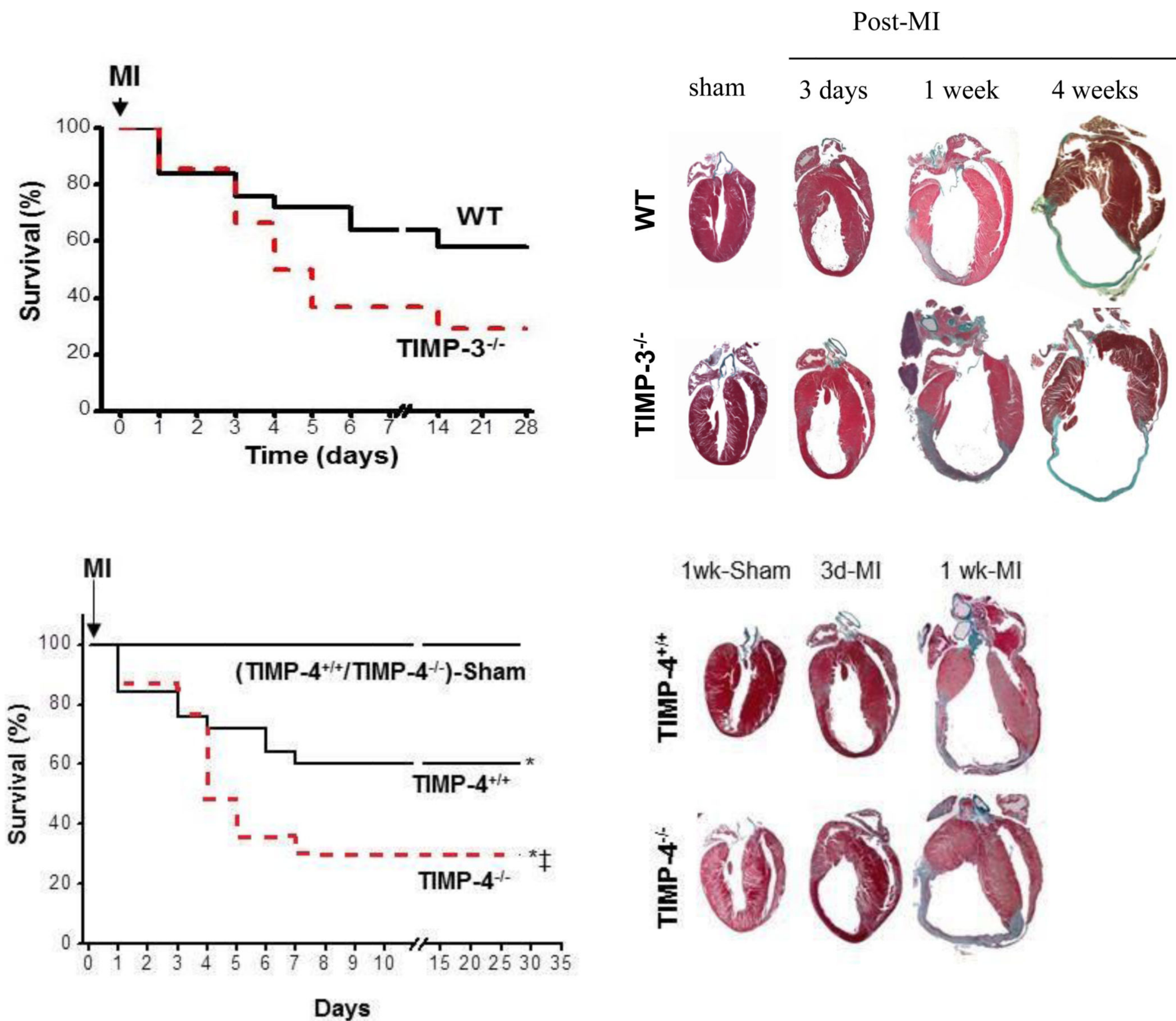

Figure 1.

The effects of transgenic deletion of specific TIMPs with respect to post-MI remodeling have been the subject of several studies by the Kassiri laboratory,[38,40,43] and representative findings from TIMP-3 gene deletion and that of TIMP-4 gene deletion are shown here. Left panels demonstrated a worsening survival in the TIMP-3 and TIMP-4 gene knockout mice following MI, but the pathophysiology for this reduced survival may be distinctly different. In the TIMP-3 null mice, increased inflammation and incidence of MI rupture were observed, whereas accelerated LV dilation and progression to HF was the observation in the TIMP-4 null mice. In both cases, adverse LV remodeling characterized by increased LV volumes, MI thinning, and expansion was observed in both the TIMP-3 and TIMP-4 null mice (right panels). Reproduced from references #40 and 43.

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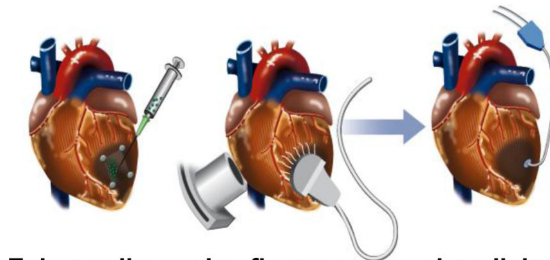

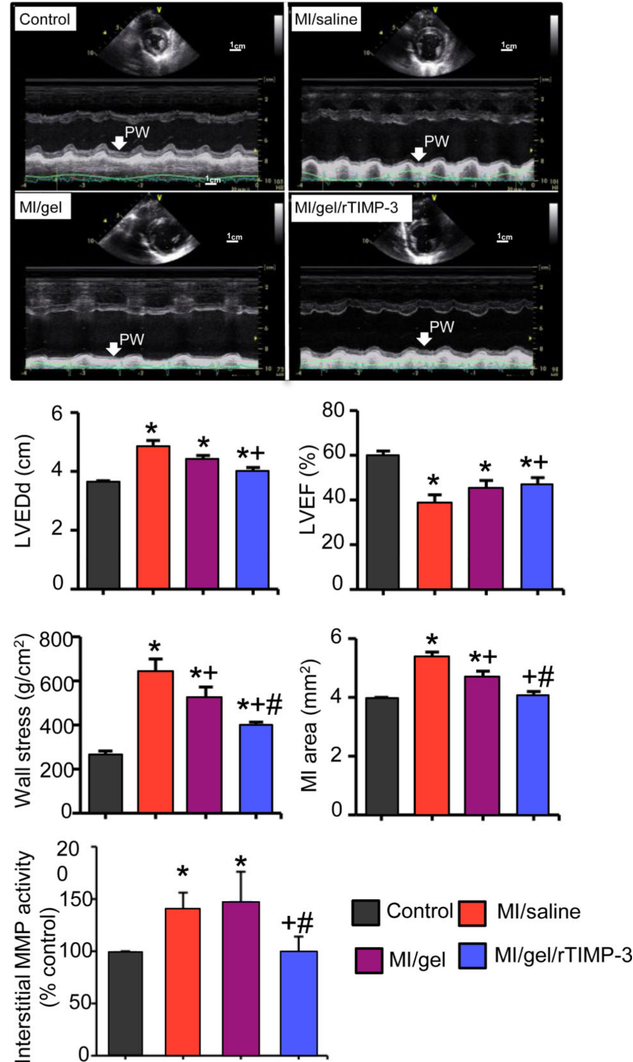

Figure 2.

(TOP PANELS) Intra-myocardial injections of a hydrogel (HA-gel) containing a recombinant full length TIMP-3 (r-TIMP-3), HA gel alone (gel), or saline, using a 9-point array pattern were performed in adult pigs at the time of MI induction. The goal of this study was to demonstrate that continuous release of a recombinant TIMP within the MI region would alter the natural history of adverse LV remodeling. Age matched, non-MI pigs were included in the study design and were considered referent controls. Serial studies of LV geometry by echocardiography and MI expansion by fluoroscopic marker localization were

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performed up to 14 days post-MI, which were then followed by microdialysis in order to measure MMP interstitial activity. (MIDDLE PANELS) Representative LV echocardiograms revealed LV dilation and poor posterior wall motion (PW) at 14 days post-MI, which were improved in the MI/gel/r-TIMP-3 group when compared to either MI and saline injections or MT and gel only injections. (LOWER PANELS) Quantitation of LV echocardiograms revealed a reduction in the degree of LV dilation as measured by LV end-diastolic dimension (LVEDd), LV peak wall stress, and MI area (reflective of infarct expansion) in the MI/gel/r-TIMP-3 group, which was associated with an improved LV pump function as measured by LV ejection fraction (LVEF). Notably, LV wall stress and MI area were also reduced, albeit to a more modest degree, in the MI/gel group underscoring that there are effects of these biomaterials alone. While interstitial MMP activity was increased in the MI region in the MI/saline and MI/gel groups, this was normalized in the MI/gel/r-TIMP-3 group. Reproduced from reference #78.

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Table 1

Differential Biological Functionality of Tissue Inhibitors of MMPs (TIMPs) Potentially Relevant to Post-MI Remodeling

	TIMP-1[3]	TIMP-2	TIMP-3	TIMP-4	References
Cell Growth/Proliferation[1]	++	−	+	−	31–34,36,37,41
Cell Apoptosis	−	+	+	+	31,36,41
Cytokine Processing			++	+	27,30
Fibroblast Transdifferentiation	+	+++		+	36,37
Pro-MMP Complex Formation	Pro-MMP-9	Pro-MMP-2	Pro-MMP-2	Pro-MMP-2[2]	27,30,35,36
Transgenic Deletion and Post-MI Remodeling	↑ LV Dilation	↑ LV Dilation	Rupture, ↑ Inflammation	LV Dilation, Accelerated HF	17,38,40,43

1) Derived from studies previously in fibroblasts / smooth muscle cell culture

2) Does not yield an activation complex

3) Weak inhibitor of membrane bound MMPs

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Table 2

Tissue Inhibitor of Matrix Metalloproteinase Dynamics in Patients with Ischemic Heart Disease and Relation to Clinical Outcomes

Subjects (n)	Biomarker(s)	Primary Observation	Reference #
233	PINP, ICTP, PIIINP, MMP-1, TIMP-1	Longitudinal changes in all ECM markers whereby ICTP associated with greater HF symptom progression	33
1009	PIIINP, MMP-1, TIMP-1, hsCRP, IL-18, IL-10	Indices of increased ECM turnover associated with functional capacity and outcomes.	34
39	MMP-1, TIMP-1 and Biopsy	Shift in MMP-1/TIMP-1 balance favoring ECM degradation as evidenced by ECM biopsy histology	26
85	Portfolio of MMPs and TIMPs serially examined	A specific cassette of MMPs and TIMPs are increased following MI and associated with progressive LV remodeling	37
100	TIMP-1, -2, -4	Early changes in plasma TIMP-4 levels associated with degree of LV dilation at 3 months post-MI	39
404	TIMP-1, MMP-9, NTproBNP	Changes in MMP-9 associated with adverse post-MI remodeling as defined by LV dilation and TIMP-1 levels demonstrated predictive risk for combined event endpoint (HF/death)	40
1313	TIMP-1,-2,-4	Univariate and composite score relationship to major advance cardiovascular events	58
30	MMP-9, TIMP-1, TIMP-2	MMp-9/TIMP-1 ratio associated with coronary plaque rupture	59
500	NTpro-BNP, TIMP-1	TIMP-1 predictive of long-term outcome in ischemic heart disease	60
389	MMP-9, TIMP-1	TIMP-1 predictive of mortality following MI	61
556	TIMP-1, TIMP-1 polymorphism	TIMP-1 associated with worsening heart failure post-MI	62
1082	MMP-9, TIMP-1	TIMP-1 associated with cardiovascular risk in elderly	63