A specific temporal pattern of MMP/TIMP occurred in post-MI patients that included an early and robust rise in MMP-9 and MMP-8 and a uniform fall in TIMP-4. These findings suggest that a specific MMP/TIMP plasma profile occurs after MI and holds both prognostic and diagnostic significance. (Circulation. 2006;114:1020-1027.)

Key Words extracellular matrix ■ matrix metalloproteinases ■ myocardial infarction ■ remodeling

An important structural event after myocardial infarction (MI) is left ventricular (LV) remodeling, which generally can be defined as changes within the cellular and extracellular constituents of the myocardial wall leading to changes in myocardial geometry subsequently leading to changes in LV volumes. The rate and extent of this post-MI remodeling process have been established to be independent predictors of morbidity and mortality. Thus, identifying those patients at the greatest risk for developing post-MI remodeling and the basic mechanisms that contribute to post-MI remodeling holds great diagnostic/therapeutic relevance. The factors that contribute to post-MI remodeling are multifactorial and include both cellular and extracellular processes. It is now becoming recognized that changes within the myocardial extracellular matrix (ECM) contribute to post-MI remodeling.1-7 Disruption within the fibrillar ECM network will cause a loss of normal structural support and continuity, resulting in myocyte fascicles being subjected to abnormal stress and strain patterns during the cardiac cycle, which in turn cause changes in myocardial geometry and function. The matrix metalloproteinases (MMPs) constitute a large family of proteolytic enzymes responsible for ECM degradation and remodeling under normal and pathological conditions.7-9 An important control point for MMP activation is binding to an endogenous family of inhibitors, the tissue inhibitors of MMPs (TIMPs).3,7,10 A clear cause-and-effect relationship between MMPs and the post-MI remodeling process has been demonstrated through the use of transgenic constructs and pharmacological MMP inhibition in animals.2-5,11 In general, these preclinical studies have demonstrated that an imbalance between MMPs and TIMPs occurs in the post-MI myocardium and that increased MMP proteolytic activity facilitates post-MI remodeling and eventually LV dilation. Moreover, these preclinical studies have demonstrated that a specific temporal pattern of MMP/TIMP expression occurs after MI that is causally related to the degree of post-MI remodeling.5,6,11 Whether and to what degree a similar MMP/TIMP profile occurs in patients after MI remains to be established, however.
from the remodeling/remodeled myocardium.3,5,6 For example, it has been reported previously that in patients with end-stage cardiomyopathy, certain MMP types increased significantly without a concomitant increase in TIMP levels.11–13 However, this approach cannot be used to construct a temporal profile of MMP/TIMP changes in patients after MI. Accordingly, our group and others have developed high-sensitivity assays to measure MMP/TIMPs in the plasma of patients.14,15 Initial studies have demonstrated that changes in MMP plasma levels occur in patients after MI.16–18 However, a more comprehensive temporal profile of changes in MMP/TIMPs after MI and the relationship of these changes to LV remodeling has not been performed. Accordingly, the present study serially measured plasma MMP/TIMP levels and LV geometry in patients up to 6 months after MI to perform temporal comparisons in these MMP/TIMP profiles and to perform comparisons with a group of age-matched, reference control subjects.

Methods

Subjects

Thirty-two patients with confirmed MI and 53 reference control subjects were enrolled in this study after providing informed consent. All of the studies described herein were reviewed and approved by the Medical University of South Carolina Institutional Review Board. ECG or a positive cardiac enzyme panel confirmed the MI. The criterion for enrollment as an MI subject was a troponin-I value that was 2.5 times greater than the laboratory reference value recorded within 48 hours from the time of presentation to the emergency department. Patients were excluded from enrollment if there was a history of MI; previous coronary revascularization surgery within past 24 months; anticipated need for emergent coronary revascularization; cardiac disease states other than ischemic heart disease; a history of active malignancy in past 3 years; significant renal or hepatic dysfunction; ongoing or active rheumatological disease requiring significant antiinflammatory agents, steroids, or immunosuppression; and a significant history of substance abuse. The timing of the studies described in the next paragraph was based on an index event, which was defined as the time of initial presentation to the emergency department. For the purposes of this study, these initial measurements were identified as post-MI day 1. Open enrollment was from fall 2001 to spring 2002 at the Medical University of South Carolina. The mean time from onset of symptoms to treatment intervention was 3.5±0.9 hours, and the time to initial study was 71±8 hours with a median time of 50 hours. In this post-MI patient cohort, 33% received thrombolytic therapy, and 89% received a percutaneous coronary intervention (angioplasty with or without stent). The distribution of the MI was 36% anterior, 61% inferior, and 3% posterior as determined by ECG. ST-segment elevation was noted in 84% of the MI patients, and a Q wave was noted in 48% of the MI patients. Peak troponin levels were 166±30 ng/mL. The mean white blood cell count at admission was slightly elevated at 10.7±0.72 10^3 cells/mm³.

The reference control group consisted of subjects with no evidence of cardiovascular disease. Cardiovascular disease was excluded by performing a complete medical history, comprehensive physical examination, ECG, and echocardiogram. Patient demographics and medication profiles for the reference control and MI subjects are shown in Table 1. For the MI patients, the medication profiles are those that were operative on post-MI day 1, continued throughout the study interval, and followed American Heart Association/American College of Cardiology guidelines. For the control subjects, β-blocker, angiotensin-converting enzyme inhibitors, and angiotensin receptor antagonists were used to treat mild increases in systolic pressure, but no evidence of hypertrophy was present in echocardiographic studies. Digitalis was present in 1 patient to treat a remote history of a single episode of atrial fibrillation. Aspirin or antiinflammatory agents were used in the reference control group as part of a routine medical management for arthritic pain.

Protocol

For the MI patients, studies were performed at the time of enrollment (post-MI day 1). An echocardiogram was performed, and blood was collected from a peripheral vein. Plasma from this blood sample was used to measure MMP/TIMP profiles at post-MI days 2 to 5 and post-MI days 28, 90, and 180. At post-MI days 5, 28, 90, and 180, an echocardiogram also was obtained. All patients fasted overnight before each study but took their morning medications as prescribed. For the control subjects, a complete study identical to that for the post-MI patients at post-MI day 1 was performed.

MMP and TIMP Profiles

For this study, representative MMPs from the different MMP classes were measured, specifically the interstitial collagenase MMP-8, the gelatinases (MMP-2 and MMP-9), and MMP-7 from the matrilysin subclass.7–9 The rationale for selecting these MMP types is that they have been identified in animal studies to be altered after MI and have been associated with matrix remodeling after myocardial injury.5,6,11,14 TIMP-1 and TIMP-2 were measured in this study because they have been successfully identified in the plasma of patients and have been shown to be altered in animal models of MI.5,6,14,15 The approach for all measurements used a 2-site ELISA (Amersham Pharmacia Biotech, Buckinghamshire, UK) using methods described previously.14,15 These were high-sensitivity assay systems with a detection range of 0.016 to 1 ng/mL. All samples were analyzed in duplicate and averaged. The intra-assay coefficient of variation for these measurements was <6%. Past studies have documented that TIMP-4 is uniquely and highly expressed within the cardiovascular system, particularly the myocardium.19,20 Moreover, past studies have documented that this specific TIMP is altered in animal models of MI.5,6 The authors of the present study have previously reported that TIMP-4 can be measured through an immunoassay approach.21 Accordingly, a high-sensitivity (0.008 ng/mL) ELISA with high specificity (no cross-reactivity with other TIMPs or proteases) was

| Table 1. Demographics for Normal Control Subjects and Patients After MI |
|-----------------------------|----------|----------|----------|
|                            | Control  | After MI | P        |
| Total, n                   | 53       | 32       | ...      |
| Men, n (%)                 | 20 (38)  | 24 (75)  | ...      |
| Women, n (%)               | 33 (62)  | 8 (25)   | ...      |
| Age, y                     | 59±1     | 58±2     | 0.65     |
| Body surface area, m²      | 1.87±0.03| 1.99±0.04| 0.07     |

Control patients had no evidence of a MI or cardiovascular disease. Post-MI patients had troponin I levels >2.5 times normal or typical ECG changes.
used (R&D Systems, Minneapolis, Minn). This assay measured both free and bound TIMP-4 with high linearity ($r^2=0.95$) over a wide range of TIMP-4 standards (0.078 to 5 ng/mL). This ELISA was also cross-calibrated and validated with a quantitative immunoassay described previously. In addition to measuring MMP-2 and MMP-9 through quantitative ELISA, semiquantitative measurements were performed through gelatin zymography.

**Echocardiographic Methods**

Transthoracic echocardiography was performed with a Sonos 5500 system with an S-4 MHz transducer (Agilent Technologies, Andover, Mass). Measurements were made with American Society of Echocardiography criteria. Two-dimensional echocardiographic studies were performed using standard short- and parasternal long-axis views to obtain measurements of LV volumes and ejection fraction. Images were coded and read in a blinded fashion, and this analysis remained unlinked to the MMP/TIMP levels until completion of the study.

**Data Analysis**

The echocardiographic and MMP/TIMP data given in this study were presented in an untransformed manner using parametric statistics. For the reference control group, blood collection and echocardiographic measurements were performed at only 1 point in time. Thus, comparisons between reference control values and post-MI day 1 values were first performed with a 2-tailed Student t test. Next, changes in LV geometry and function and MMP/TIMP levels in time in the post-MI patients were examined by use of a 2-way ANOVA for repeated measures in which reference control/MI was the first treatment level and time after MI was the second treatment level. After the ANOVA, mean separation was performed by Bonferroni bounds; this resulted in a $6 \times 6$ matrix of pairwise comparisons for the echocardiographic indexes and a $9 \times 9$ matrix of pairwise comparisons for the MMP/TIMP comparisons. In addition, the potential effects of gender were first examined with respect to control and post-MI day 1 values by 1-way ANOVA and then through the use of a multiway ANOVA model to test for interactions. The relationships between changes in MMP/TIMP levels and LV volumes in the post-MI period were examined by linear regression methods. Because the peak troponin levels were not normally distributed (Shapiro-Wilk W test, $P=0.001$), associations between changes in MMP levels and LV volumes were performed with the Spearman correlation approach. Values of $P<0.05$ were considered significant. All values are presented as the mean and SEM. All statistical procedures were performed with Stata Statistical Software (StataCorp, release 8.0, College Station, Tex).

The authors had full access to the data and take full responsibility for their integrity. All authors have read and agree to the manuscript as written.

**Results**

Measurements of LV geometry and function and of systemic blood pressure and heart rate obtained at the initial study for age-matched control and post-MI patients are summarized in Table 2. At this early post-MI time point, LV end-diastolic volume was increased and systemic arterial blood pressure was decreased compared with reference control subjects. As shown in Figure 1, LV end-diastolic volume increased in a time-dependent manner in the post-MI group. LV end-diastolic volumes increased from post-MI day 1 values at post-MI day 28. Although LV dilation occurred in the post-MI group, LV ejection fraction increased slightly early after MI and then fell to within the reference control range for the remainder of the post-MI study period.

Absolute values for plasma levels of MMP-2, -7, -8, and -9 and TIMP-1, -2, and -4 obtained at the initial study point are summarized for the reference control and post-MI groups in Table 3. These measurements also were computed as a percent change from reference control values. MMP-2 levels were lower than reference control values at post-MI day 1. In contrast, MMP-8 and MMP-9 levels were significantly higher at post-MI day 1 compared with reference control values. For example, plasma MMP-9 levels were $>200\%$ higher than reference control values at post-MI day 1. Plasma TIMP-1 levels were higher at post-MI day 1, whereas TIMP-2 and TIMP-4 levels were unchanged from reference control values. To examine the stoichiometric relation between changes in relative MMP-9 and TIMP levels, the ratios of MMP-9 to TIMP were computed (Table 3). The ratio of MMP-9 to TIMP-1 increased by $>2$-fold, whereas the ratios of MMP-9 to TIMP-2 and of MMP-9 to TIMP-4 increased by $>200\%$ at post-MI day 1 compared with reference control values. When the MMP/TIMP levels were stratified across gender, a gender effect was observed by ANOVA for TIMP-1 ($P=0.03$) in reference control male versus female subjects (1101±73 versus 937±37 ng/mL) and in male versus female subjects at post-MI day 1 (1508±125 versus 1231±117 ng/mL). However, no significant gender interaction with respect to TIMP-1.

![Figure 1](http://circ.ahajournals.org/doi/fig/1/)

**Table 1.** LV Structure and Function Data in Reference Control Subjects and Post-MI Patients

<table>
<thead>
<tr>
<th></th>
<th>Control*</th>
<th>Post-MI Day 1†</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>LV end-diastolic volume, mL</td>
<td>96±2</td>
<td>111±5</td>
<td>0.004</td>
</tr>
<tr>
<td>LV end systolic volume, mL</td>
<td>33±1</td>
<td>35±3</td>
<td>0.54</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>65±1</td>
<td>69±2</td>
<td>0.035</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>70±1</td>
<td>68±2</td>
<td>0.47</td>
</tr>
<tr>
<td>Arterial systolic pressure, mm Hg</td>
<td>126±2</td>
<td>119±3</td>
<td>0.06</td>
</tr>
<tr>
<td>Arterial diastolic pressure, mm Hg</td>
<td>75±1</td>
<td>67±2</td>
<td>0.0008</td>
</tr>
</tbody>
</table>

*Reference control subjects; n=53.
†Initial measurements within 72 hours from index event; n=32.

Table 3. These measurements also were computed as a percent change from reference control values. MMP-2 levels were lower than reference control values at post-MI day 1. In contrast, MMP-8 and MMP-9 levels were significantly higher at post-MI day 1 compared with reference control values. For example, plasma MMP-9 levels were $>200\%$ higher than reference control values at post-MI day 1. Plasma TIMP-1 levels were higher at post-MI day 1, whereas TIMP-2 and TIMP-4 levels were unchanged from reference control values. To examine the stoichiometric relation between changes in relative MMP-9 and TIMP levels, the ratios of MMP-9 to TIMP were computed (Table 3). The ratio of MMP-9 to TIMP-1 increased by $>2$-fold, whereas the ratios of MMP-9 to TIMP-2 and of MMP-9 to TIMP-4 increased by $>200\%$ at post-MI day 1 compared with reference control values. When the MMP/TIMP levels were stratified across gender, a gender effect was observed by ANOVA for TIMP-1 ($P=0.03$) in reference control male versus female subjects (1101±73 versus 937±37 ng/mL) and in male versus female subjects at post-MI day 1 (1508±125 versus 1231±117 ng/mL). However, no significant gender interaction with respect to TIMP-1.
TABLE 3. MMP and TIMP Data

<table>
<thead>
<tr>
<th></th>
<th>Control, Day 1</th>
<th>Post-MI Day 1</th>
<th>Change from control, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-2, ng/mL</td>
<td>1387±39</td>
<td>1005±48*</td>
<td>-27±3*</td>
</tr>
<tr>
<td>MMP-7, ng/mL</td>
<td>2.5±0.2</td>
<td>3.5±0.8</td>
<td>41±32</td>
</tr>
<tr>
<td>MMP-8, ng/mL</td>
<td>2.8±0.6</td>
<td>5.5±0.9*</td>
<td>95±32*</td>
</tr>
<tr>
<td>MMP-9, ng/mL</td>
<td>13±3</td>
<td>47±6*</td>
<td>255±47*</td>
</tr>
<tr>
<td>TIMP-1, ng/mL</td>
<td>997±36</td>
<td>1436±99*</td>
<td>44±10*</td>
</tr>
<tr>
<td>TIMP-2, ng/mL</td>
<td>44±4</td>
<td>52±5</td>
<td>19±12</td>
</tr>
<tr>
<td>TIMP-4, ng/mL</td>
<td>1.9±0.1</td>
<td>1.9±0.1</td>
<td>3.6±7.4</td>
</tr>
<tr>
<td>MMP-9/TIMP-1, ×10⁻³</td>
<td>14±3</td>
<td>37±6*</td>
<td>162±45*</td>
</tr>
<tr>
<td>MMP-9/TIMP-2, ×10⁻³</td>
<td>388±88</td>
<td>1233±233*</td>
<td>218±60*</td>
</tr>
<tr>
<td>MMP-9/TIMP-4</td>
<td>8±1</td>
<td>28±4*</td>
<td>247±50*</td>
</tr>
</tbody>
</table>

*P<0.05 vs control (n=53).

levels and the presence of an MI was detected by ANOVA (P=0.56).

The MMP profiles measured over time in the post-MI patients are shown in Figure 2. Plasma levels for the proform of MMP-2 (proMMP-2) remained decreased from relative control values. Plasma levels for total MMP-7 remained comparable to reference control values for the entire study period. MMP-8 levels were significantly elevated at post-MI day 1 and spiked again at post-MI day 3. Plasma levels for proMMP-9 remained significantly elevated at all post-MI time points but trended downward by post-MI day 28. Plasma samples were subjected to gelatin zymography, and a clear proteolytic band was observed at 92 kDa, likely reflecting MMP-9 levels (Figure 3). Zymographic activity at this 92-kDa region increased relative to reference normal controls at the early post-MI time points. A 72-kDa proteolytic band, reflecting MMP-2, appeared to be increased at 28 days after MI but remained within normal reference values at all other post-MI time points.

Serial plasma measurements of TIMP profiles are shown in Figure 4. TIMP-1 levels remained substantially elevated throughout the post-MI study period, and TIMP-2 levels increased from reference control values at post-MI day 180. TIMP-4 plasma levels remained lower than reference control values at all post-MI time points, with a significant reduction at post-MI day 5. The relations between the time-dependent changes in MMP-9 and TIMP-4 are shown in Figure 4. The ratio of MMP-9 to TIMP-4 increased significantly at all post-MI time points.

Individual response plots for changes in plasma MMP-9 levels from post-MI day 1 to day 5 are shown in Figure 5. A mixed response in individual MMP-9 levels occurred within this time frame; therefore, individual responses were computed as a percent change from day 1 post-MI values. These values were then placed in relationship to changes in LV

*P<0.05 vs normal reference range.

**Figure 2.** The proform of MMP-2 was decreased in post-MI patients, whereas MMP-7 remained within the normal range. MMP-8 levels increased at post-MI days 1 and 3. MMP-9 levels remained elevated throughout the post-MI period. *P<0.05 vs normal reference range.

**Figure 3.** Top, Gelatin zymography demonstrated an increased 92-kDa band, indicative of MMP-9, through post-MI day 28. Bottom, A lower-molecular-weight band (72 kDa) indicative of MMP-2 also was observed. A small but significant increase in relative levels was observed at post-MI day 28. *P<0.05 vs normal values set to 100%.

In Table 3, the data for MMP and TIMP are presented in a tabular format. The table shows the levels of MMP-2, MMP-7, MMP-8, MMP-9, TIMP-1, TIMP-2, and TIMP-4, along with the change from control values. The changes are calculated as a percentage of the control values, and some values are marked with an asterisk (*) to indicate statistical significance (P<0.05 vs control). The table also includes the ratio of MMP-9 to TIMP-1, MMP-9 to TIMP-2, and MMP-9 to TIMP-4, with their changes from control values.

In Figure 2, the proform of MMP-2 was decreased in post-MI patients, whereas MMP-7 remained within the normal range. MMP-8 levels increased at post-MI days 1 and 3. MMP-9 levels remained elevated throughout the post-MI period. *P<0.05 vs normal reference range.

In Figure 3, the gelatin zymography demonstrated an increased 92-kDa band, indicative of MMP-9, through post-MI day 28. A lower-molecular-weight band (72 kDa) indicative of MMP-2 also was observed. A small but significant increase in relative levels was observed at post-MI day 28. *P<0.05 vs normal values set to 100%.

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end-diastolic volumes at post-MI day 28 (Figure 5). In those patients with persistently elevated or increased MMP-9 levels at post-MI day 5, a much greater increase in LV end-diastolic volume occurred at day 28. The relative magnitude of the early change in plasma MMP-9 levels was stratified on the basis of a 35% increase in MMP-9 levels from day 1 to day 5 after MI. In those patients in whom plasma MMP-9 levels increased further from day 1 post-MI values, a greater percent change in LV end-diastolic volume occurred at 90 days after MI (Figure 6). No significant relationships were observed between early changes in MMP-2, MMP-7, and MMP-8 or TIMP-1 and TIMP-2 levels and the degree of LV dilation (r = 0.27, 0.10, 0.04, -0.20, -0.24, respectively; all P > 0.20). However, there was a significant relationship between early changes in MMP-9 and that of LV dilation. Specifically, a more robust change in MMP-9 levels detected between post-MI days 1 and 3 was associated with a greater degree of LV dilation at post-MI day 90 (r = 0.63, P = 0.03).

The peak troponin level was not associated with the early changes in MMP-9 levels (r = 0.01, P = 0.94), nor was it related to changes in LV end-diastolic volume (r = -0.32, P = 0.17). With respect to other covariates, there was no significant difference in MMP/TIMP levels when stratified across MI location or post-MI medications (P > 0.40). When gender was introduced as a factor in a multivariate ANOVA, no significant interactions were observed between MMP/TIMP levels within the post-MI sampling period.

Discussion

The unique and significant findings from this study were twofold. First, a distinct temporal pattern of MMP and TIMP release occurred in patients after MI. Specifically, an acute rise in plasma MMP-9 and MMP-8 occurred after MI, but other MMP types such as MMP-7 and MMP-2 remained unchanged or were reduced from reference control values. Plasma TIMP-1 levels were increased, but cardiac-specific TIMP-4 was reduced after MI. Second, a relationship was observed between early increases in a certain MMP type,
MMP-9, and the degree of LV dilation that occurred late after MI. These results demonstrated that dynamic changes occur in MMP and TIMP levels in patients after MI and that stochastic profiling of this proteolytic system may hold clinical utility with respect to adverse LV remodeling after MI.

The MMPs have been categorized into subfamilies or classes that originally were predicated on similarities in proteolytic substrates.7–10 These classes are the interstitial collagensases, which include MMP-8; the gelatinases, which comprise MMP-2 and MMP-9; the matrilysins, which include MMP-7; and the membrane-type MMPs. MMP types representative of each class except the membrane-type MMPs were measured in the present study. Past clinical studies have reported a significant change in specific MMP types such as MMP-9 at a specific time point after MI.17–19 The present study builds on these reports by demonstrating that changes in MMP and TIMP types are not uniform in patients after MI and therefore measurement of a single MMP or TIMP type may not reflect the dynamic nature of this proteolytic system. Furthermore, this study is the first to demonstrate that measuring the changes in the profiles of specific MMP types such as MMP-9 early after MI was associated with a later manifestation of adverse myocardial remodeling, LV dilation.

There were distinct and differential changes in the plasma profiles of MMPs belonging to the gelatinase subclass in the post-MI period. Specifically, MMP-2 levels were reduced and plasma MMP-9 levels were elevated after MI. The basis for these differences in MMP-2 and MMP-9 profiles in the post-MI patients is likely the differences in transcriptional regulation and cell sources for these MMP types. MMP-9 contains a number of transcription factor binding domains within the promoter region such as the AP-1 binding site that are absent in the MMP-2 promoter region.23 Cytokines such as tumor necrosis factor are elaborated in the early post-MI period and have been demonstrated to induce MMP-9 transcription in vitro.23,24 However, a similar robust increase in cytokine-mediated MMP-2 transcription has not been reported. Thus, the elaboration of critical signaling molecules in the post-MI period would likely cause differential MMP induction. Although all cell types, such as myocytes and fibroblasts, can express MMP-9, an important source of MMP-9 is the neutrophil. Thus, the robust increase in MMP-9 levels observed in the initial post-MI period probably was due to the localized recruitment and degranulation of neutrophils.

Past animal studies have demonstrated a robust increase in MMP myocardial interstitial activity within 1 hour after ischemia-reperfusion.25 Plasma levels of MMP-9 have been identified in patients as early as 12 hours after myocardial injury.18 The present study began MMP profiling almost 3 days after the initial coronary intervention; therefore, the relative magnitude of MMP release that may have occurred during the initial reperfusion period was not assessed. It must also be recognized that the immunoassay technique used in the present study measured only proMMP-2. Thus, the fall in MMP-2 plasma levels may have been the result of reduced synthesis and/or enhanced conversion of proMMP-2 to the active form. Gelatin zymography revealed some discordant findings with respect to MMP-2 levels. First, the proMMP-2 levels observed by zymography were relatively unchanged from normal reference values. MMPs circulating within the bloodstream are protein-bound to either TIMPs or high-molecular-weight proteins such as α-macroglobulin and therefore can be considered biochemically inert. Accordingly, performing zymography on plasma samples requires detergent treatment, electrophoretic separation of bound proteins, and incubation under ideal conditions. Therefore, absolute measurements in crude plasma and after electrophoretic separation may not necessarily assess the same total MMP protein pool.

Past basic studies have provided a cause-effect relation between MMP-9 and adverse LV myocardial remodeling after MI.2–6,11 The results from these basic studies and the present clinical report suggest that the robust increase in plasma MMP-9 levels observed early after MI likely reflects the initiation of an adverse myocardial structural remodeling process that is manifested as LV dilation in the later post-MI period. In the present study, this LV remodeling was not associated with a significant compromise in systolic function as evidenced by no change in LV ejection fraction. The increase in LV ejection fraction observed in the early time points probably was due to increased neurohormonal system activation. However, quantitative assessment of LV wall motion abnormalities and how they may have affected indices of LV ejection performance was beyond the focus of the present study and was not undertaken.

An early increase in plasma levels of the collagenase MMP-8 was detected in patients after MI. MMP-8 is primarily synthesized and released by inflammatory cells such as neutrophils and macrophages; therefore, the early increase in MMP-8 was likely reflective of an acute inflammatory process. A second peak, while highly variable, occurred 3 days after MI and likely reflects the influx of macrophages that occurs during this phase of the MI healing process. Other MMPs, including MMP-1 and MMP-13, belong to the interstitial collagenase subclass. Large animal studies of MI and human studies of end-stage cardiomyopathy have reported a reduction in myocardial levels of MMP-1.5,6,13 In the present study, preliminary results using the ELISA approach did not yield detectable plasma levels of MMP-1 in a subset of post-MI patients. For these reasons, quantification of MMP-1 was not pursued further. Another member of the interstitial collagenase subclass, MMP-13, has been identified within the myocardium of patients with end-stage cardiomyopathy.13 However, the role of MMP-13 in the myocardial remodeling process remains uncertain. This is the first study to demonstrate that plasma levels of MMP-7 were similar to those of normal age-matched subjects throughout the 6-month post-MI period. A past animal study demonstrated no change in MMP-7 myocardial levels at 8 weeks after MI.6 This provides clinical correlative support to past in vitro findings that demonstrated differential regulation of MMP expression after MI.5,6,12,13

The TIMPs are a family of low-molecular-weight proteins that bind to the active catalytic domain of all MMPs and thereby inhibit the proteolytic activity of the enzyme. Although this was originally considered to be the sole function of these low-molecular-weight proteins, it is now recognized that TIMPs have a wide range of additional biological...
properties, including having effects on cell growth and viability and participating in the MMP activational cascade. In the present study, plasma levels for TIMP-1 were significantly increased in patients after MI throughout the 6-month follow-up period. Past animal studies have demonstrated that with an established infarct, myocardial TIMP-1 levels are decreased within the MI region but are either stable or increased within the viable remote myocardium.5,6 This regional heterogeneity in myocardial TIMP-1 levels would favor matrix turnover and remodeling within the MI region and matrix accumulation within the viable myocardium. The increased plasma levels of TIMP-1 observed in patients after MI are likely reflective of this heterogeneous ECM remodeling process. The present study demonstrated that ratios of MMP-9 to TIMP-1 or TIMP-2 remained elevated early in the post-MI period, which would favor prolonged MMP activational states, but that these stoichiometric relationships normalized or were reversed at later post-MI time periods. However, it also has been demonstrated that TIMP-2 participates in the MMP activational cascade by forming a complex with proMMP-2 and the membrane type-1 MMP, yielding active MMP-2.26 Thus, although the present study demonstrated increased plasma levels of both TIMP-1 and TIMP-2 in the later post-MI periods, it cannot be assumed that this simply reflects a profibrotic process occurring throughout the myocardium but rather reflects a diverse number of cellular and extracellular events. One unique aspect of the present study was that it was the first to measure TIMP-4 in post-MI patients, a specific TIMP highly expressed in the myocardium.20,21 Plasma TIMP-4 levels were reduced and the relative ratios of MMP-9 to TIMP-4 were increased compared with age-matched control subjects. These findings provide further supportive evidence that significant and prolonged alterations in myocardial MMP inhibitory control occur in patients after MI. Although the present study is the first to demonstrate that plasma levels for MMPs and TIMPs yield a unique temporal signature in patients after MI, there are inherent limitations that must be recognized. Plasma-profiling MMPs and TIMPs are at best only surrogate markers for local myocardial levels and cannot provide information on regional distribution and proteolytic activity. However, clinical and experimental data demonstrate that a major source of the changes in MMPs and TIMPs observed in the plasma after MI is the myocardium.14,15,25 In the present study, patients with other disease processes that would constitute an alternative source for plasma MMPs and TIMPs were excluded. Taken together, it would appear reasonable to conclude that the temporal changes in MMP and TIMP levels observed in the plasma of the post-MI patients included in this study reflect the dynamic changes occurring within the myocardium. Reports derived from the Framingham Heart Study have demonstrated a relationship between changes in MMP/TIMP profiles and adverse LV remodeling.27,28 In post-MI patients, Sundstrom and colleagues27 demonstrated that the relationship of MMP-9 to adverse LV remodeling was predominant in men. The present study demonstrated an association between early changes in MMP-9 profiles and later changes in LV geometry after MI. However, because of the small sample size and the unequal gender distribution, the present study could not address whether the early change in MMP-9 was associated with gender-specific adverse LV remodeling in the later post-MI periods. In a community-based sample, TIMP-1 levels were higher in male subjects, but when adjusted for potential gender differences, TIMP-1 levels remained an independent factor for LV remodeling.28 Consistent with these past findings, the present study demonstrated higher TIMP-1 levels in reference control and post-MI male subjects. In this patient sample, significant gender interactions could not be demonstrated in the post-MI period with respect to TIMP-1. Thus, whether the gender-dependent differences in TIMP-1 profiles may be associated with different patterns of LV remodeling in the post-MI period remains to be established. These limitations notwithstanding, the present study demonstrated that a unique and temporally diverse plasma profile of MMPs and TIMPs can be quantified in post-MI patients that may hold prognostic and diagnostic utility.

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Despite significant improvements in reperfusion strategies for acute coronary syndromes, myocardial injury/infarction (MI) is a common event that results in changes in the structure and geometry of the left ventricle called post-MI remodeling. Although a number of biochemical and physical factors likely contribute to adverse post-MI remodeling, basic studies have identified that the emergence and activation of an intrinsic proteolytic system, the matrix metalloproteinases (MMPs), contribute to this process. There are a number of MMP subtypes within the myocardium, and MMP activity is regulated in part by the tissue inhibitors of the MMPs (TIMPs). However, it remained unclear whether a specific MMP/TIMP pattern of MMPs and TIMPs occurred in patients in the early (days) and late (months) post-MI period. The present study demonstrated that a unique MMP/TIMP plasma signature could be identified in post-MI patients and that this profile was time dependent. Moreover, the early change in certain MMP levels was associated with the degree of left ventricular dilation at 3 months after MI. These unique findings demonstrated that a specific temporal MMP/TIMP plasma profile occurs after MI and likely holds both prognostic and diagnostic significance.
Specific Temporal Profile of Matrix Metalloproteinase Release Occurs in Patients After Myocardial Infarction: Relation to Left Ventricular Remodeling


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