Region- and Type-Specific Induction of Matrix Metalloproteinases in Post–Myocardial Infarction Remodeling

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Background—Induction of matrix metalloproteinases (MMPs) contributes to adverse remodeling after myocardial infarction (MI). Whether a region- and type-specific distribution of MMPs occurs within the post-MI myocardium remained unknown.

Methods and Results—Ten sheep were instrumented with a sonomicrometry array to measure dimensions in 7 distinct regions corresponding to the remote, transition, and MI regions. Eight sheep served as reference controls. The relative abundance of representative MMP types and the tissue inhibitors of the MMPs (TIMPs) was quantified by immunoblotting. Segment length increased from baseline in the remote (24.9±5.4%), transition (18.0±2.9%), and MI (53.8±11.0%) regions at 8 weeks after MI (P<0.05) and was greatest in the MI region (P<0.05). Region- and type-specific changes in MMPs occurred after MI. For example, MMP-1 and MMP-9 abundance was unchanged in the remote, fell to 3±2% in the transition, and was undetectable in the MI region (P<0.05). MMP-13, MMP-8, and MT1-MMP increased by >300% in the transition and MI regions (P<0.05). TIMP abundance decreased significantly in the transition region after MI and fell to undetectable levels within the MI region.

Conclusions—The unique findings of this study were 2-fold. First, changes in regional geometry after MI were associated with changes in MMP levels. Second, a region-specific portfolio of MMPs was induced after MI and was accompanied by a decline in TIMP levels, indicative of a loss of MMP inhibitory control. Targeting the regional imbalance between specific MMPs and TIMPs within the post-MI myocardium holds therapeutic potential. (Circulation. 2003;107:2857-2863.)

Key Words: metalloproteinases • myocardial infarction • remodeling

Although significant extracellular remodeling occurs after myocardial infarction (MI), the basis for this remodeling process remains poorly understood. The matrix metalloproteinases (MMPs) are a family of proteolytic enzymes that contribute to left ventricular (LV) remodeling.1,2 Endogenous MMP activity is controlled by the tissue inhibitors of metalloproteinases (TIMPs).1–5 Past clinical studies have demonstrated alterations in MMPs and TIMPs after MI.6,7 Experimental studies have provided evidence for a cause-and-effect relationship between MMP induction and the post-MI myocardial remodeling process.1,8–10 A large number of MMPs exist and have been classified into general categories that include the collagenases, the gelatinases, the stromelysins/matrilysins, and the membrane-type MMPs. However, the regional distribution of these myocardial MMP types in relation to TIMP levels after MI and the relationship of this proteolytic system to regional remodeling remains to be established. Accordingly, the goal of the present study was to examine the regional distribution of myocardial MMPs and TIMPs in a post-MI animal model and to test the hypothesis that a direct relationship exists between changes in regional geometry and MMP and TIMP abundance in the post-MI myocardium.

Methods

Animal Instrumentation
Adult Dorset hybrid sheep (n=10; Thomas Morris, Reisterstown, Md) were anesthetized and instrumented as described previously.11 Briefly, snares were placed around the left anterior descending and second diagonal coronary arteries. Sonomicrometry transducers were placed as described below. The animals were allowed to recover for a minimum of 10 days before the induction of an MI. All animals

Received December 19, 2002; revision received February 27, 2003; accepted March 1, 2003.
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Circulation is available at http://www.circulationaha.org DOI: 10.1161/01.CIR.0000068375.40887.FA
region circumscribed by transducer locations, resulting in the 7 regions shown in Figure 1. The LV was maintained rigorously at 4°C during this sampling, and the sections were immediately flash-frozen. Parallel transmural sections (1 cm) were immediately immersed in a buffered 4% formalin solution for histochemistry. Normal myocardial samples were obtained from age-matched control Dorset hybrid sheep (n=8). Because a region-specific demarcation of the MI zone in the sheep model has been demonstrated, the sonomicrometric chord pairs were presented in a geometric relationship to the LV and categorized as remote, transition, and MI regions.

MMP and TIMP Abundance
For this study, the relative abundances of MMPs corresponding to each class were examined by quantitative immunoblotting, which has been described in detail previously. Specifically, regional myocardial abundance of the interstitial collagenases (MMP-1, MMP-13, and MMP-8), the gelatinases (MMP-9, MMP-2), the matrixins (MMP-3, MMP-7), and the membrane-type MMP (MT1-MMP) were examined from LV myocardial extracts (2.0 μg) loaded onto 4% to 12% BisTris gels and subjected to electrophoretic separation. The separated proteins were then transferred to a nitrocellulose membrane. After a blocking and washing step, the membranes were incubated in antisera (0.2 μg/mL) corresponding to MMP-1 (IM35L, Oncogene Research Products), MMP-13 (AB8114, Chemicon International), MMP-8 (PC493, Oncogene), MMP-9 (IM10L, Oncogene), MMP-2 (MAB3308, Chemicon), MMP-3 (IM35L, Chemicon), MMP-7 (AB8118, Chemicon), and MT1-MMP (AB815, Chemicon). The TIMPs were measured in identical fashion using specific antisera for TIMP-1 (AB8116, Chemicon), TIMP-2 (IM11L, Oncogene), TIMP-3 (IM43L, Oncogene), and TIMP-4 (AB816, Chemicon).

After incubation with a secondary antibody, immunoreactive signals were detected by chemiluminescence (Western Lightning Chemiluminescence Reagent Plus, Perkin Elmer). Recombinant standards (Chemicon or Oncogene) were included in all immunoblots as positive controls. The immunoblots were analyzed by densitometric methods. All measurements were performed in triplicate, and preliminary studies confirmed that these assays were linear with increasing amounts of protein (1 to 4 μg).

MMP Substrate Activity
LV myocardial extracts (5 μg protein) were subjected to electrophoresis followed by either gelatin (Novex 10% zymogram gel, 0.1% gelatin, Invitrogen) or casein (Novex 4% to 16% zymogram gel, 0.1% casein, Invitrogen) zymography. The size-fractionated MMP proteolytic regions were quantified by densitometry (Gel Pro Analyzer, Media Cybernetics).

Myocardial Histology
Qualitative histology of the remote, transition, and MI regions with respect to tissue type and cell types was examined in paraaffin-embedded sections (5 mm) stained with hematoxylin and eosin or were subjected to immunohistochemistry for α-smooth muscle actin (1:100 dilution, Sigma).

Data Analysis
End-systolic chord length at 8 weeks after MI was normalized to respective baseline values and computed as a percentage. End-systolic chord length data were subjected to ANOVA and pairwise separation with the Bonferroni adjusted t test. Myocardial MMP and TIMP levels were computed as a percentage of reference control values. MMP and TIMP levels were compared by 2-way ANOVA in which the main effects were the presence of MI and region. Pairwise contrasts were performed by post hoc Bonferroni comparisons. All statistical procedures were performed with Systat (SPSS). Results are presented as mean±SEM. Values of P<0.05 were considered statistically significant.
**TABLE 1. Regional LV End-Systolic Chord Lengths at Baseline and 8 Weeks After MI**

<table>
<thead>
<tr>
<th>Region</th>
<th>Remote</th>
<th>Transition</th>
<th>MI</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>2–4</td>
<td>4–6</td>
<td>6–8</td>
</tr>
<tr>
<td>Baseline, mm</td>
<td>14.7±1.0</td>
<td>14.6±1.8</td>
<td>11.9±1.0</td>
</tr>
<tr>
<td>Post-MI, mm</td>
<td>18.7±2.1</td>
<td>16.9±2.3</td>
<td>14.7±1.4</td>
</tr>
<tr>
<td>Change from baseline, %</td>
<td>20.5±3.5 †</td>
<td>15.1±3.1 †</td>
<td>23.3±4.5 †</td>
</tr>
</tbody>
</table>

Values are presented as mean±SEM. *P<0.05 vs baseline; †P<0.05 vs region 2–4; ¶P<0.05 vs region 10–12; §P<0.05 vs region 8–10; |P<0.05 vs remote regions (2–4, 4–6, and 6–8); ||P<0.05 vs region 12–14; |||P<0.05 vs remote regions (2–4, 4–6, and 6–8).

**Results**

Baseline (pre-MI) LV end-diastolic volumes were 64±3 mL and increased to 104±6 mL at 8 weeks after MI (P<0.05). Significant increases in chord lengths that were directionally greater within the MI region (chords 12 to 16) occurred in all regions at 8 weeks after MI (Table 1).

An immunoreactive signal was observed in normal LV myocardium for all MMP types examined (Figure 2). Region-specific changes in MMP types occurred in the post-MI samples (Table 2). Collagenases (MMP-1, MMP-8, and MMP-13) were differentially expressed at 8 weeks after MI. Specifically, MMP-1 levels were not detectable within the MI region, but MMP-8 levels increased by >6-fold, and MMP-13 levels increased by nearly 3-fold. With respect to the gelatinases, MMP-2 levels increased substantially within the transition and MI regions, but MMP-9 levels fell to undetectable levels within the MI region. Immunoreactive signals for stromelysin (MMP-3) appeared as high- and low-molecular-weight forms (Figure 2), with the lower-molecular-weight form being reduced within the MI region (Table 2). Matrilysin (MMP-7) fell within the transition and MI regions. The membrane type 1 MMP, MT1-MMP, increased by >3-fold within the transition and MI regions. An immunoreactive signal was detected for all TIMPs (Figure 3), with the strongest signal observed for TIMP-4. TIMP-4 levels fell by ≈50% within the MI region. TIMP-1, TIMP-2, and TIMP-3 levels were undetectable within the MI region (Table 2).

**TABLE 2. Regional Abundance of Myocardial MMPs and TIMPs at 8 Weeks After MI**

<table>
<thead>
<tr>
<th>Region</th>
<th>Remote</th>
<th>Transition</th>
<th>MI</th>
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<tbody>
<tr>
<td></td>
<td>2–4</td>
<td>4–6</td>
<td>6–8</td>
</tr>
<tr>
<td>Collagenases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-1</td>
<td>92±21</td>
<td>124±29</td>
<td>102±17</td>
</tr>
<tr>
<td>MMP-8</td>
<td>91±10</td>
<td>100±11</td>
<td>78±9*</td>
</tr>
<tr>
<td>MMP-13</td>
<td>100±16</td>
<td>121±16</td>
<td>107±20</td>
</tr>
<tr>
<td>Gelatinases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-2</td>
<td>71±10*</td>
<td>100±41</td>
<td>64±10*</td>
</tr>
<tr>
<td>MMP-9</td>
<td>178±30*</td>
<td>185±24*</td>
<td>175±31*</td>
</tr>
<tr>
<td>Stromelysins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP-3 (HMW)</td>
<td>115±24</td>
<td>100±20</td>
<td>92±25</td>
</tr>
<tr>
<td>MMP-3 (LMW)</td>
<td>163±58</td>
<td>172±62</td>
<td>114±35</td>
</tr>
<tr>
<td>MMP-7</td>
<td>61±9*</td>
<td>101±28</td>
<td>76±12</td>
</tr>
<tr>
<td>Membrane-type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MT1-MMP</td>
<td>100±11</td>
<td>103±16</td>
<td>100±15</td>
</tr>
<tr>
<td>TIMPs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIMP-1</td>
<td>54±9*</td>
<td>89±23</td>
<td>78±13</td>
</tr>
<tr>
<td>TIMP-2</td>
<td>68±9*</td>
<td>80±14*</td>
<td>72±8*</td>
</tr>
<tr>
<td>TIMP-3</td>
<td>62±9*</td>
<td>76±15*</td>
<td>73±11*</td>
</tr>
<tr>
<td>TIMP-4</td>
<td>98±11</td>
<td>109±12</td>
<td>101±9</td>
</tr>
</tbody>
</table>

Values are computed as a percentage of control myocardial values and are presented as mean±SEM. ND indicates not detectable; HMW, LMW, high- and low-molecular-weight bands, respectively. *P<0.05 vs normal myocardium; †P<0.05 vs Region 8–10; §P<0.05 vs Region 10–12; ‡P<0.05 vs Region 8–10; ¶P<0.05 vs Region 12–14; |P<0.05 vs remote regions (2–4, 4–6, and 6–8).
Gelatin zymographic activity increased by 4-fold within the transition and MI regions, whereas caseinolytic activity increased by ~3-fold within all myocardial regions (Table 3).

The relative levels of MMPs and TIMPs within the post-MI myocardium with respect to LV region are demonstrated in Figure 4. Linear regression analysis was performed to determine whether changes in regional LV geometry were correlated to localized changes in MMP and TIMP levels. MT1-MMP levels were associated positively with changes in LV end-systolic chord lengths, whereas TIMP-4 levels were associated negatively with changes in LV end-systolic chord lengths (Figure 5).

Qualitative histological analyses revealed that the transition zone contained islands of viable myocytes interspersed with large bundles of fibrous tissue. Other sections revealed a high density of inflammatory cells (Figure 6). Within the MI region, fibrous tissue was observed and regions of inflammatory cells could be noted. α-Smooth muscle actin staining revealed a high degree of positively stained cells forming a neovascular network, whereas in other regions, clusters of positively stained spindle-shaped cells were observed. Within the MI region, bundles of elongated cells were positive for α-smooth muscle actin.

**Discussion**

LV remodeling after MI is a common structural event and contributes to increased morbidity and mortality. The MMPs have been demonstrated to contribute to LV remodeling after MI. However, whether and to what degree a regional distribution of MMP types or the endogenous TIMPs exists within the post-MI myocardium remained to be examined. The present study used a post-MI sheep model to examine regional levels of myocardial MMPs and TIMPs.

The new and important findings of this study were 2-fold. First, there was a significant induction of MMPs within the post-MI myocardium, which was type and region specific. Second, the abundance of TIMPs declined in a region-specific manner after an established MI. These findings demonstrated that a specific portfolio of MMPs is induced within the MI region and the viable transition zone and is not paralleled by an induction of TIMPs.

The present study builds on these past reports in several important ways. First, through the measurement of a wide range of MMP and TIMP species, the present study demonstrated that a specific portfolio of MMPs was induced in a large-animal MI model of established MI. Second, relative levels of MMPs and TIMPs were altered in a region-specific manner. Increased levels of certain MMPs after MI were accompanied by a substantial reduction in TIMP levels in the transition and MI regions. Quantitative data are presented in Table 2.
MI regions. In the present study, gelatinolytic and caseino-
lytic activities were increased within the MI regions. On the
basis of the immunoblotting results, the increased gelatin
zymographic activity most likely reflects the increased
MMP-2 levels, whereas the increased caseinolytic activity
probably reflects that of MMP-3.15–17 Although these meth-
ods cannot be extrapolated directly to in vivo MMP activity,
the imbalance in MMP/TIMP stoichiometry would suggest
that MMP proteolysis would be heightened within the tran-
sition and MI regions. Nevertheless, neither the role nor the
regional distribution of MMP/TIMP complexes in post-MI
myocardial remodeling was examined in the present study.
Future studies that examine these issues may be appropriate.

The present study demonstrated discordant changes in
MMPs within the post-MI myocardium. Although the basis
for these divergent patterns of MMP expression are likely to
be multifactorial, differences in the gene promoter regions of
MMP types probably contributed to these observations.20
Bioactive molecules such as cytokines, endothelin, and cate-
cholamines can alter MMP levels in vitro.14,21 However, the
same extracellular stimuli may not yield a uniform induction
of MMPs. Extracellular stimuli can cause the formation of
transcription factors, which will bind to the differing numbers
and positions of the promoter elements on MMP genes.20 It
has also been demonstrated that physical stimuli may also
modify MMP and TIMP expression.12,22 Thus, it is likely that
a summation of specific biological and physical stimuli
causd the region- and type-specific expression of MMPs and
TIMPs in the post-MI myocardium.

The present study measured myocardial MMPs and TIMPs
at 2 months after MI. Thus, MMP and TIMP levels were
measured well after the resolution of the initial myocardial
injury; therefore, the MMPs and TIMPs induced during the
acute phase of the MI process were not examined. The early
post-MI period has been associated with induction of MMPs
secondary to the acute inflammatory/injury response.1,5,23,24
Most notably, MMP-9 release from endogenous myocardial
cells and neutrophils has been documented early after
MI.19,23,24 In the present study, MMP-9 levels were reduced
significantly within the transition and MI regions at 8 weeks
after MI.19 This is probably because MMP-9 is associated with
early post-MI events.1,23 In contrast to the acute MI setting, a
different set of MMPs emerged at 8 weeks after MI, which
may have contributed to the LV remodeling process. Inter-
estingly, MMP-8, also localized to neutrophils, was increased
within the transition and MI regions at 8 weeks after MI.

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### TABLE 3. Regional Gelatinolytic and Caseinolytic Levels of Myocardial MMPs at 8 Weeks After MI

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<thead>
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<th>Remote</th>
<th>Transition</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2–4</td>
<td>4–6</td>
<td>6–8</td>
</tr>
<tr>
<td>Gelatinolytic</td>
<td>116±19</td>
<td>137±24</td>
<td>86±9</td>
</tr>
<tr>
<td>Caseinolytic</td>
<td>391±27</td>
<td>312±28†</td>
<td>352±35*</td>
</tr>
</tbody>
</table>

Values are computed as a percentage of control myocardial values and are presented as mean±SEM.

*P<0.05 vs normal myocardium; †P<0.05 vs Region 2–4; ‡P<0.05 vs Region 6–8; §P<0.05 vs Region 8–10; †P<0.05 vs all remote regions (2–4, 4–6, and 6–8).
Histological examination of the transition and MI regions revealed a cellular infiltrate that most likely reflected macrophages and other cell types associated with a more chronic inflammatory response. In a longitudinal study in rats, MMP-8 levels were not increased until weeks after MI induction. Consistent with recent reports in human post-MI myocardium, the present study identified cells positive for \( \alpha \)-smooth muscle actin within the transition and MI regions. These cells probably reflect smooth muscle cells within areas of neovascularization and active myofibroblasts. Thus, the potential cellular sources for the MMPs induced within the transition and MI regions included residual viable myocytes, smooth muscle cells, fibroblasts, and macrophages. However, it is likely that the cell types responsible for the elaboration of MMPs within the post-MI myocardium are time dependent. Future studies that examine the temporal expression profile of MMPs and TIMPs in the post-MI period are warranted.

The present study demonstrated increased levels of MMP-13 and MT1-MMP after MI, which may have particular significance with respect to pathological remodeling. Specifically, MMP-13 is increased in end-stage cardiomyopathies and aggressive breast carcinomas. MMP-13 degrades fibrillar collagens and therefore may contribute to myocardial extracellular remodeling. Increased MT1-MMP levels within the transition and MI regions may have particular significance, for 2 reasons. First, MT1-MMP degrades a wide range of extracellular matrix proteins. Second, MT1-MMP can proteolytically process soluble pro-MMPs, such as pro-MMP-13, and thereby amplify local proteolytic activity. The LV regions in which this local induction of MT1-MMP and MMP-13 occurred were paralleled by decreased TIMP levels. The present study demonstrated that increased MT1-MMP levels and decreased TIMP-4 levels were correlated to the extent of regional LV remodeling. This regional imbalance between these specific MMPs and TIMPs probably contributed to continued regional expansion in the post-MI myocardium. Future studies that directly modify regional expression of MMPs and TIMPs will be necessary to define the relationship between MMP activation and expression and regional expansion after MI.

In post-MI animal models, a cause-and-effect relationship between myocardial MMP activation and remodeling has been demonstrated through the use of broad-spectrum pharmacological MMP inhibition. However, broad-spectrum MMP inhibition may not be a practical approach for modifying post-MI remodeling clinically because of possible systemic effects. The present study clearly demonstrated that a specific induction of MMPs occurred late in the post-MI period. For example, MMP-1 levels decreased in the transition region and were undetectable in the MI region. A recent study demonstrated that an MMP inhibition strategy in which MMP-1 activity was spared effectively reduced LV dilation in a rabbit post-MI model. Taken together, these results suggest that targeting specific MMPs that are induced locally within the post-MI myocardium may represent a promising and novel therapeutic target.

Acknowledgments

This study was supported in part by National Institutes of Health grants HL-59165, HL-36308, HL-63594, and PO1-HL-48788-08 and grants from the Mary L. Smith Charitable Trust, Newtown Square, Pa, and the W.W. Smith Charitable Trust, Newtown Square, Pa.
References


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Circulation. 2003;107:2857-2863; originally published online May 27, 2003;
doi: 10.1161/01.CIR.0000068375.40887.FA
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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