Targeted Imaging of the Spatial and Temporal Variation of Matrix Metalloproteinase Activity in a Porcine Model of Postinfarct Remodeling

Relationship to Myocardial Dysfunction

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Background—Matrix metalloproteinases (MMPs) are known to modulate left ventricular (LV) remodeling after a myocardial infarction (MI). However, the temporal and spatial variation of MMP activation and their relationship to mechanical dysfunction after MI remain undefined.

Methods and Results—MI was surgically induced in pigs (n = 23) and cine magnetic resonance (MR) and dual-isotope hybrid single-photon emission CT (SPECT)/CT imaging obtained using thallium-201 and a technetium-99m-labeled MMP targeted tracer (99mTc-RP805) at 1, 2, and 4 weeks post-MI along with controls (n = 5). Regional myocardial strain was computed from MR images and related to MMP zymography and ex vivo myocardial 99mTc-RP805 retention. MMP activation as assessed by in vivo and ex vivo 99mTc-RP805 imaging and retention studies was increased nearly 4-fold within the infarct region at 1 week post-MI and remained elevated up to 1 month post-MI. The post-MI change in LV end-diastolic volumes was correlated with MMP activity (y = 31.34e^{0.48x}, P = 0.04). MMP activity was increased within the border and remote regions early post-MI, but declined over 1 month. There was a high concordance between regional 99mTc-RP805 uptake and ex vivo MMP-2 activity.

Conclusions—A novel, multimodality, noninvasive hybrid SPECT/CT imaging approach was validated and applied for in vivo evaluation of MMP activation in combination with cine MR analysis of LV deformation. Increased 99mTc-RP805 retention was seen throughout the heart early post-MI and was not purely a reciprocal of thallium-201 perfusion. The 99mTc-RP805 SPECT/CT imaging may provide unique information regarding regional myocardial MMP activation and predict late post-MI LV remodeling. (Circ Cardiovasc Imaging. 2011;4:381-391.)

Key Words: matrix metalloproteinases • ventricular remodeling • imaging

Left ventricular (LV) remodeling after myocardial infarction (MI) is characterized by infarct expansion, progressive LV dilation, hypertrophy of noninfarct zones, and overall global ventricular remodeling.1-4 Clinical and animal studies have demonstrated an association between the development of adverse LV remodeling after MI and poor outcomes.1,2,5,6 The changes in anatomic structure or function associated with post-MI LV remodeling are routinely evaluated with imaging modalities, including echocardiography, MRI, and cine radiograph CT, or even conventional nuclear-based approaches, such as single-photon emission CT (SPECT). Although changes in LV size and shape appear to be a direct measurement of remodeling, these indices do not provide information on the underlying molecular processes, which then may be used as surrogate markers of LV remodeling.1

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A cause-and-effect relationship has been established between LV remodeling and activation of the matrix metalloproteinases (MMPs).7-12 However, defining the spatial and temporal variation of MMP activation after MI in relationship to LV remodeling has been difficult because of the lack of a direct in vivo approach to assess MMP activity. Studies have demonstrated the feasibility of targeted noninvasive imaging with a technetium-99m-labeled MMP-targeted radiotracer...
MMP activity and LV geometry and mechanics is critical to better understanding the mechanism underlying LV remodeling after MI. Accordingly, the aims of the current study were 2-fold: (1) validate an MMP-targeted radiotracер approach for in vivo evaluation of MMP activation after MI, comparing radiotracer-derived indices of regional MMP activation with quantitative MMP zymography, and (2) use hybrid SPECT/CT imaging of MMP activation in combination with MRI-derived indices of regional myocardial deformation to relate temporal changes in regional MMP activation with changes in regional myocardial deformation.

Methods

Surgical Preparation

MI was surgically induced in male Yorkshire pigs (n = 23; weight, 25 kg) as previously described. Briefly, MI was induced through the ligation of the first 2 obtuse marginal branches of the circumflex artery, which results in a uniform MI size involving \( \approx 22\% \) of the LV in a region positioned on the posterior-lateral wall. After a 7- to 10-day recovery period, the pigs were randomly assigned to undergo cine 3D cardiac MRI and in vivo SPECT/CT imaging, with euthanasia at 1 week (n = 9), 2 weeks (n = 6), or 4 weeks (n = 8) after MI. An additional group of nonoperated controls (n = 5) were used for reference imaging and biochemical studies. All experimental protocols were approved by the Institutional Animal Care and Use Committees at the Medical University of South Carolina and the Yale University School of Medicine. Studies were performed according to the National Institutes of Health guidelines for Care and Use of Laboratory Animals (1996).

Imaging Protocol

MRI

Images were acquired on a Siemens Sonata 1.5-T magnet using a multiple-phase echocardiography-triggered true fast imaging with steady-state precession sequence (repetition time, 47.2 ms; echo time, 1.58 ms; field of view, 340 mm; slice thickness, 5 mm; interslice gap, 1 mm; flip angle, 60\(^\circ\)). After orienting the acquisition plane along both long axes of the heart, contiguous 5-mm-thick short-axis images were obtained with an end-expiratory breathhold achieved by transient disconnection of the ventilator. The final image resolution was 1.5 x 1.5 x 5 mm. The raw images were then converted to a 4D DICOM format.

MRF Analysis

The MRIs were semiautomatically segmented from below the membranous septum down to the apex from end diastolic to end systole using a modified version of ITK-SNAP\(^\text{16}\) and BioImage Suite.\(^\text{17}\) Local shape properties of the endocardial and epicardial surfaces were used to derive 3D trajectories for a dense field of points on the surface of the heart over the cardiac cycle. These trajectories were then used to deform a mesh that represented the LV myocardial volume through a continuum biomechanics model. This integrated shape-based approach for tracking regional myocardial deformation has been validated previously in both 2 and 3 dimensions.\(^\text{17}\)

In Vivo SPECT/CT Imaging and Reconstruction

SPECT/CT images were acquired (GE Infinia Hawkeye) ungated with a 360\(^\circ\) acquisition orbit (32 stops/head, 20 seconds/stop), zoom factor of 1.3, and 128 x 128 matrix size. In vivo SPECT/CT imaging was performed at 112 \pm 14 minutes after intravenous injection of 99mTc-RP805 (25 mCi), a macrocyclic compound that binds to the catalytic domain on MMPs\(^\text{11}\) to show localization of regions where MMPs are activated. This procedure was followed by dual-isotope imaging 15 minutes after intravenous injection of thallium-201 (201TI) (3 mCi). Images were obtained using 15\% windows centered on the photopeaks of 201TI (75 \pm 5 keV) and 99mTc (140 \pm 5 keV). SPECT/CT transaxial images were reconstructed as described previously.\(^\text{14}\) The 201TI images were used for reorientation of the heart into standard short- and long-axis views. The 201TI and 99mTc-RP805 images were batch reconstructed to ensure exact orientation.

Ex Vivo Imaging and Sampling

Immediately after in vivo SPECT/CT imaging, pigs were euthanized and the heart extracted to measure 201TI and 99mTc radioactivity as previously described. Briefly, the hearts were filled with an alginate material and then cut into 5-mm-thick short-axis slices. One slice containing the infarct region was divided into 8 epicardial and endocardial radial sectors and flash frozen in liquid nitrogen for subsequent MMP zymography. The remaining LV slices were placed on the collimator of a gamma camera (GE Millenium VG), and planar images were acquired using the same energy peaks of 201TI and 99mTc. These LV slices also were divided into 8 epicardial and endocardial radial sectors (16 total sectors) for gamma well counting. All reported values for regional myocardial 201TI and 99mTc-RP805 retention were derived from gamma well counting of tissue samples. Absolute myocardial uptake was computed as a percentage of the injected dose per gram tissue after correcting for background activity and radioactive decay. The radial segments from the slice used for zymographic analysis were compared with the corresponding segments above and below those analyzed for radiotracer activity.

MMP Zymography, Immunoblotting, and Activity Assay

The radial sections were maintained at \(-80\)\(^\circ\)C to allow for radioactive decay (\(\approx 30\) days), and then LV extracts were prepared for MMP zymographic analysis. Briefly, LV homogenates (2 \(\mu\)g protein) were subjected to electrophoresis using gels containing denatured collagen, and the proteolytic bands corresponding to proMMP-9 (92 kDa), proMMP-2 (72 kDa), and the active form of MMP-2 (64 kDa) were digitized. MMP-7 (primary antibody Ab38996) (Abcam) and membrane type 1 (MT1)-MMP (Open Biosystems) levels were determined by immunoblotting.

A specific global MMP fluorogenic substrate (Enzo Life Sciences BML-P126) (excitation, 330 nm; emission, 405 nm) was used to measure MMP activity within the tissue homogenate. Briefly, 30 \(\mu\)mol/L of the fluorogenic MMP substrate was incubated with myocardial tissue extract (25 \(\mu\)g), and fluorescence of the cleaved substrate was recorded (FLUOstar; BMG Labtech). The fluorescence signal was converted to MMP activity (nanogram per gram wet weight) on the basis of a calibration curve determined using active MMP-2 catalytic domain (Enzo Life Sciences BML-SE237) and the initial wet tissue weight of the homogenate.

Data Analysis

End-diastolic volumes were indexed to body mass, and values at each post-MI time point were compared with ANOVA, using time as the independent variable and adjusted for multiple comparisons using Dunnett test, with the non-MI pigs designated as the comparison control group. Selected midventricular MRIs were manually aligned and registered with the postmortem slices using structural features. The LV radial segments analyzed by zymography were aligned by sector with adjacent segments from the wall count. To develop a relationship among perfusion, MMP tracer uptake, and MMP zymography, a 2D histogram was constructed. For each LV section, the MMP tracer values and zymographic values were normalized on a scale from 0 to 100, and these values were assigned to each of the 16 sectors and displayed in a concentric circle format for the endocardial and epicardial regions using a colorimetric

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\(^\text{99mTc-RP805}\) for the in vivo detection of regional myocardial MMP activation after MI. The current study extended these initial findings to a relevant preclinical post-MI porcine myocardial deforma-
registration. Regional 201TI and 99mTc-RP805 uptake and MMP expression and activity were subjected to 2-way ANOVA, using time post-MI and LV region as independent factors. Given that the myocardial samples for each region were obtained from the same pigs, an adjustment for a within-group effect was performed by including each pig as a random effect variable within the ANOVA design. Post hoc separation of means was performed using Bonferroni-adjusted pairwise comparison of means (STATA module prcomp). Measured 99mTc-RP805 activity was correlated with quantitative estimates of total MMP activity for each MMP subspecies derived from the zymography, using standard curve fitting.

Average radial strain was then computed for the infarct, border, and remote regions for each animal and compared between groups using ANOVA, which included animal as a random-effects variable. Means for each region were analyzed using Welch t test. Statistical analyses were performed using STATA version 8.0 (Stata Corp; College Station, TX) or BMDP (Statistical Solutions; Saugus, MA) software. Values are presented as mean±SEM. P<0.05 was considered statistically significant.

Results

Post-MI Model
Animals demonstrated comparable hemodynamics during the serial MRI and SPECT imaging. LV end-diastolic volumes indexed for body mass in the control pigs was 1.43±0.10 mL/kg. Consistent with LV remodeling after MI,9,10 LV end-diastolic volume index was increased by 59±8% over control values by 1 week post-MI (2.17±0.12 mL/kg, P<0.05), remained elevated over control values at 2 weeks post-MI (2.09±0.14 mL/kg, P<0.05), and was further increased by 4 weeks post-MI (2.24±0.11 mL/kg, P<0.05) versus controls.

Dual-Isotope In Vivo SPECT/CT Imaging and Ex Vivo Planar Imaging
In vivo dual-isotope (99mTc-RP805 and 201TI) SPECT/CT images from representative MI pigs at 1 week, 2 weeks, and 4 weeks post-MI showed a 201TI perfusion defect in the posterolateral wall with corresponding focal 99mTc-RP805 uptake within the perfusion defect (Figure 1A). The maximal 99mTc-RP805 uptake was observed at 2 weeks post-MI. Ex vivo planar dual-energy imaging of LV slices confirmed the colocalization of the perfusion defect and the region of maximal increase in MMP tracer uptake (Figure 1B), although 99mTc-RP805 uptake also was seen in remote regions at 1 and 2 weeks post-MI. There was an excellent correspondence between the in vivo and ex vivo images (Figure 1C).

Gamma Well Counting of 201TI and 99mTc-RP805 Radioactivity
Myocardial segments were segregated into infarct, border, and remote regions based on the regional quantitative 201TI activity from well counting as illustrated in Figure 2. Specifically, myocardial segments in which 201TI uptake was <60% of the remote region were designated as the infarct region, and the 2 myocardial segments immediately adjacent to the infarct region (on either side of the infarct region) were designated as the border region. Time-dependent changes in 201TI and 99mTc-RP805 retention are summarized in Table 1, and the relationship between 201TI and 99mTc-RP805 activity in the infarct, border, and remote regions is shown in Figure 3. At 1 and 2 weeks post-MI, there was increased in 99mTc-RP805 retention in all myocardial regions, with a >4-fold increase in 99mTc-RP805 activity within the infarct region compared with controls. By 4 weeks post-MI, 99mTc-RP805 retention returned to control levels in the remote region but remained higher than control levels in the infarct and border regions.

Comparison of 201TI and 99mTc-RP805 Activity With MMP Zymography
Changes in the levels of MMP2, MMP-7, MMP-9, and MT1-MMP and MMP activity are summarized in Table 2. There were distinct spatial and temporal patterns in the expression of the various MMP types in the post-MI period. For example, MMP-9 levels within the MI region were elevated in the earlier post-MI time points and then were lower than 1-week post-MI values at 4 weeks post-MI. MMP-7 and MT1-MMP levels within the border region were elevated at 1 week post-MI but were similar to control levels by 4 weeks post-MI. MMP activity, which was measured as a function of cleavage of an MMP substrate,20 was increased within the MI region at 1 week post-MI and was further elevated at 4 weeks post-MI. To provide a representation of the spatiotemporal changes in MMP-targeted radiotracer retention and MMP zymographic levels, the normalized distribution of average values were computed for the endocardial and epicardial regions for each LV sector and are pictorially depicted in Figure 4. A clear spatial and temporal concordance was observed among the perfusion defect, 99mTc-RP805 retention, and MMP levels, particularly for the active form of MMP-2 (Figure 5). The segment-by-segment correlation coefficient between the active form of MMP-2 and absolute 99mTc-RP805 retention was excellent at 1 week post-MI (r=0.78, P<0.01), although this relationship continued to declined between 2 weeks (r=0.72) and 4 weeks (r=0.59) post-MI. There was an exponential relationship between the post-MI change in LV end-diastolic volume and MMP activity (y=31.34e0.48x, r=0.38, P=0.04) (Figure 6).

Comparison of 99mTc-RP805 Retention With Regional Myocardial Strain
The interaction between altered LV mechanics and MMP activation was assessed by comparing regional myocardial 99mTc-RP805 retention with regional myocardial thickening (radial strain) at different time points after MI (Figure 7). At 1 week post-MI, there was an 4-fold increase in 99mTc-RP805 retention in the dyskinetic infarct region compared with referent control values. Although the border region initially demonstrated a similar degree of dysfunction to the infarct region with near-zero radial strain, 99mTc-RP805 retention was much lower than in the central infarct region. There was also increased 99mTc-RP805 retention in the remote regions of the heart, suggesting global activation of MMPs early after MI associated with the global remodeling process. At 2 weeks post-MI, the infarct and border regions remained dysfunctional, and again, a significant increase in 99mTc-RP805 retention was seen in all 3 regions. By 4 weeks post-MI, regional 99mTc-RP805 retention increased only in the infarct region, which remained akinetic and dyskinetic.
Animal models have demonstrated a clear cause-and-effect relationship between MMP activation and LV remodeling after MI. In addition, clear changes in plasma MMP profiles occur in patients after MI, which are related to indices of post-MI outcomes. However, plasma profiling is a surrogate marker, and a direct relationship to myocardial MMP activation after MI and to the LV remodeling process remains to be established. Thus, critical translational research development would include an approach for noninvasively
detecting and quantifying in vivo MMP activity after MI. Accordingly, the present study used a combined molecular-mechanical approach to define the relationship between regional changes in MMP activity and regional mechanics in a well-established and clinically relevant porcine model of post-MI remodeling. The significant and unique findings of this study were 2-fold. First, a validated MMP-targeted radiotracer was used for in vivo evaluation of MMP activation and allowed comparison of quantitative radiotracer-based indices of regional MMP activation with quantitative MMP zymography. Second, a hybrid SPECT/CT imaging approach for quantifying MMP activation was used in combination with MRI-derived myocardial strains to quantify the temporal and spatial variation of MMP activation with regional myocardial strain during the initial 4 weeks post-MI. This noninvasive, multimodality approach demonstrated that

Table 1. Regional and Time-Dependent Changes in Gamma Well Counting After MI

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Remote</th>
<th>Border</th>
<th>MI</th>
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<th>MI</th>
<th>Remote</th>
<th>Border</th>
<th>MI</th>
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<tbody>
<tr>
<td><strong>Perfusion</strong> (201TI) $\times 10^{-2}$</td>
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<tr>
<td>Endo</td>
<td>107.3±2.9</td>
<td>110.0±1.8</td>
<td>100.7±2.1</td>
<td>46.9±4.8††</td>
<td>109.4±1.9</td>
<td>94.0±5.2††</td>
<td>42.6±4.7††</td>
<td>108.8±3.0</td>
<td>93.5±2.2 ††</td>
<td>41.9±3.4 ††</td>
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<tr>
<td>Epi</td>
<td>95.7±2.1</td>
<td>88.9±2.2</td>
<td>83.2±3.1</td>
<td>40.0±3.3† ††</td>
<td>86.9±2.8</td>
<td>94.6±6.9§</td>
<td>46.7±6.3††</td>
<td>94.6±2.4</td>
<td>83.7±2.7††</td>
<td>38.9±4.4 ††</td>
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<td>Total</td>
<td>101.5±2.5</td>
<td>100.0±1.4</td>
<td>91.8±2.3 † †</td>
<td>43.2±2.3 † †</td>
<td>98.2±2.2</td>
<td>92.3±3.2 †</td>
<td>47.2±5.4 † †</td>
<td>101.7±1.4</td>
<td>90.9±1.1 † †</td>
<td>40.7±3.9 † †</td>
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</table>

| **MMP tracer** $^{99mTc}$-RP805, $\times 10^{-4}$ |         |        |        |          |        |        |          |        |        |          |
| Endo             | 11.3±2.5 | 13.7±1.3 | 16.6±2.1 | 40.0±5.3 † † | 15.6±1.9 | 17.9±2.4 | 52.8±8.5 † † § | 6.8±1.5 | 10.1±1.9 | 26.1±3.7 † † § |
| Epi              | 11.6±2.1 | 14.9±1.6 | 19.4±2.2 | 38.6±4.7 † † | 17.7±2.1 | 21.3±1.7 | 42.5±7.7 † † | 7.6±1.5 | 10.4±1.8 † † | 24.5±3.2 † † |
| Total            | 11.5±2.3 | 13.8±1.5 | 18.3±2.0 | 39.7±4.8 † † | 16.7±1.9 | 21.3±1.7 | 42.5±7.8 † † | 7.2±1.5 | 9.8±1.8 † † | 24.5±2.7 † † |

Sample size, n 5 9 9 9 6 6 6 8 8 8

Data are presented as mean±SEM. All data are corrected for tissue weight (milligrams). Endo indicates endocardial; Epi, epicardial; MI, myocardial infarction; $^{99mTc}$, technetium-99m; 201TI, thallium-201.

$^*P<0.05$ vs remote (same week post-MI).
$^†P<0.05$ vs control.
$^‡P<0.05$ vs border (same week post-MI).
$§P<0.05$ vs 1 week post-MI (same region).
$^||P<0.05$ vs 2 weeks post-MI (same region).
Spatial and Temporal Changes in 99mTc-RP805 Retention in Relationship to Regional MMP Activation

An earlier study from our laboratory using a rodent model of MI provided proof of concept that 99mTc-RP805 could be used to provide in vivo localization of myocardial MMP activity.

Table 2. Regional and Time-Dependent Changes in Levels of MMPs After MI

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<th>Control</th>
<th>Remote</th>
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<tr>
<td>Endo</td>
<td>90±17</td>
<td>222±107</td>
<td>510±135</td>
<td>3543±1130†‡</td>
<td>41±11</td>
<td>68±31</td>
<td>2210±854†‡</td>
<td>142±78</td>
<td>344±148</td>
<td>750±465§</td>
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<tr>
<td>Epi</td>
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<td>326±154</td>
<td>574±203</td>
<td>2097±505†‡</td>
<td>42±17</td>
<td>62±18</td>
<td>1704±1111†‡</td>
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<td>208±79</td>
<td>657±235§</td>
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<tr>
<td>Total</td>
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<td>318±144</td>
<td>539±144</td>
<td>2596±596†‡</td>
<td>42±13</td>
<td>200±86</td>
<td>1604±789†‡§</td>
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<td>107±13</td>
<td>462±129</td>
<td>3190±629†‡</td>
<td>73±30</td>
<td>150±48</td>
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<td>735±284</td>
<td>1743±419†‡§</td>
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<td>Epi</td>
<td>63±25</td>
<td>132±23</td>
<td>571±217</td>
<td>3387±717†‡</td>
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<td>535±145</td>
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<td>362±99</td>
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<td>434±126</td>
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<tr>
<td>Endo</td>
<td>1.5±0.2</td>
<td>8.1±3.0*</td>
<td>15.9±9.6‡</td>
<td>12.5±6.8*</td>
<td>6.1±2.5*</td>
<td>9.8±5.1*</td>
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<td>MT1-MMP</td>
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<tr>
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<td>27.1±5.3*</td>
<td>34.0±6.4*</td>
<td>20.8±5.3*</td>
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<td>25.8±4.7*§</td>
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<td>11.1±2.6</td>
<td>12±9.3*§</td>
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<tr>
<td>Total</td>
<td>9.9±0.7</td>
<td>18.0±2.1*</td>
<td>21.5±3.1*</td>
<td>26.2±4.2*</td>
<td>17.2±2.9*</td>
<td>28.7±6.1*</td>
<td>30.9±5.3*</td>
<td>12.6±1.4</td>
<td>13.7±1.9</td>
<td>19.5±5.0*</td>
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<td>MMP activity, ng/mg per h x 10^-1</td>
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<tr>
<td>Endo</td>
<td>2.3±0.4</td>
<td>5.5±0.7</td>
<td>4.9±0.9</td>
<td>6.3±0.8*</td>
<td>4.8±0.5</td>
<td>5.1±0.5</td>
<td>6.2±1.1</td>
<td>3.3±0.5</td>
<td>4.7±1.0</td>
<td>10.4±2.0‡§</td>
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<tr>
<td>Epi</td>
<td>2.0±0.3</td>
<td>4.2±0.2</td>
<td>3.9±0.4</td>
<td>5.2±0.6</td>
<td>3.4±0.4</td>
<td>4.3±0.5</td>
<td>7.4±1.3*</td>
<td>2.9±0.3</td>
<td>3.4±0.4</td>
<td>8.2±1.8*‡§</td>
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<tr>
<td>Total</td>
<td>2.2±0.3</td>
<td>4.8±0.4*</td>
<td>4.3±0.6</td>
<td>5.6±0.6*</td>
<td>4.1±0.3</td>
<td>4.8±0.4</td>
<td>6.6±1.0*</td>
<td>3.1±0.2</td>
<td>3.9±0.6</td>
<td>9.3±1.7*‡§</td>
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</table>

Data are presented as mean±SEM. All data are corrected for tissue weight (milligrams). MMP indicates matrix metalloproteinase; MT1, membrane type 1. Other abbreviations as in Table 2.

*P<0.05 vs control.
†P<0.05 vs remote (same week post-MI).
‡P<0.05 vs border (same week post-MI).
§P<0.05 vs 1 week post-MI (same region).
P<0.05 vs 2 weeks post-MI (same region).

Figure 3. Gamma well counting of 201Tl and 99mTc-RP805 radioactivity. Correlation of the average relative myocardial 201Tl activity and 99mTc-RP805 activity for infarct, border, and remote myocardial regions in pig hearts at 1, 2, and 4 weeks after myocardial infarction (MI) as determined by gamma well counting. Notice that the relative 201Tl perfusion deficit does not change over time, although there are dramatic time-dependent changes in 99mTc-RP805 activity. These data suggest that 99mTc-RP805 imaging may provide unique information after MI. *P<0.05 vs remote. †P<0.05 vs border region. #P<0.05 vs 1 week post-MI. *P<0.05 vs 2 weeks post-MI. Abbreviations as in Figure 1.
activation in the setting of LV remodeling after MI. However, it must be recognized that significant differences exist with respect to processes that contribute to LV remodeling after MI in rodents compared with humans. First, the portfolio of MMPs expressed in mice is notably different from that in larger mammalian species, including humans. Second, MI sizes in mice often are considerably larger than that in other larger mammalian species, which may activate a different set of MMPs during the progression of LV remodeling after MI. Finally, given the high intrinsic heart rate in mice, it is technically difficult to determine relationships between regional MMP activation and mechanical function after MI. Accordingly, the findings of the present study build on those of the past rodent study by demonstrating that 99mTc-RP805 may be effectively used to localize MMP activation in a clinically relevant, large-animal MI model using a conventional clinical SPECT imaging system.

Changes in the myocardial levels of several MMP types occur with distinct temporal trajectories in the post-MI period. For example, MMP-9 levels achieve peak values early in the post-MI period and decline over longer durations. In contrast, the increase in MMP-2 levels is sustained over longer post-MI periods. Consistent with these findings, levels of both MMP-2 and MMP-9 in the present study were increased within the infarct region at all post-MI time points. However, although the increase in MMP-2 levels was sustained through 4 weeks post-MI, there was a time-dependent decline in MMP-9 levels at the later post-MI time points. This observation is in agreement with a previous study that demonstrated temporal and spatial changes of MMP-2,
MMP-8, MMP-13, and MT1-MMP levels in border, remote, and infarct regions.\(^\text{18}\) In the present study, the greatest retention of the MMP-targeted radiotracer \(^{99m}\text{Tc-RP805}\) was observed within the infarct region at 2 weeks post-MI. \(^{99m}\text{Tc-RP805}\) retention correlated with changes in the levels of active MMP-2, MMP-7, MMP-9, and MT1-MMP at early post-MI time points. Myocardial \(^{99m}\text{Tc-RP805}\) retention remained elevated within the infarct and border regions at 2 and 4 weeks post-MI and correlated with MMP-2 zymographic levels. The significance of these findings is 2-fold. First, gamma well counting of \(^{99m}\text{Tc-RP805}\) demonstrates a complex spatial and temporal variation of MMP activity after MI. Second, the correlation between in vivo \(^{99m}\text{Tc-RP805}\) retention and ex vivo measurements of MMP levels validates the use of targeted radiotracers as means to longitudinally track fundamental molecular processes that contribute to LV remodeling after MI.

**Relationship Between Regional Deformation and MMP Activation**

Mechanical dysfunction after MI has been hypothesized to cause increased MMP activation, potentially leading to further infarct expansion and exacerbation of LV dilation after MI.\(^\text{2,9}\) Some in vitro models have shown that fibroblasts grown on mechanically relaxed collagen I produced high levels of MMP-2 and MT1-MMP,\(^\text{26}\) whereas others have shown that cyclic stretching of myofibroblasts causes increased MT-MMP activity.\(^\text{27}\) Altering the mechanical activation pattern of a region of the LV free wall in vivo increased MMP-9 levels, interstitial MMP activity, and increased collagen degradation.\(^\text{19,28}\) Rohde et al\(^\text{8}\) reported that increased wall stress in the border zones was associated with increased MMP-9 activity. The present study builds on these previous investigations by directly correlating regional LV strain with MMP activation at selected time points after MI. Specifically,
at 1 week post-MI there was a significant correlation between MMP activation and mechanical dysfunction in the infarct and border regions, although our analysis of the relationship between changes in regional MMP activation and mechanical functioning after MI was restricted to systolic strain. One may hypothesize that other mechanical events, including localized shear forces and diastolic dysfunction, may contribute more to MMP activation and LV remodeling. Nevertheless, our results support a complex relationship between regional LV mechanics and MMP activation.

**Prediction of LV Remodeling**

LV remodeling after MI can vary from patient to patient in terms of LV dilation and changes in pump function. Studies have provided evidence that several factors, including, but not limited to, infarct size, the transmurality of injury, extent of myocardial viability, and post-MI wall stress, are correlated with the degree of LV dilation after MI. Targeted imaging of MMPs, which are central to LV remodeling, provides an attractive approach to follow the process of LV remodeling in vivo and a potential means to predict late outcome after MI. Our earlier work demonstrated the feasibility of $^{99m}$Tc-RP805 for target imaging of MMP activation in vivo in a mouse model of MI. The present study extends those observations to a more clinically relevant porcine model of transmural infarction and defines the regional spatial and temporal changes in $^{99m}$Tc-RP805 retention in relation to changes in myocardial strain and global LV geometry. These dynamic changes in $^{99m}$Tc-RP805 retention were not directly associated with specific regional changes in $^{201}$TI perfusion or myocardial strain, suggesting that $^{99m}$Tc-RP805 imaging might provide unique information about the LV remodeling process after MI. The retention of $^{99m}$Tc-RP805 was not purely a reciprocal of $^{201}$TI activity and may provide unique information about regional MMP activation that can be derived in vivo with noninvasive SPECT imaging. Perhaps more importantly, a significant increase in $^{99m}$Tc-RP805 retention was observed in the remote regions, which may provide a better predictor of late global remodeling than other estimates of MI size or molecular events within the infarct region.

**Limitations**

In the present study, the MI was created surgically through a left lateral thoracotomy that resulted in significant chest wall inflammation and increased focal retention of $^{99m}$Tc-RP805 in the chest wall immediately over the injured region of the heart, complicating the evaluation of myocardial radiotracer retention. Therefore, clinical $^{99m}$Tc-RP805 SPECT/CT images are likely to be of higher quality than those in the pigs. In our correlation of $^{99m}$Tc-RP805 retention with MMP activity, we were restricted to analyzing radial segments from a single short-axis slice of the heart with segments from immediately adjacent slices that were used for gamma well counting of myocardial $^{99m}$Tc-RP805 activity. Additional analyses at even earlier time points (1 day post-MI and 3 days post-MI) or in the setting of ischemia/reperfusion and nontransmural infarction may further help to elucidate the factors involved in postinfarct LV remodeling. In the present study, changes in LV geometry and MMP activation were determined during terminal examination at each of the designated...
post-MI time points; therefore, longitudinal post-MI changes in LV geometry and MMP activation could not be determined. Finally, the dose of $^{201}$TI and $^{99m}$Tc-RP805 was selected based on standard clinical dosing for imaging with our clinical SPECT/CT camera, and ratios of the 2 radiotracers were selected to optimize in vivo dual-isotope imaging and facilitate reliable gamma well counting. However, these doses would represent approximately 3 times the standard clinical dose used (based on subject weight). These doses per subject weight could be easily reduced for clinical translation, particularly with application of newer solid-state multidetector imaging technology that provides $\approx$5 times the sensitivity of a conventional camera.

Conclusions
A novel, multimodality, noninvasive hybrid SPECT/CT imaging approach was validated and applied for in vivo evaluation of MMP activation in combination with cine MRI analysis of LV deformation in a clinically relevant porcine model to quantify the temporal and spatial variation of MMP activation and regional myocardial strain during the initial 4 weeks after MI. Past studies have provided evidence of cause-and-effect relationships between MMP activation and LV remodeling after MI. $^{7-9}$ Moreover, plasma profiling of MMPs has been suggested to hold prognostic value in terms of predicting LV remodeling after MI. $^{21-23}$ However, plasma levels of MMP can represent spillover from multiple compartments and may not directly reflect net myocardial MMP activity. The present study provides proof of concept that direct high-quality in vivo imaging of myocardial MMP activation is feasible on a standard clinical hybrid SPECT/CT system and may provide a clinically applicable means to determine the effects of MMP activation within the context of LV remodeling after MI.

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We are grateful to the many people at both Yale University and the Medical University of South Carolina without whose diligence and help the experiments presented in this article would not have been possible. In particular, we thank Patti Cavaliere, Christi Hawley, Jennifer Hu, Grace Chung, Donna Dione, and Christopher Weyman from Yale University and G. Patricia Escobar, Stuart M. Saunders, and Julie E. McLean from the Medical University of South Carolina.

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Disclosures
None.

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**CLINICAL PERSPECTIVE**

Left ventricular (LV) remodeling after myocardial infarction (MI) remains an important cause of morbidity and mortality. Although changes in LV geometry can be clinically assessed using a variety of imaging modalities, these images do not address fundamental underlying mechanisms that contribute to LV dilation after MI. A number of animal studies have demonstrated a causal relationship between LV dilation after MI and activation of the matrix metalloproteinases (MMPs). In the present study, a technetium-99m-labeled compound (99mTc-RP805) designed to bind to the catalytic domain of a number of MMPs was used to image in vivo MMP activation after MI. The myocardial spatial distribution and specificity of this MMP-targeted compound was validated through direct comparisons with ex vivo immunoblotting and zymography for the determination of regional MMP levels and activity. Regional in vivo MMP activation also was correlated with temporal changes in regional myocardial strain and post-MI remodeling. The post-MI change in LV end-diastolic volumes correlated with MMP activity. High-quality in vivo images of 99mTc-RP805 retention were obtained on a standard clinical hybrid single-photon emission CT/CT system, suggesting that this approach holds potential for extension to imaging MMP activation in patients after MI, with potential value for risk stratification and directing therapy. Thus, 99mTc-RP805 single-photon emission CT/CT imaging may provide unique information regarding regional myocardial MMP activation and predict late post-MI LV remodeling.
Targeted Imaging of the Spatial and Temporal Variation of Matrix Metalloproteinase Activity in a Porcine Model of Postinfarct Remodeling: Relationship to Myocardial Dysfunction

Zakir H. Sahul, Rupak Mukherjee, James Song, Jarod McAteer, Robert E. Stroud, Donald P. Dione, Lawrence Staib, Xenophon Papademetris, Lawrence W. Dobrucki, James S. Duncan, Francis G. Spinale and Albert J. Sinusas

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