Noninvasive Targeted Imaging of Matrix Metalloproteinase Activation in a Murine Model of Postinfarction Remodeling

Haili Su, MD; Francis G. Spinale, MD, PhD; Lawrence W. Dobrucki, PhD; James Song, MD; Jing Hua, MD; Sarah Sweeterlitz, BS; Donald P. Diene, BS; Patti Cavaliere, BS; Conroy Chow, BS; Brian N. Bourke, MS; Xiao-Yu Hu, MD; Michael Azure, PhD; Padmaja Yalamanchili, PhD; Richard Liu, PhD; Edward H. Cheesman, PhD; Simon Robinson, PhD; D. Scott Edwards, PhD; Albert J. Sinusas, MD

Background—Time-dependent activation of matrix metalloproteinases (MMPs) after myocardial infarction (MI) contributes to adverse left ventricular (LV) remodeling; however, noninvasive methods to monitor this process serially are needed.

Methods and Results—MMP-targeted radiotracers were developed that displayed selective binding kinetics to the active MMP catalytic domain. Initial nonimaging studies were performed with a $^{111}$In-labeled MMP-targeted radiotracer ($^{111}$In-RP782) and negative control compound ($^{111}$In-RP788) in control mice (Ctrl) and in mice 1 week after surgically induced MI. Localization of $^{111}$In-RP782 was demonstrated within the MI by microautoradiography. A 334±44% increase ($P<0.001$ versus Ctrl) in relative retention of $^{111}$In-RP782 was confirmed by gamma well counting of myocardium. Subsequent high-resolution dual-isotope planar and hybrid micro–single-photon emission computed tomography/CT imaging studies with an analogous $^{99m}$Tc-labeled MMP-targeted radiotracer ($^{99m}$Tc-RP805) and $^{201}$Tl demonstrated favorable biodistribution and clearance kinetics of $^{99m}$Tc-RP805 for in vivo cardiac imaging, with robust retention 1 to 3 weeks after MI in regions of decreased $^{201}$Tl perfusion. Gamma well counting yielded a similar $\approx 300\%$ increase in relative myocardial retention of $^{99m}$Tc-RP805 in MI regions (Ctrl, 102±9%; 1 week, 351±77%; 2 weeks, 291±45%; 3 weeks, 292±41%; $P<0.05$ versus Ctrl). Myocardial uptake in the MI region was also significantly increased $\approx 5$-fold when expressed as percentage injected dose per gram tissue. There was also a significant 2-fold increase in myocardial activity in remote regions relative to control mice, suggesting activation of MMPs in regions remote from the MI.

Conclusions—This novel noninvasive targeted MMP radiotracer imaging approach holds significant diagnostic potential for in vivo localization of MMP activation and tracking of MMP-mediated post-MI remodeling. (Circulation. 2005;112:3157-3167.)

Key Words: myocardial infarction • metalloproteinases • remodeling • radionuclide imaging

The adverse changes in left ventricular (LV) structure and geometry that occur after a myocardial infarction (MI) have been called myocardial remodeling. This remodeling process can give rise to LV dysfunction and progressive heart failure.1,2 The relative rate and extent of this adverse remodeling process have been demonstrated to be independent predictors of morbidity and mortality in post-MI patients. Although the mechanisms for post-MI remodeling are multifactorial, it has now been established that a family of proteolytic enzymes, the matrix metalloproteinases (MMPs), contribute to this process.3–5 Specifically, past animal studies have demonstrated a cause-and-effect relationship between MMP induction and the post-MI remodeling process through the use of MMP inhibition and transgenic modification of MMP expression.6–10 However, understanding the interrelationship between MMP activation and the post-MI remodeling process has been hampered by the inability to directly measure MMP activity in vivo. To date, profiling MMP activation after MI has been possible only through tissue sampling and biochemical methods.11–13 These past in vitro approaches remove the MMPs from natural substrates and interaction with an endogenous family of tissue inhibitors.
of MMPs. Therefore, defining the spatial and temporal profile of MMP activation in the post-MI setting using these in vitro approaches is problematic. Accordingly, the overall goal of this study was to develop a novel and highly sensitive MMP-targeted imaging approach that could be used in a murine model of post-MI remodeling. We hypothesized that this approach could be used to examine spatial and temporal changes in MMP activation after MI.

**Methods**

The studies described below were performed in a stepwise manner using adult (2-month-old) inbred C57BL/6 mice of both sexes. The first set of studies was to confirm binding and specificity of the MMP radiotracer in normal control and post-MI mice. The second set of studies used targeted imaging of post-MI mice at discrete intervals after MI. All studies were performed with the approval of the Institutional Animal Care and Use Committee and according to the guiding principles of the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals (National Research Council, Washington, DC, 1996).

**Surgical Induction of MI**

An anterolateral MI was induced by surgical ligation of the main left coronary artery via a left lateral thoracotomy as described previously. Under isoflurane anesthesia (3% in oxygen), mice were placed in the supine position, and the trachea was intubated and ventilated at a tidal volume of 1 mL and 200 cycles/min. Under sterile technique, a left thoracotomy was performed in the fourth intercostal space. After the pericardium had been opened, the left anterior descending artery was ligated near its origin with 6-0 Prolene and an atrumatic needle (Ethicon, K801). The incisions were then closed. The surgical mortality rate (either because of sudden death or a requirement for euthanasia because of deteriorating status) was 15%. It has been demonstrated previously that this approach results in a 40% to 45% infarct size of the LV free wall.

**MMP-Targeted Radiotracers**

Initial nonimaging studies were performed with a 111In-labeled MMP-targeted radiotracer (111In-RP782) and a 111In-labeled negative control enantiomeric compound (111In-RP788). The chemical structures of these 111In-labeled compounds are shown in Figure 1A and 1B. Subsequent imaging studies were performed with a similar MMP-targeted 99mTc-labeled compound (99mTc-RP805). The structure of 99mTc-RP805 is shown in Figure 1C. The portion of this compound that binds to the exposed catalytic domain of MMP is structurally similar to the 111In-labeled compounds. These macrocyclic compounds were developed by Bristol-Myers Squibb Medical Imaging on the basis of the previously reported structures of MMP inhibitors. On the basis of the enzymatic assays performed previously and those described below, the structure of these MMP tracers bound specifically to MMPs and not other proteases. These compounds bind to the activated catalytic domain and therefore exhibit enzyme inhibitory profiles consistent with MMP inhibition.

**MMP Inhibitor Assays**

We performed inhibition assays of the nonradiolabeled ligand of RP805 (50686-095A) for MMP-2, -3, -7, -9, -12, and -13. For this assay, a selected human MMP (R&D Systems) was activated at 37°C in the presence of 1 mmol/L aminophenylmercuric acetate for 1 hour. The level of active MMP was quantified by active site titration in the presence of 1 mmol/L aminophenylmercuric acetate for 1 hour. Residual MMP activity was determined by measuring fluorescence with monitoring at \( \lambda_{\text{ex}} \) 320 nm and \( \lambda_{\text{em}} \) 395 nm. Nonlinear regression was used to determine the \( K_i \) (enzyme inhibition constant, mmol/L) of the nonradiolabeled ligand.

**Binding of 99mTc-RP805 to MMP-2**

Active MMP-2 (0.05 nmol/L) was preincubated with 99mTc-RP805 (0.25 \( \mu \)Ci) at 37°C for 1 hour in 200 \( \mu \)L of 50 mmol/L Tris-HCl (pH 7.5), 10 mmol/L CaCl2, and 150 mmol/L NaCl. The unbound 99mTc-RP805 was removed by filtering through Centrifree columns. To determine nonspecific binding to the filters, 99mTc-RP805 was incubated in reaction buffer with no MMP-2. Active MMP-2 (0.05 nmol/L) was preincubated with 10 \( \mu \)mol/L of one of several commercially available MMP inhibitors (GM6001, MMPI-III, or MMP-II, Calbiochem, EMD Biosciences) for 15 minutes at 4°C, and 99mTc-RP805 (0.25 \( \mu \)Ci) was added to the reaction mixture. The reaction mixture was incubated at 37°C for 1 hour. The unbound 99mTc-RP805 was removed by filtering through Centrifree columns. The MMP-2–bound 99mTc-RP805 was determined by counting the activity in the column in a gamma well counter.

**Experimental Protocols**

For the terminal studies, the mice were anesthetized as previously described, and the left jugular vein was isolated for placement of a polyethylene catheter (PE-10, Becton-Dickinson) to facilitate serial injection of radiotracers.

**Stage I**

Initial nonimaging studies were performed in normal control mice \((n = 9)\) and mice 1 week after MI \((n = 13)\), injected intravenously with either 0.77 ± 0.05 mCi of 111In-RP782 or 1.04 ± 0.02 mCi of 111In-RP788. Group IA mice were injected with 111In-RP782 (post-MI, \(n = 9)\); control mice, \(n = 5)\), and group IB mice were injected with 111In-RP788 (post-MI, \(n = 4); control mice, \(n = 4)\). Hearts were excised 90 minutes after radiotracer injection for postmortem tissue microautoradiography and gamma well counting.
Stage II
99mTc-RP805 was injected in control mice (n=4), sham-operated mice (n=3), and separate groups of mice at 1 week (n=16), 2 weeks (n=12), and 3 weeks (n=13) after MI. In this stage of the protocol, imaging studies were attempted in a total of 48 mice. All mice had a static 15-minute planar pinhole image or pinhole micro-single-photon emission computed tomography (SPECT)/CT imaging performed at 75 minutes after radiotracer injection. The mice included in the initial phase of stage II analysis (n=26) were injected intravenously with 1.5±0.1 mCi of 99mTc-RP805 for both in vivo pinhole planar and/or hybrid pinhole micro-SPECT x-ray CT pinhole imaging and postmortem tissue analysis. This was followed by intravenous injection of 0.25±0.05 mCi of 201Tl and repeat dual-isotope static planar pinhole or pinhole micro-SPECT/CT imaging 15 minutes after 201Tl injection. In this protocol, 9 of 26 mice underwent additional serial imaging after the initial injection of 99mTc-RP805 to evaluate the biodistribution and clearance of the MMP-targeted radiotracer. After completing the imaging, hearts were excised and prepared for gamma well counting, immunohistochemistry, and in situ zymography.

An additional series of mice had dual-isotope micro-SPECT/CT imaging performed at either 1 week (n=5) or 3 weeks (n=3) after MI with 99mTc-RP805 (2.0±0.4 mCi) and 201Tl (0.30±0.06 mCi) after the intravenous administration of 0.15 mL of an x-ray contrast agent (FenestraVC, AlerionBio) to provide improved definition of the myocardium. The CT scans were acquired as outlined below. These SPECT images were acquired with an upgraded micro-SPECT detector (matrix size, 82×82) with improved spatial resolution.

Postmortem Tissue Preparation and Analyses
The mice were euthanized, and the hearts were quickly excised and maintained in a chilled saline solution. The excised hearts were filled with dental molding material (alginite impression material, type II, non-set; Quala Dental Products) by retrograde infusion through the pulmonary artery and aorta to facilitate cutting. The hearts were cut into short-axis slices (~1 mm thick). One selected slice from each heart was frozen in embedding compound (optimal cutting temperature [O.C.T.]). Tissue-Tek (Sakura) using a bath of 2-methylbutane cooled on dry ice and cut into 15-μm and 5-μm sections for microautoradiography and histological studies, respectively. The remaining heart slices were cut into 4 transmural sections and weighed for gamma well counting.

In Situ Zymography and Immunofluorescence
Using the previously prepared LV frozen sections, in situ zymography was performed as described previously. A solution of 0.1 mg/mL of a custom-synthesized quenched fluorescent substrate of gelatin (DQ gelatin, D12054; Molecular Probes) contained within a ×1 developing buffer (50 mM/L Tris base, 40 mM/L HCl, 200 mM/L NaCl, 5 mM/L CaCl2, 2 H2O, and 0.2% [wt/vol] Brij 35) and 50 mM/L phenylmethanesulfonyl fluoride was placed onto the myocardial sections. Sections were incubated at 37°C for 3 hours, washed 3 times to remove unbound gelatin, and visualized by use of an FITC excitation filter mounted on a Zeiss Axioptik-2. LV sections were imaged at a final magnification of ×25 and digitized (AxioCam MR, Zeiss). Parallel sections were treated in an identical manner, but 500 mM/L of EDTA was included and served as negative controls.

In addition, LV frozen sections were subjected to immunofluorescent colocalization of MMP-2 and MMP-9 by use of methods described previously. LV frozen sections were immersed in acetone for 30 seconds, washed twice in ice-cold PBS, and then incubated at 4°C with the primary anti-sera at a 1:100 dilution (goat anti-MMP-2; Santa Cruz [sc-8825]; rabbit anti-MMP-9, Chemicon [AB19047]) overnight. The primary anti-sera were validated for specificity by initial immunoblotting studies, and the concentrations used for these studies were determined by initial titration experiments. The selection of these antisera were predicated on minimizing cross-reactivity to murine myocardium. After vigorous washing, the sections were then incubated with conjugated fluorescent secondary antibodies (Alexa 488 donkey anti-goat, Alexa 647 donkey anti-rabbit; Molecular Probes) at 1:200 at 4°C for 2 hours. The sections were then imaged at ×25 with a confocal fluorescence microscope (Leica Microsystems Inc, TCS SP2A0BS, MUSC HCC Molecular Imaging Facility).

Microautoradiography
Microautoradiography was performed in group I mice after injection of the 111In-labeled compounds. Slices were dried at −20°C and exposed to high-resolution x-ray film (BioMax, Eastman Kodak Co) for 48 hours. Microautoradiographs were digitized for qualitative analyses and correlation with images derived from comparable stained histological sections.

Gamma Well Counting
Unstained heart slices were then cut into 4 segments for gamma well counting (Cobra Packard) of 201Tl, 99mTc, and 111In activity using appropriate energy windows (201Tl, 50 to 90 keV; 99mTc, 120 to 160 keV; 111In, 170 to 300 keV). Raw counts were corrected for spill-up/spill-down, background, decay, and weight. Corrected counts were converted to mCi/g by use of a previously determined counter efficiency.

Relative regional myocardial activity was determined by normalizing activity in selected segments within the central infarct area to the average activity of the remote region and expressed as a percentage of nonischemic area. Activity in each myocardial segment was also calculated as percentage of injected dose (%ID). For this calculation, the tissue activity was corrected for decay to the time of radiotracer injection. The calculated %ID was computed by dividing corrected tissue counts (mCi/g) by the corrected injected dose (mCi) and expressed in %ID per gram tissue.

In Vivo Pinhole Planar and Micro-SPECT/CT Imaging
Planar Image Acquisition
The mice were placed in an anteroposterior position for planar imaging using a large-field-of-view gamma camera (GE Millennium, General Electric) and a planar pinhole collimator with a 1-mm aperture. This collimator provided ×10-fold magnification. Selected animals underwent serial dynamic planar pinhole images acquired every 30 seconds for the initial 15 minutes after injection of 99mTc-RP805, followed by serial 15-minute images over the following 60 minutes. All group II mice underwent late 99mTc-RP805 imaging 75 minutes after injection and again after injection of 201Tl. Images were acquired by use of an energy window of 78 keV ±7.5% for 201Tl and 140 keV ±10% for 99mTc. The late images were acquired for 15 minutes for better image definition.

Micro-SPECT/CT Acquisition
A subset of mice from each experimental group in the initial phase of stage II were injected with 99mTc-RP805 via the jugular catheter for micro-SPECT/CT imaging using a dedicated high-resolution small-animal hybrid imaging system (GammaMedica, X-SPECT) as an alternative to the planar imaging. This imaging approach permitted better localization of the radioactivity with the myocardium, as well as separation from surrounding structures. After the injection of 99mTc-RP805, ungated CT imaging was performed (energy, 50 kVp/600 μA; matrix, 512×512; distance to the center of rotation, 64 mm) to identify the anatomic structure. One hour after the initial injection of 99mTc-RP805, micro-SPECT images were acquired by use of the following acquisition parameters: 32 projections, 30 seconds per projection, 4 cm to the center of rotation, 140 keV photopeak ±10% window). After completion of the 99mTc-RP805 micro-SPECT imaging, 201Tl was injected to define regional myocardial perfusion and delineate the area of infarction. Five minutes later, another micro-SPECT image was acquired (32 projections, 60 seconds per projection, 4 cm to the center of rotation, 78 keV photopeak ±15% window). Immediately after acquisition of the 201Tl micro-SPECT image, the animals were euthanized.
Planar Image Analyses
Dynamic images were analyzed by use of the Xeleris Functional Imaging Workstation (General Electric). Several regions of interest (ROIs) were placed in locations over selected organs to calculate the dynamic $^{99m}$Tc-RP805 average activity per pixel of heart, lung, liver, gallbladder, and kidneys. For each animal, the activities of each organ were normalized by the final lung activity per pixel measured at 15 minutes after injection.

Micro-SPECT/CT Image Reconstruction and Analyses
The CT projection images were reconstructed by use of commercial software (Cobra, Exxim Computing Corp) that implemented a cone-beam reconstruction algorithm. Pinhole $^{201}$TI and $^{99m}$Tc-RP805 micro-SPECT images were reconstructed by use of system software (LumaGEM, Segami Corp) and filtered after reconstruction by use of a Butterworth filter (cutoff, 0.4 Nyquist frequency; order, 6). Reconstructed micro-SPECT images were reoriented according to the CT anatomic images, fused, and exported in “Analyze” format (Analyze, Mayo Clinic) for further processing using Amide Medical Imaging Data Examiner (amide.sf.net).

Statistical Analyses
All data are presented as mean±SEM. Comparisons between the control and MI groups were made by use of either a paired or unpaired Student $t$ test, as appropriate. Multiple comparisons were performed by ANOVA followed by an adjusted $t$ test. Differences between groups were considered significant at a value of $P<0.05$ (2-tailed).

Results
Inhibition Assays
Inhibition assays were performed with the nonradiolabeled ligand of $^{99m}$Tc-RP805 by use of several MMPs known to play an important role in post-MI remodeling. The individual $K_i$ values derived for the nonradiolabeled compound for the specific MMPs evaluated are as follows: MMP-2, 10.5 nmol/L; MMP-3, 14 nmol/L; MMP-7, <6.4 nmol/L; MMP-9, 7.4 nmol/L; MMP-12, <6 nmol/L; and MMP-13, 7.3 nmol/L. The inhibition curve generated for MMP-2 is shown in Figure 2A. Additional inhibition assays were performed for MMP-2 with $^{99m}$Tc-RP805, the radiolabeled compound that was used for in vivo imaging in this study. These results are summarized in Figure 2B. The specificity of binding of $^{99m}$Tc-RP805 to MMP-2 was determined by competition with known MMP-2 inhibitors.

Experimental Protocols
At terminal study, a clearly observable MI region could not be detected in 7 mice, and these mice were excluded from further analysis. Six additional mice were excluded. Three of these mice died during radiotracer injection or imaging, and an additional 3 mice were excluded for technical reasons related to problems with tracer injection or tissue sectioning. The exclusions outlined above resulted in a total of 21 mice included in the stage I protocol (Control, n=9; 1 week MI, n=12) and 26 mice in the stage II protocol (Control, n=4; Sham, n=3; 1 week MI, n=7; 2 weeks MI, n=6; 3 weeks MI, n=6). The presence of post-MI remodeling is evident by the dramatic changes in heart weight. The average heart weight in the control mice (stages I and II) was $87±11$ mg, whereas

Figure 2. Binding characteristics of $^{99m}$Tc-RP805 and nonradiolabeled ligand. Inhibition of MMP-2 activity by the nonradiolabeled ligand (50686-118A) of $^{99m}$Tc-RP805 is shown (A). Binding of $^{99m}$Tc-RP805 to MMP-2 was studied by use of purified activated human MMP-2 (B). Specificity of binding of MMP-2 to $^{99m}$Tc-RP805 was demonstrated by competition with known MMP-2 inhibitors (GM6001, MMPI III, MMPI II).

Figure 3. Morphology and $^{111}$In-RP782 and $^{111}$In-RP788 micro-autoradiography of short-axis myocardial sections from post-MI and control mice from group I. The gross morphological sections demonstrate the extensive infarct size and LV dilation seen in mice at 1 week after MI (a and c) compared with normal control mice (e and g). Microautoradiographs demonstrate focal retentions of $^{111}$In-RP782 in the infarct regions of post-MI mice (b), whereas no focal $^{111}$In-RP788 retention is seen in the infarct region (d). Control mice demonstrated uniform retention of both $^{111}$In-labeled radiotracers (f and h).
heart weight in MI mice was significantly increased (1 week, 113 ± 26 mg; 2 weeks, 127 ± 35 mg; 3 weeks, 127 ± 22 mg; 
P < 0.05 versus control). Gross histology revealed decreased wall thickness of the central MI region and significant LV dilation. The MI region demonstrated infiltration of inflammatory cells on hematoxylin and eosin–stained sections and extensive fibrosis with Masson’s trichrome staining.

Stage I

Microautoradiography

Increased focal retention of 111In-RP782, the MMP-targeted compound, was seen within the infarct region on microautoradiography of infarcted mouse heart sections. However, there was no focal increase in retention of the negative control compound, 111In-RP788, within the infarct region of MI mice. Control mice demonstrated a uniform uptake of both 111In-labeled radiotracers. Figure 3 provides an example of typical microautoradiographs obtained from this portion of the study.

Gamma Well Counting

The significant increase in 111In-RP782 activity within the infarct region that was observed by microautoradiography was confirmed by gamma well counting of myocardial tissue sections (Figure 4A). The retention of both radiotracers within the MI region was first expressed as a percentage of the remote noninfarcted (%NI) region. 111In-RP782 activity within the MI region was 3- to 4-fold higher than in remote regions at 7 days after MI. In contrast, no significant increase in relative 111In-RP788 activity (negative control) was seen within the MI area. In the control mice, there was no significant increase in either 111In-RP782 or 111In-RP788 retention from the anterior wall relative to the posterior wall. Myocardial 111In-RP782 and 111In-RP788 activity in the central infarct region and remote noninfarcted region was also expressed as a %ID/g. These results are summarized in Figure 4B and 4C. Myocardial 111In-RP782 activity, quantified by use of this alternative approach, was increased nearly 3-fold in the infarct region (2.9 ± 0.8%ID/g) at 1 week after MI, compared with activity (0.9 ± 0.1%ID/g) in the same territory in control mice (Figure 4B). Regional uptake of 111In-RP788 was similar in all regions of both infarcted and control mice (Figure 4C).

Stage II

Gamma Well Counting

In mice injected with 111In-RP805, the relative myocardial retention of 99mTc-RP805 was significantly increased in the MI region at all time points after MI compared with the non-MI remote region. In contrast, relative myocardial 201Tl activity was significantly reduced within the MI region compared with non-MI remote values. A 351% increase in the retention of 99mTc-RP805 was observed at 1 week after MI, which was slightly higher than that observed at 2 weeks and 3 weeks after MI. However, the differences between the infarct groups did not achieve statistical significance (P = NS). These results are summarized in Figure 5A. Re-
Regional myocardial $^{99m}$Tc-RP805 activities in infarcted and remote noninfarcted regions were also expressed as a %ID per gram of tissue and compared with those of control mice to evaluate MMP activation in remote noninfarcted regions. Data from control mice that did not undergo a thoracotomy and sham-operated mice were not different, and therefore, these groups were combined to create a single control group ($n=7$). These data are summarized in Figure 5B. By use of this quantitative approach, myocardial $^{99m}$Tc-RP805 activity in the infarct region was increased approximately 5-fold over that of control mice. Myocardial $^{99m}$Tc-RP805 activity in the remote noninfarcted region was increased nearly 2-fold over that of control mice; however, these differences achieved statistical significance only at 2 and 3 weeks after MI.

**In Situ Zymography**
Representative in situ zymograms from a control normal LV section (non-MI) and after 7 or 14 days after MI are shown in Figure 6. In the non-MI sections, minimal gelatinase activity could be documented. However, in the post-MI sections, significant green fluorescence, consistent with gelatinolytic activity, was observed within the MI region. Interestingly, by 14 days after MI, regions of gelatinolytic activity could be observed beyond the MI region and extended into the viable border regions.

**Immunofluorescence**
MMP-2 and MMP-9 colocalization by use of immunofluorescence is shown in Figure 6. In normal control (non-MI) LV sections, minimal staining for MMP-2 could be appreciated, with no staining for MMP-9. However, significant and robust staining for both MMP-2 and MMP-9 occurred after MI, and significant colocalization could be appreciated within the MI region. This staining pattern paralleled the in situ zymography results. In addition, MMP-2 and MMP-9 could be localized to remote regions of the LV, indicative of an upregulation of these MMPs after MI. The distribution of these specific MMPs was similar to that observed on the
microautoradiographs obtained with $^{111}$In-RP782, the $^{111}$In-labeled radiotracer targeted at broad-spectrum MMP activation.

**Dynamic Planar Imaging**

The early dynamic in vivo planar pinhole images demonstrated favorable initial biodistribution and clearance kinetics of $^{99m}$Tc-RP805 for cardiac imaging of MMP activation (Figure 7A). $^{99m}$Tc-RP805 cleared rapidly from the blood and was excreted primarily by the kidneys. Faint uptake of $^{99m}$Tc-RP805 was seen in the heart of infarcted mice, although image interpretation is complicated by uptake of the radiotracer in the chest wall after the thoracotomy. About half of these animals showed prominent uptake in the gallbladder, although very little uptake was seen in the liver, even as early as 15 minutes after injection. Initial clearance kinetics from all critical organs was quantified by placing ROIs on the dynamic image sets, and values were normalized by activity in the lung at 15 minutes after injection. The average clearance curves derived from all 9 mice that underwent dynamic imaging are shown in Figure 7B.

The late $^{99m}$Tc-RP805 planar pinhole images, at 90 minutes after radiotracer injection, demonstrate faint uptake of tracer in the area of the $^{201}$Tl perfusion defect (not shown). However, some $^{99m}$Tc-RP805 uptake was also seen in the overlying chest wall at the site of the thoracotomy. It was difficult to separate myocardial $^{99m}$Tc-RP805 uptake from uptake in the chest wall on these in vivo planar images, and therefore, additional animals underwent micro-SPECT/CT imaging.

**Late Dual-Isotope Micro-SPECT/CT Imaging**

Static dual-isotope micro-SPECT/CT imaging was performed at 90 minutes after injection of $^{99m}$Tc-RP805. Representative images are shown in a control mouse and separate mice at 1 week and 3 weeks after MI (Figure 8A). $^{201}$Tl images demonstrated a large anterolateral perfusion defect in all animals, whereas uniform $^{201}$Tl perfusion was seen in control mice. The late $^{99m}$Tc-RP805 pinhole micro-SPECT images demonstrate focal uptake of tracer in the area of the $^{201}$Tl perfusion defect, as well as in the chest wall at the site of the thoracotomy. This overlap was best seen by creating color and black-and-white fusion images (Figure 8A). These in vivo micro-SPECT/CT images clearly demonstrated localization of $^{99m}$Tc-RP805 to the site of the $^{201}$Tl perfusion defect and therefore colocalization to the region of the MI.

**Late Dual-Isotope Micro-SPECT/CT Imaging After X-Ray Contrast Administration**

Static dual-isotope micro-SPECT/CT imaging was performed at 90 minutes after injection of $^{99m}$Tc-RP805 in an additional group of 8 mice, after the administration of FenestraVC, an intravascular contrast agent, to better define the right ventricular and LV myocardium. Representative images are shown in separate mice at 1 week (Figure 8B) and 3 weeks (Figure 8C) after MI. The administration of FenestraVC did improve definition of the myocardial edges and demonstrated LV enlargement and myocardial wall thinning in the infarct territory. In all 8 of these mice, the $^{99m}$Tc-RP805 pinhole micro-SPECT images demonstrated focal uptake of the MMP-targeted radiotracer in the infarct territory, as well as in the thoracotomy site. These in vivo micro-SPECT/CT images with contrast provide better localization of $^{99m}$Tc-RP805 to the site of MI. The use of CT with contrast permitted reconstruction of the MMP-targeted images without the reference $^{201}$Tl perfusion image. This may provide an important advantage over dual-isotope micro-SPECT imaging, because in several mice, the $^{201}$Tl images were of suboptimal quality. Reconstruction of the $^{99m}$Tc-RP805 SPECT images in these mice was extremely difficult without the reference CT image.

**Discussion**

LV remodeling after an MI is a structural milestone in the progression to LV dysfunction and eventually failure. Although the underlying mechanisms responsible for post-MI remodeling are multifactorial, the family of proteolytic enzymes, the MMPs, have been demonstrated to be causally

---

**Figure 6.** Representative in situ zymograms (left) and MMP-2/-9 colocalization using immunofluorescence (right) in control (non-MI) LV sections and in sections after either 7 or 14 days after MI. In situ zymography revealed minimal gelatinolytic activity in non-MI sections but robust activity in post-MI sections. The highest levels of gelatinolytic activity were observed in the MI region (demarcated by dashed circle) but extended beyond this region in many of the 14-day post-MI sections. The insets reflect parallel negative control sections demonstrating specificity of the in situ zymography. In non-MI sections, minimal staining for MMP-2 (green) was observed, with no staining for MMP-9 (red). However, clear and robust colocalization for MMP-2/-9 was observed in the post-MI LV sections, particularly in the MI region (dashed circle). MMP-2 and MMP-9 staining was also observed to extend beyond the MI region, particularly in the 14-day post-MI sections.
related to this process. However, direct in vivo visualization and assessment of relative MMP activation within the myocardium after MI has never been attempted. In the present study, MMP-targeted radiotracers were developed that displayed selective binding kinetics to the active MMP catalytic domain. In a murine model of MI, the present study established through in situ autoradiography, zymography, and immunochemistry that significant and specific binding occurred after MI. Regional localization of the \(^{111}\text{In}\)-labeled MMP-targeted radiotracer (\(^{111}\text{In}-\text{RP782}\)) to immunohistochemical signals for MMP-2 and MMP-9 was established. In addition, the use of a negative control enantiomeric \(^{111}\text{In}\)-labeled MMP radiotracer construct (\(^{111}\text{In}-\text{RP788}\)) abolished selective myocardial retention. Studies were then performed with a \(^{99m}\text{Tc}\)-labeled MMP-targeted radiotracer (\(^{99m}\text{Tc}-\text{RP805}\)). In vitro gamma well counting demonstrated an inverse relation between relative \(^{99m}\text{Tc}\)-RP805 retention and \(^{201}\text{Tl}\) perfusion. Additional analyses of \(^{99m}\text{Tc}\)-RP805 retention as a percentage of the injected dosage demonstrated MMP activation early within the central MI, with subsequent MMP activation in remote noninfarcted regions. High-resolution dual-isotope planar pinhole and hybrid micro-SPECT/CT gamma camera imaging demonstrated a very favorable biodistribution and clearance kinetics for in vivo cardiac imaging. The \(^{99m}\text{Tc}\)-labeled MMP-targeted radiotracer demonstrated a robust retention at 1, 2, and 3 weeks after MI in regions that corresponded to decreased myocardial perfusion, as well as increases in myocardial activity in remote noninfarcted regions at later time points.

**Role of MMP Activation in Post-MI Remodeling**

MMPs constitute a family of proteolytic enzymes that degrade a broad spectrum of ECM proteins and play important roles in normal tissue repair as well as pathological processes such as post-MI remodeling. MMPs are tightly regulated through both transcriptional and posttranscriptional processes and require proteolytic processing to expose the catalytic domain. Therefore, the development of MMP-targeted tracers has been focused on constructs that will selectively bind to the active MMP catalytic domain. Chen et al recently demonstrated the feasibility of ex vivo imaging of MMP activity after MI using a near-infrared fluorescent probe that is activated by the cleavage of 2 of the gelatinases (MMP2 and MMP9). However, application of MMP-targeted tracers or near-infrared fluorescent probes for in vivo myocardial imaging after an MI has not been performed previously. The present study used an MMP-targeted radiolabeled peptidomimetic to visualize time-dependent changes in myocardial activity within the intact mouse after MI. The MMP-targeted radiotracer should not induce significant pharmacological inhibition, on the basis of the observed pharmacokinetics. The dose of the radiotracer (1 mCi/mouse) that we administered to the mice in this study would translate to a picomolar concentration in the blood, which would be 1000-fold lower than the calculated \(K_i\), because a greater than 5 nmol/L concentration was required at the site of action for 50% inhibition of MMPs. In addition, current clinical application of MMP inhibitors requires oral administration multiple times daily over extended periods of time to
achieve significant biological effects.\textsuperscript{10,16} Taken together, the MMP radiotracer will not affect the course of MMP activation within the myocardium and could be used serially to evaluate MMP activation. Past studies have demonstrated that a specific profile of MMP types is induced within the myocardium after MI.\textsuperscript{7,10,15,20,21} Thus, on the basis of the findings from the present study and these past reports, developing methods to visualize which MMP types were specifically activated within the post-MI myocardium is warranted.

Experimental studies have demonstrated that MMP up-regulation evolves over time after MI and contributes directly to LV remodeling.\textsuperscript{9,13} MMP-9 activation has been demonstrated within the myocardial interstitium within 1 hour of ischemia but was reduced significantly at 8 weeks after MI, whereas MMP-8, MMP-13, MMP-2, and MT1-MMP demonstrated a >3-fold increase within the myocardium at 8 weeks after MI. This is consistent with the fact that the induction of some types of MMPs in the early post-MI period are associated with acute inflammation/injury response, whereas the other set of MMPs is involved in the LV remodeling process. This phenomenon was recently confirmed by Chen et al.,\textsuperscript{20} who demonstrated that MMP-9 expression peaked at 2 to 4 days after MI, whereas MMP-2 expression peaked 1 to 2 weeks after MI. In the present study, there was a 3- to 4-fold increase in retention of the MMP radiotracer from 1 week to 3 weeks after MI, which most likely represents activation of both of these sets of MMPs.

The results from the in situ zymography and immunofluorescent studies were not surprising, in that increased MMP abundance and regional activity was demonstrated after MI, consistent with past findings.\textsuperscript{9,10,15,16} The magnitude increase of the MMP-targeted radiotracer within the MI and remote noninfarcted regions was similar to the degree of regional MMP activation obtained in previous experimental studies using other more invasive methodologies.\textsuperscript{9,15,22}

After the in situ and in vitro characterization of the MMP radiotracer, dynamic in vivo pinhole planar and hybrid micro-SPECT/CT imaging was performed and demonstrated that the 99mTc-MMP radiotracer provided a favorable biodistribution and kinetics for cardiac imaging, with the principal clearance of the radiotracer through the kidneys and rapid clearance from the blood. High-resolution dual-isotope hybrid pinhole micro-SPECT/CT

![Figure 8](http://circ.ahajournals.org/)
imaging using this MMP-targeted radiotracer and $^{201}$TI perfusion demonstrated discrete and focal myocardial distribution. Specifically, the MMP radiotracer was localized to the MI region with reduced perfusion. This in vivo imaging pattern for the MMP-targeted radiotracer and perfusion was obtained consistently for different MI mice and at different time points after surgical induction of the MI. We demonstrated that in vivo targeted MMP imaging can be performed in mice either by application of a dual-isotope approach or by hybrid SPECT/CT imaging with an intravenous contrast agent. These results provide the potential for mapping changes in MMP activation in murine models of myocardial injury as well as potentially expanding these studies to larger animals as well as to humans.

Conclusions
The present study demonstrates the feasibility of targeting noninvasive imaging for serial evaluation of regional MMP activation after MI and provides an initial validation of the approach. The application of target radiotracer imaging of regional MMP activation as proposed in the present study holds the potential to directly quantify the extent and localization of MMP activation in vivo and relate these biological events to the post-MI remodeling process. More importantly, the proposed noninvasive imaging approach offers the opportunity to track novel therapeutic interventions directed at MMP inhibition and reduction of post-MI remodeling.

Therapeutic strategies that are targeted at ECM remodeling after MI must take into account the absolute requirement to maximize ECM stability within the infarct region and temporally identify the processes that may contribute to the adverse LV remodeling process. Although surrogate markers for ECM remodeling such as plasma MMPs and collagen degradation products have been described in patients after MI,33–36 real-time spatiotemporal profiling of myocardial MMP activation would provide a novel diagnostic tool in assessing determinants of ECM degradation. However, noninvasive serial quantitative analysis of absolute regional myocardial MMP activation from images would require application of hybrid imaging systems that combine MMP-targeted SPECT or PET imaging with x-ray CT imaging to provide better spatial localization and correction for photon attenuation and partial-volume errors. This type of hybrid noninvasive targeted MMP imaging will probably hold important clinical implications for evaluating new therapeutic interventions designed to reduce post-MI remodeling.

Acknowledgments
This study was supported by NIH grants R01-HL-65662 (Dr Sinusas), R01-HL-59165 (Dr Spinaile), and P01-HL-48788 (Dr Spinaile); a Career Development Award from the Veterans’ Affairs Health Administration (Dr Spinaile); and a basic research grant from Bristol-Myers Squibb Medical Imaging (Drs Sinusas and Spinaile). The radiotracers used in this study were provided by Bristol-Myers Squibb Medical Imaging. We gratefully acknowledge the technical assistance of Patricia Escobar, DVM, Jennifer Hendrick, and Jeffrey Sample. We also acknowledge the guidance of James G. Cama- rano, DMD, in our application of dental molding material for postmortem tissue processing.

Disclosure
Drs Spinaile and Sinusas have sponsored research agreements with Bristol-Myers Squibb Medical Imaging, North Billerica, Mass. Drs Azure, Yalamanchili, Liu, Cheesman, Robinson, and Edwards were employees of Bristol-Myers Squibb Medical Imaging at the time this study was conducted.

References


**CLINICAL PERSPECTIVE**

It is well recognized that left ventricular (LV) remodeling is an independent determinant of morbidity and mortality in patients after myocardial infarction (MI) and is associated with important changes within the myocardial extracellular matrix (ECM). Through both clinical and experimental studies, it is apparent that strategies that alter the course of ECM remodeling after MI can favorably affect the extent of LV remodeling and potentially clinical outcome. The matrix metalloproteinases (MMPs) constitute a large family of proteolytic enzymes responsible for ECM degradation and LV remodeling. Although surrogate markers for ECM remodeling, such as plasma MMPs and collagen degradation products, have been described in patients after MI, real-time in vivo spatiotemporal profiling of myocardial MMP activation would provide both diagnostic and prognostic potential in patients after MI. The present study demonstrates that enhanced expression/activation of specific MMPs can be tracked noninvasively with MMP-targeted radiotracers and multimodality micro–single-photon emission computed tomography/CT imaging and correlated with adverse LV remodeling after MI. The application of target radiotracer imaging of regional MMP activation as proposed in the present study holds the potential to directly quantify the extent and localization of MMP activation in vivo and relate these biological events to the post-MI remodeling process. This novel targeted MMP imaging approach may permit early risk stratification of patients after MI. More importantly, the proposed noninvasive imaging approach offers the opportunity to track novel therapeutic interventions directed at MMP inhibition and reduction of post-MI remodeling.
Noninvasive Targeted Imaging of Matrix Metalloproteinase Activation in a Murine Model of Postinfarction Remodeling


_Circulation_. 2005;112:3157-3167; originally published online November 7, 2005;
doi: 10.1161/CIRCULATIONAHA.105.583021
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/112/20/3157

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/