Assessment of Myocardial Infarction and Postinfarction Scar Remodeling With an Elastin-Specific Magnetic Resonance Agent

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Background—To prospectively evaluate an elastin-specific MR contrast agent (ESMA) for in vivo targeting of elastic fibers in myocardial infarction (MI) and postinfarction scar remodeling.

Methods and Results—MI was induced in C57BL/6J mice (n=40) by permanent ligation of the left anterior descending coronary artery. MRI was performed at 7 and 21 days after MI. The merits of gadolinium-based ESMA (Gd-ESMA) were compared with gadopentetic acid (Gd-DTPA) for infarct size determination, contrast-to-noise ratio (CNR), and enhancement kinetics. Specific binding in vivo was evaluated by blocking the molecular target using nonparamagnetic lanthanum-ESMA. In vivo imaging results were confirmed by postmortem triphenyltetrazolium chloride staining, elastica van Gieson staining, and Western blotting. Delayed enhancement MRI revealed prolonged enhancement of Gd-ESMA in the postischemic scar compared with Gd-DTPA. Infarct size measurements showed good agreement between Gd-ESMA and Gd-DTPA and were confirmed by ex vivo triphenyltetrazolium chloride staining. Preinjection of the blocking lanthanum-ESMA resulted in significantly lower CNR of Gd-ESMA at the infarct site (P=0.0019). Although no significant differences in CNR were observed between delayed enhancement imaging and Gd-DTPA between days 7 and 21 (1.8± versus 3.8; P=ns), Gd-ESMA showed markedly higher CNR on day 21 after MI (14.1 versus 4.9; P=0.0032), which correlated with increased synthesis of tropoelastin detected by Western blot analysis and histology. Higher CNR values for Gd-ESMA further correlated with improved ejection fraction of the mice on day 21 after MI.

Conclusions—Gd-ESMA enables targeting of elastin within the infarct scar in a mouse model of MI. The imaging properties of Gd-ESMA allow quantification of intrascar elastin content in vivo and thereby provide potential for noninvasive characterization of postinfarction scar remodeling. (Circ Cardiovasc Imaging. 2014;7:321-329.)

Key Words: magnetic resonance imaging ■ molecular imaging ■ myocardial infarction ■ ventricular remodeling

Postinfarction scar remodeling after myocardial infarction (MI) can result in a progressive loss of heart function and can ultimately lead to heart failure. The structural pattern of left ventricular (LV) scar remodeling and evidence of scarring on cardiac MRI have been shown to be of prognostic value for the development of heart failure and survival after MI.1 Besides the assessment of infarct size, ventricular architecture, chamber volume, and ventricular function, in vivo targeting of cellular and molecular processes within the injured myocardium by means of molecular imaging may provide novel insights into the pathophysiology of myocardial injury and healing.2 During wound healing and subsequent formation of the fibrotic scar, the synthesis of extracellular matrix (ECM) proteins is upregulated. Of the specific ECM proteins, collagen type I has been found to be a major constituent of scar formation and stabilization. Besides collagen, elastin has been identified as a key ECM protein decisive for infarct stabilization and preservation of ventricular function.3-5

Although the remote myocardium contains elastin only to a negligible degree within the interstitium and within the coronary vasculature, elastic fibers increase within the myocardial scar in the first weeks after ischemic injury and continue to form a dense network among remaining viable myocytes, myofibroblasts, and smooth muscle cells during maturation of the infarct scar.3 MRI, thus, could prove to be a powerful tool for detecting elastic fibers because of its excellent soft-tissue...
contrast and high spatial and temporal resolution. The abundance of elastin within the myocardial scar makes this ECM protein a promising imaging biomarker for molecular MRI. Elastin is expected to be present in the forming scar tissue in high enough concentrations to be detected by an elastin-specific contrast agent. We hypothesized that a novel gadolinium-based elastin-specific MR agent (Gd-ESMA), already successfully applied for elastin targeting within the vessel wall in atherosclerosis, would generate persistent enhancement of the myocardial scar because of binding to elastin fibers. This would allow for noninvasive assessment and monitoring of scar maturation and remodeling and potentially enable the evaluation of novel cardioprotective therapies.

Methods

Animal Model

MI was induced in 40 female C57BL/6J mice by permanent ligation of the left anterior descending artery, leading to a transmural infarct. In brief, mice were intubated endotracheally and mechanically ventilated. A left thoracotomy was performed in the fourth intercostal space, the pericardium removed, and the left anterior descending artery ligated permanently with an 8-0 nylon suture. After thoracotomy, subcutaneous tissue and skin were closed in separate layers and the animal weaned from the ventilator. Imaging was performed on postoperative day (POD) 7 and POD 21 after MI. Mice were anesthetized for all surgical and imaging procedures by general inhalation anesthesia (isofluorane 1.5%–2.5% vol plus 1 L O2). Analgesia was performed by preemptive application of buprenorphine at 0.05 mg/kg SC and carprofen at 4 mg/kg SC. Analgesia was continued for 72 hours after surgery. After completion of the imaging study, mice were euthanized under deep anesthesia, and the hearts were excised and prepared for further ex vivo analysis. All animal experiments were approved by the local subcommittee on Research Animal Care (protocol number 79-09) and performed in accordance with the US National Institutes of Health (NIH publication No. 85-23, revised 1996).

Elastin-Specific MR Agent

ESMA is a gadolinium-based MRI agent (C16H16N6O2Gd) that has been shown to bind with high specificity to elastin (Figure 1). The molecular mass is 855.95 Da. ESMA was prepared from D-homophenylalanine, 4-aminomethylbenzoic acid, and 2-(bis((tert-butyl)oxy)-carbonyl)methyl)-amino)ethyl)amino)acetic acid according to a published patent [PCT Int. Appl. WO 2007/005491 A1, PCT/US2006/025298 (2007)]. Bound and unbound relaxivity of the agent, as well as in vitro/ex vivo binding assays, has been reported previously. It has been shown that binding of radiolabeled Gd-ESMA to plaque-laden rabbit aortas decreased inversely when incubated with increasing concentrations of nonradioactive ESMA. In addition, x-ray spectra of tissue-bound Gd-ESMA were acquired via transmission electron microscopy, which showed a strong colocalization of the targeted gadolinium with elastin fibers.

MRI

MRI was performed on a 1.5-T clinical imaging system (Achieva; Philips Healthcare, Best, The Netherlands) equipped with a standard clinical gradient system (30 mT/m, 200 mT/m per millisecond). Image acquisition was performed under free-breathing conditions and with prospective ECG triggering using a small animal monitoring and gating system (model 1025; SA instruments Inc, Stony Brook, NY). Subcannulated electrodes for detection of the ECG signal were implanted in the region of the chest to obtain a strong QRS signal. Animals were examined in prone position with the heart positioned over the center of a 23-mm surface coil (Philips Healthcare, Best, The Netherlands). During imaging, the animals’ temperature was maintained at 38°C using an MR-compatible heating pad (Bruker Biospin, Ettlingen, Germany). The heating pad was placed below the radiofrequency coil with the mouse lying in a prone position on the coil. This setup has proven as robust to maintain the animals’ core temperature as well as the circulation (blood pressure and heart rates) at stable levels. Calculation of LV function parameters was performed using Osirix (Osiris Foundation, Geneva, Switzerland). After the endocardial borders were traced, the end-diastolic and end-systolic phases were identified, and LV end-systolic volume, LV end-diastolic volume, and LV ejection fraction (EF) were computed from the traced borders. Epicardial contours were similarly traced at the end-diastolic phase to compute the LV mass. LV mass is obtained by multiplying the LV volume with the specific gravity of myocardium $\gamma=1.055$ g/cm$^3$.

After acquisition of standard scout scans, 2-chamber, 4-chamber, and short-axis views were obtained for planning the slice orientations for the assessment of late-gadolinium enhancement (LGE) of the heart. Short-axis cine views were acquired as a 7-slice stack to cover the entire ventricle and for determination of functional parameters. Cine image acquisition was synchronized with every R wave. Sequence parameters for cine image acquisition included the following: field of view 35×35 mm, matrix 160×160, slice thickness 1 mm, repetition time/echo time 18/7.4 ms, flip angle 30°, 1 line/RR interval, temporal resolution 18 ms, in-plane resolution 220×220 μm, slice thickness 1 mm, and number of signal averages 2. An average of 12 cardiac cycles was acquired per slice. CINE imaging was performed in each animal at every time point. For delayed enhancement imaging, we used a segmented 2-dimensional phase-sensitive inversion recovery sequence. The concept of phase-sensitive inversion recovery imaging for LGE has been introduced by Kellman et al and has been successfully adapted to small animal imaging. The 2-dimensional phase-sensitive inversion recovery image acquisition was synchronized with every second and every fourth R wave, respectively. The inversion time was set to 200 ms for all time points, and this has proven as the most robust value to obtain stable image quality with high contrast between infarcted and remote myocardium. Other sequence parameters included the following: FOV 50×50 mm, matrix 128×125 (in-plane resolution 390×400 μm), slice thickness 1 mm, TR/TE 13/6.1 ms, flip angle 25°/5° (imaging data/reference data), 10 lines/RR interval, and number of signal averages 2. During reconstruction, images were normalized to account for differences in coil sensitivity. To analyze
the contrast-to-noise ratio (CNR) kinetic behavior of Gd-ESMA and Gd-DTPA, subsequent dynamic LGE scans were performed for 90 and 45 minutes, respectively. In each dynamic phase, a 7-slice short-axis stack covering the entire LV was performed with a slice thickness of 1 mm. The temporal resolution of the 7-slice stack was ≈150 s.

For comparison of the enhancement kinetics between Gd-ESMA and Gd-DTPA, on day 7 after MI, mice were first injected 0.6 mmol Gd-DTPA per kg body weight via the tail vein, and dynamic imaging was performed for a period of 45 minutes. After an additional 45 minutes break to allow complete washout of the Gd-DTPA, Gd-ESMA was injected at 0.2 mmol/kg body weight, and dynamic imaging was performed for 90 minutes at the exact same slice orientations. After imaging, mice were euthanized for triphenyltetrazolium chloride (TTC) staining of the heart. In a second set of experiments, mice were preinjected with lanthanum-ESMA (La-ESMA) to block the specific binding structures of Gd-ESMA. La-ESMA was used at 10× higher dose at 2 mmol/kg body weight and injected 1 hour before Gd-ESMA imaging. One hour after La-ESMA injection, dynamic delayed enhancement imaging was performed similarly as described above for a period of 90 minutes, and the CNR values were compared between mice with and without preinjection of the blocking La-ESMA.

In a last set of MR imaging experiments, additional 6 mice were consecutively imaged at days 7 and 21 after MI to determine differences in infarct size and CNR in the course of scar formation and maturation. Dynamic LGE imaging was similarly performed for 90 minutes. After imaging on days 7 or 21 after MI, mice were euthanized for histological and molecular analysis and TTC staining of the heart.

**Histology**

After completion of MR imaging, mice were euthanized under deep anesthesia, and hearts were excised.

For TTC staining, hearts were cut in 1 mm slices using a specialized mouse heart slicer (Zivic Instruments, Pittsburgh, PA) that allows preparation of 1-mm-thick slices in a short-axis orientation perpendicular to the long axis, resembling the short-axis views obtained by MRI. Heart slices were then incubated with 1% TTC solution (37°C; pH=7.4) for 20 minutes followed by formalin fixation (4% paraformaldehyde for 10 minutes at room temperature). Slices were photographed with a high-resolution digital camera and planimetered using Image J software (http://rsbweb.nih.gov/ij/). Segmentation of thresholding was performed with manual corrections, where necessary, after conversion of the RGB image into a 256-color gray-scale image. Sizes of nonischemic and ischemic area were calculated for each slice to allow comparison with MRI data. Coregistration errors between the 1-mm tissue sections and 1-mm MI short-axis planes were minimized by using unique landmarks, but in some cases, exact colocalization was challenging.

For histology, excised hearts were formalin-fixed and paraffin-embedded. The embedded hearts were cut into 4-μm-thick sections and mounted onto poly-l-lysine–coated glass slides. Hematoxylin and eosin and elastica van Gieson (EvG) stainings were performed according to standard procedures.

All stained slides were scanned at ×20 objective magnification using a Mirax Desk digital slide scanner (Carl Zeiss MicroImaging, Munich, Germany). For each of the resulting digital slides, subsets (regions of interest) were defined from areas of MI and analyzed using commercially available software (Definiens Enterprise Image Intelligence Suite, Definiens AG, Munich, Germany). A specific rule set was developed to detect and quantify the EvG-stained elastic fibers and whitespaces within the tissue based on staining intensity, morphology, neighborhood, and special color features. The relative areas of the whitespaces in comparison with the total tissue area and the relative red staining intensities of the elastic fibers were calculated.

**Western Blot Analysis**

Parts of infarcted and control hearts were cut at days 7 and 21 after ligation. Tissues were cut on ice, immediately snap-frozen in liquid nitrogen, and stored at −80°C until use. Tissue lysates were prepared in protein lysis buffer (150 mmol/L Tris-HCl, pH 7; 100 mmol/L NaCl, 1% nonyl phenoxypolyethoxylethanol), denatured in Laemmli buffer, and resolved on 10% SDS-PAGE according to standard protocol. Western blot was performed by tank blotting, and elastin was identified with rabbit anti-mouse Elastin antibody (Abcam). Anti-GAPDH was used as loading control.

**Statistics**

A total of 40 mice was used for the study: 27 mice were studied at POD 7 after MI and 13 mice at day 21. On POD 7, 16 mice were used for comparison of enhancement kinetics of both Gd-DTPA and Gd-ESMA (Figure 2), infarct distribution (Figure 3A), and infarct size determination (Figure 3C and 3E). Of these 16 mice studied for enhancement kinetics (single-slice scans), 10 mice were scanned covering the entire ventricle, allowing for determination of infarct volumes (Figure 3E). After euthanization of these 10 mice, 5 were used to establish histological stainings and the remaining 5 mice were used for TTC stainings to compare infarct sizes in vivo versus ex vivo (Figure 3C). Seven mice were used for competition experiments with La-ESMA (Figure 4), and an additional 4 mice were used for histology (Figure 5D, top row). Of the 7 mice scheduled for infarct size distribution on POD 21, 1 mouse died before reaching day 21; therefore, 6 mice were used for this purpose (Figure 3B, 3D, and 3F). In addition, 6 mice were used for consecutive measurements on days 7 and 21 (Figures 5 and 6). On POD 21 after completion of imaging, these mice were euthanized; 3 mice were used for histology and the remaining 3 for Western blot analysis. Samples for Western blot analysis on POD 7 (n=3) were taken from the 16 mice studied for enhancement kinetics.

Values are presented as bar graphs with mean±SD or dot plot diagrams with mean and 95% confidence interval. Because of the normal distribution of the data, comparison of measurements over time between different groups was performed using repeated-measures ANOVA followed by Bonferroni post hoc testing for multiple comparisons. Comparison of contrast-to-noise values with LVEF was performed using the Pearson correlation. A value of P<0.05 (2-sided) was considered statistically significant.

**Results**

**Enhancement Kinetics of ESMA Compared With Gd-DTPA**

Gd-ESMA accumulates within the infarcted LV wall (Figure 1). After intravenous injection on day 7, Gd-ESMA showed increased and prolonged enhancement of the infarct scar compared with Gd-DTPA when tracked over time (Figure 2A and 2B). Although peak enhancement of Gd-ESMA and Gd-DTPA was similarly observed at 5 minutes after injection, Gd-ESMA revealed significantly increased signal-to-noise ratio (P<0.001) and CNR values (P<0.001) throughout the course of dynamic image acquisition (Figure 2C and 2D).

**Infarct Size**

Infarct visualization and size were comparable between Gd-DTPA– and Gd-ESMA–enhanced imaging (Figure 3A). Infarct area at a midventricular plane determined by LGE on day 7 revealed no significant differences between Gd-ESMA and Gd-DTPA as well as in comparison with ex vivo TTC staining (Figure 3B; P=ns). The optimal time point after contrast administration for LGE-based infarct size measurement was at 7.5 to 10 minutes after injection for Gd-DTPA (Figure 3C) and at 7.5 to 15 minutes for Gd-ESMA because at these time points, agreement between infarct volume determined by LGE and ex vivo TTC was best. There were no differences in infarct volume assessment between days 7 and 21 after MI (Figure 3D) for...
both Gd-DTPA and Gd-ESMA ($P=\text{ns}$). Infarct size determined by Gd-ESMA and Gd-DTPA showed strong correlation both on day 7 ($r=0.96; P<0.0001$) and on day 21 ($r=0.90; P=0.0067$) and only minor differences between both methods in the corresponding Bland–Altman analysis (Figure 3E and 3F).

**Specific In Vivo Binding of Gd-ESMA**

To confirm the in vivo specificity of Gd-ESMA, we performed competition experiments with Gd-ESMA and La-ESMA (Figure 4). Preinjection of a 10-fold higher dose of nonparamagnetic La-ESMA (2 mmol/kg body weight) resulted in a significant decrease of CNR during the 90-minute period after Gd-ESMA injection (0.2 mmol/kg body weight) compared with Gd-ESMA alone ($P=0.0019$). Competition experiments were performed at day 7 in 7 mice.

**Gd-ESMA for Assessment of Postinfarction Remodeling of the Myocardial Scar**

Because the prolonged binding characteristics of Gd-ESMA, together with the results from the competition experiments, suggested specific binding to elastin-containing fibers in vivo, we evaluated the use of this agent for assessment of remodeling of myocardial scar. Therefore, 6 mice were investigated consecutively on days 7 and 21 after MI with Gd-DTPA and Gd-ESMA (Figure 5A). Although CNR showed no significant differences between days 7 and 21 for Gd-DTPA ($P=\text{ns}$), CNRs significantly increased at day 21 for Gd-ESMA compared with day 7 during the entire scan period ($P=0.0001$). At 15 minutes after injection, mean CNR for Gd-DTPA was 1.8 on day 7 and 3.8 on day 21 after MI ($P=0.100$), whereas mean CNR for Gd-ESMA significantly increased from day 7 to day 21 (4.9 versus 14.1; $P=0.0032$; Figure 5B). Higher CNR values with Gd-ESMA on day 21 compared with day 7 were corroborated by increased tropoelastin content on day 21 on Western blot analysis (Figure 5C). In agreement with the above observations, EvG staining of the infarct at days 7 and 21 after MI revealed an increase in elastic fibers at day 21 (Figure 5D). Relative red intensity was significantly higher on day 21 compared with day 7 after MI ($P=0.0498$; Figure 5E, top). Quantification of the fraction of whitespace in-between the elastic fibers simultaneously decreased from day 7 to day 21 ($P=0.0323$; Figure 5E, bottom), indicating a progressive organization of the elastic fibers on day 21. This increasing formation of elastic fibers compared with relatively unorganized elastin/tropoelastin on day 7 can be observed in the highly magnified EvG stains (×630 in Figure 5D) and can be regarded as dynamic maturation to stabilize the myocardial scar.

**Relationship Between LV Function and Contrast-to-Noise Ratio**

LV function parameters were determined from short-axis cine images before, at POD 7, and at POD 21 after MI (Figure 6A).

**Figure 2.** Enhancement kinetics of gadolinium-based elastin-specific MR contrast agent (GD-ESMA) compared with Gd-DTPA on postoperative day 7 after myocardial infarction. A, After contrast agent injection, Gd-DTPA is quickly washed out from the infarct, whereas Gd-ESMA (B) shows prolonged enhancement of the left ventricular scar with significantly increased signal-to-noise ratios (SNRs; C) and contrast-to-noise ratios (CNRs; D), $P<0.001$ for both (repeated measures ANOVA). Bars show SD of the mean. Experiment was performed in 16 mice.
Within 21 days, end-diastolic volume showed no change over time after MI (day 0: 69.8±9.7 μL; day 7: 62.9±6.8 μL; day 21: 64.3±24.8 μL), whereas the end-systolic volume increased (day 0: 21.3±3.2 μL; day 7: 37.7±11.1 μL; day 21: 40.6±18.9 μL). The EF decreased from day 0 to POD 7 and further to POD 21 (day 0: 69.3±3.8%; day 7: 40.8±12.4%; day 21: 37.9±7.8%). The LV mass, however, decreased only moderately (day 0: 121.7±15.7 mg; day 7: 109.2±17.8 mg; day 21: 98.5±15.0 mg).

When correlating the CNR values obtained by both contrast agents on POD 7 and POD 21 (Figure 6B), Gd-DTPA values showed no significant correlation with the LVEF on POD 7 ($r^2=0.38; P=0.186$) and POD 21 ($r^2=0.07; P=0.602$). For Gd-ESMA, the correlation between CNRs and EF showed only a weak correlation on POD 7 ($r^2=0.33; P=0.229$); however, on POD 21 there was a significant correlation between Gd-ESMA enhancement and LV function ($r^2=0.70; P=0.037$).

**Discussion**

Postinfarction scar remodeling affects both the infarcted area and the adjacent remote myocardium. It is characterized by significant alterations in ventricular geometry, such as ventricular dilation, loss of the elliptical chamber configuration, and increased chamber volumes. On a histological level, these changes are accompanied by cardiomyocyte hypertrophy, myocyte apoptosis, myofibroblast proliferation, ECM synthesis, and progressive fibrosis. The severity of myocardial injury, respectively infarct size is associated with a greater degree of adverse remodeling both in rodent models and in patients, and the degree of remodeling is highly correlated with heart function and outcome after acute MI. Besides collagen, elastin has been identified as a key protein in scar remodeling and successful infarct healing. Myocardial elastin is an insoluble ECM component that consists of a core of tropoelastin surrounded by fibrillin and microfibrils. The elastin fiber network is crucial to preserve elasticity and resilient recoil.
and to maintain the architecture against repeated expansion. Within the first days after MI, elastic fibers begin to form and increase in size and number with maturation of the scar. Within weeks, the elastic fibers become interdigitated with stumps of viable myocytes and form close contacts with myofibroblasts and nonvascular smooth muscle cells. Recent studies have suggested that cardiac function after MI can be improved by modifying the composition of myocardial scar tissue. A higher ratio of elastin compared with fibrotic collagen tissue is able to alter the composition of the myocardial scar in a way to preserve the elasticity of the infarcted heart, thereby avoiding progressive heart failure. Previous work by Mizuno et al has demonstrated that increased expression of elastin within the myocardial scar using transfected endothelial cells reduces infarct expansion and preserves ventricular function after experimental MI. Lichtenauer et al have shown that increasing the expression of elastin within the infarct scar via intravenous or intramyocardial injection of apoptotic white blood cells attenuates ventricular remodeling in a rat model.

Thus, because elastin is a major constituent of the healing heart, we thought to investigate the feasibility of a noninvasive assessment of elastin after MI using molecular MRI. We expected that the elastin concentration within the scar is high enough to be captured by a targeted contrast agent within an MR approach. The extracellular localization of elastin makes it easily accessible because the contrast agent does not need to cross cell membranes.

In our study, we successfully report delayed enhancement molecular MRI of myocardial elastin in a mouse model of MI and postinfarction scar remodeling. Using Gd-ESMA, we were able to visualize and quantify elastin expression within the infarct scar and monitor the progression of myocardial healing on a morphometric level. Performance of the elastin-specific Gd-ESMA was compared with gadopentetate dimeglumine (Gd-DTPA) in the same animals. Gd-ESMA generated a higher and more persistent contrast between the infarcted and remote myocardium with higher SNR and CNR values compared with Gd-DTPA. This is attributed both to the high density of the target protein and to the increased intrinsic relaxivity (16 versus 4.1 mmol/L⁻¹·s⁻¹) of Gd-ESMA compared with Gd-DTPA. The infarct size determined by Gd-ESMA was in good agreement with the measurements with Gd-DTPA as well as with postmortem TTC staining. This indicates that the ESMA targets elastin fibers within the scar but does not reach interstitial elastin in the remote myocardium or has too low sensitivity for visualizing those. The prolonged enhancement kinetics of Gd-ESMA also facilitates delayed enhancement imaging in rodents because imaging with Gd-DTPA is possible only within a restricted time frame.

In vivo competition experiments with nonparamagnetic La-ESMA resulted in marked decrease in infarct CNR, which is consistent with a target-specific contrast agent. Serial imaging demonstrated a significant increase in CNR during the course of 3 weeks, which was accompanied by a significant increase in tropoelastin detected by Western blot analysis and increased elastin staining on EvG stains. EvG histology not only showed an increase in tropoelastin/elastin-containing fibers on day 21 but also demonstrated an increased order of elastic fibers oriented in parallel directions along the myocardial scar. This observation is in accordance with previous electron microscopy studies investigating the formation of elastic fibers and their possible interaction with cardiomyocytes and fibroblasts. Bassett and Wakefield described an increase in de novo synthesized elastin fibers from day 4 to day 24 in a rat model of MI and found that both collagen and elastic fibers progressively interdigitated with the stumps of viable myocytes, giving resilience and strength to the attachments of the myocardial scar. The authors proposed that the elastic fibers at the scar–myocyte interface could act as a coupling mechanism between the inextensible collagen network of the scar and constantly contracting and relaxing myocardium. The increased synthesis and organization of...
elastin fibers are, therefore, regarded as a decisive mechanism to maintain the structure and elasticity of the infarct scar, thereby avoiding a progressive loss of biomechanical function. We show that the process of increased elastin synthesis can be tracked noninvasively by molecular MRI, which may help to better evaluate the healing response after MI. Interestingly, the increased CNR obtained with Gd-ESMA on day 21 after MI correlated significantly with LVEF, indicating that higher MRI signal for elastin may be associated with improved heart function. The results obtained hereby are in accordance with experimental studies by Mizuno et al., who found a beneficial effect of increased elastin expression on heart function and survival in ischemically injured hearts in a rat model of MI. These observed effects could now be followed noninvasively by MRI.

Recently, a gadolinium-based contrast agent targeted against collagen, another important ECM protein, has been studied for delayed enhancement imaging of MI and for myocardial perfusion imaging. While collagen targeting informs on the degree of fibrosis, an important process during scar remodeling, targeting elastin is able to inform on the expression of a cardioprotective ECM protein. In the context of postinfarction scar remodeling, Gd-ESMA may be able to monitor the effects of novel therapeutic regimens aiming to alter the composition of the ECM. For example, elastin overexpression via cell-based gene therapy has been recently introduced as potential tool to prevent cardiac dilatation and support regeneration in a rat model. Gd-ESMA now provides a potential noninvasive tool to track the kinetics of elastin formation over time and space in living animals. Although targeting collagen in the injured heart may inform about an adverse effect of scar remodeling and thereby provide a predictive value about heart failure, imaging elastin may report on a beneficial healing effect occurring after MI. The presented imaging approach is potentially

Figure 5. Consecutive imaging with gadolinium-based elastin-specific MR contrast agent (Gd-ESMA) on postoperative day (POD) 7 and POD 21 after myocardial infarction (MI). A, Six mice were consecutively imaged on days 7 and 21 first with Gd-DTPA and subsequently with Gd-ESMA. Although contrast-to-noise ratio (CNRs) after injection of Gd-DTPA (A, first graph) showed no significant differences between POD 7 and POD 21 during the time course after injection (P=0.09), CNRs after injection of Gd-ESMA (A, second graph) were significantly higher on POD 21 during the 90-minute period after injection (P<0.0001). Bars show SD of the mean. B, Dot plot diagram shows CNR values (mean and 95% confidence interval) on days 7 and 21 after MI at 15 minutes after injection. Although CNR values for Gd-DTPA do not differ significantly (P=0.100), CNR values for Gd-ESMA are significantly higher on day 21 compared with day 7 after MI (P=0.0032). C, Western blot analysis showed increased tropoelastin (75 kDa) synthesis on day 21 compared with day 7 after myocardial infarction (n=3). D, Infarcted hearts from animals euthanized on POD 7 (n=4) and POD 21 (n=3) were processed for hematoxylin and eosin staining (HE) and elastica van Gieson staining (EvG). An area of infarcted myocardial tissue was detected within the left ventricular wall in all mice investigated; a representative HE of a 4-chamber view is shown in the left. Magnified views of HE and EvG stains from hearts harvested on POD 7 (top row) and POD 21 (bottom row) are shown. An increase of elastin fibers on day 21 compared with POD 7 could be detected on the EvG stains. E, Quantification of the relative red intensity confirmed increased elastin presence on POD 21 (P=0.0498; Figure 5E, top) but also showed a significant decrease of the whitespace area on POD 21 compared with POD 7 (P=0.0323; Figure 5E, bottom), as a measurement for the progressive orientation of the elastic fibers in parallel bands along the scar, as seen in the magnified EvG images. Bar graphs show mean±SD. *P<0.05. ns indicates nonsignificant.
eral weeks after MI, we chose to use female C57BL/6J mice because our intent was to conduct serial MR imaging for several parameters show the temporal development of myocardial remodeling with respect to end-diastolic volume (EDV), end-systolic volume (ESV), ejection fraction (EF), and LV myocardial mass from day 0, postoperative day (POD) 7, and POD 21. B, Contrast-to-noise ratio (CNR) values obtained from Gd-DTPA- and gadolinium-based elastin-specific MR contrast agent (Gd-ESMA)-enhanced MR images of the consecutively investigated mice (n=6) on day 7 and day 21 were correlated with the corresponding parameters including left-ventricular ejection fraction. On POD 21, CNR values from Gd-ESMA correlated significantly with LV function (P=0.037). No correlation was found between contrast agent enhancement on LGE-MRI and ESV, EDV, EF, as well as mass.

translatable to human cardiac MRI. Since its initial presentation, delayed enhancement imaging using gadolinium chelates has been implemented in the daily work-up of patients with MI. New T1 mapping sequences that are increasingly used to determine the extracellular volume after MI and in other cardiac diseases are able to accurately quantify the molecular MRI signal, thereby further improving standardization.

Gd-ESMA has been successfully applied to assess atherosclerotic plaque burden in a model of apolipoprotein E–deficient mice, as well as to investigate arterial wall remodeling after stent placement in a swine model of coronary injury. Preliminary data have also suggested that Gd-ESMA may be suitable for the quantification of elastin in liver fibrosis. Although no specific toxicity studies for Gd-ESMA were performed yet, the previous reports published on Gd-ESMA, as well as our study, did not report any adverse effects.

The prolonged trapping of Gd-ESMA within the myocardial scar allows infarct imaging with high CNR within the first hour of injection and even beyond. The delayed washout, however, may be suitable for the quantification of elastin expression and deposition within the myocardial scar so that the difference in Gd-EMSA imaging might be even more pronounced with the use of male mice.

Figure 6. Left ventricular (LV) function and contrast-to-noise ratio. A, Dot plot diagrams (mean and 95% confidence interval) of LV function parameters show the temporal development of myocardial remodeling with respect to end-diastolic volume (EDV), end-systolic volume (ESV), ejection fraction (EF), and LV myocardial mass from day 0, postoperative day (POD) 7, and POD 21. B, Contrast-to-noise ratio (CNR) values obtained from Gd-DTPA- and gadolinium-based elastin-specific MR contrast agent (Gd-ESMA)-enhanced MR images of the consecutively investigated mice (n=6) on day 7 and day 21 were correlated with the corresponding parameters including left-ventricular ejection fraction. On POD 21, CNR values from Gd-ESMA correlated significantly with LV function (P=0.037). No correlation was found between contrast agent enhancement on LGE-MRI and ESV, EDV, EF, as well as mass.

In conclusion, we demonstrate the feasibility of noninvasive detection of myocardial elastin after MI using a novel ESMA, Gd-ESMA. The use of Gd-ESMA may not only allow for the precise assessment of cardioprotective elastin expression after myocardial injury but also facilitate monitoring of the beneficial effects of novel therapeutic regimens aiming at the preservation of myocardial structure and function after MI.

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Disclosures
D.C. Onthank, R.R. Cesati, and S.P. Robinson are employees of Lantheus Medical Imaging, MA. The other authors report no conflicts.

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remodeling and coronary atherosclerosis in a single MRI examination. 

Cardiac MRI is today an established technique for the assessment of patients with myocardial infarction. Cardiac MRI allows sequentially mobilizes two monocyte subsets with divergent and complementary functions. J Exp Med. 2007;204:3037–3047.

Clinical perspective. Cardiac MRI is today an established technique for the assessment of patients with myocardial infarction. Cardiac MRI allows for simultaneous determination of cardiac function, morphology, and viability, the latter after administration of gadolinium. Although unspecific gadolinium chelates inform only about the extent of myocardial injury, targeted molecular contrast agents can report on specific biological processes of myocardial healing and remodeling. Collagen-targeted molecular MRI is able to quantify the extent of fibrosis after experimental myocardial infarction. Besides collagen, elastin is an important extracellular matrix protein. It has been shown that elastin exerts cardioprotective effects on the healing myocardium because it helps to preserve elasticity of the left ventricle after myocardial infarction, thereby improving heart function and survival. Thus, elastin-targeted MRI may be a useful tool for noninvasive assessment of myocardial healing and the evaluation of cardioprotective therapies. In this report, we demonstrate the feasibility of the noninvasive detection and quantification of elastin-mediated myocardial healing in a mouse model of myocardial infarction using an elastin-specific MR contrast agent. The reported elastin-targeted MR contrast agent has been used previously for in vivo assessment of vascular remodeling in atherosclerosis. Together with the results presented here, this agent may allow for simultaneous investigations of myocardial remodeling and coronary atherosclerosis in a single MRI examination.
Assessment of Myocardial Infarction and Postinfarction Scar Remodeling With an Elastin-Specific Magnetic Resonance Agent

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