Molecular Magnetic Resonance Imaging of Myocardial Perfusion With EP-3600, a Collagen-Specific Contrast Agent
Initial Feasibility Study in a Swine Model

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Background—Cardiac magnetic resonance (MR) perfusion imaging during the first pass after intravenous administration of extracellular contrast agents is hampered by the spatial and temporal resolution achievable and by the artifacts seen in ultrafast MR imaging. Furthermore, time-consuming quantitative data analysis is often added. The use of molecular MR imaging with a target-specific contrast agent with perfusion-dependent binding to myocardium may enable prolonged visualization of perfusion defects and thus may help to overcome limitations of currently used first-pass extracellular MR imaging. EP-3600 is a new gadolinium-containing molecular contrast agent that binds reversibly to myocardial collagen.

Methods and Results—A significant but nonocclusive coronary artery stenosis was modeled in 7 domestic swine with an undersized MR-compatible balloon positioned in the left anterior descending artery as verified by x-ray angiography. Two animals died before contrast injection as a result of arrhythmias. In 5 swine, high-spatial-resolution gradient echo imaging (≈1×1 mm² in-plane resolution) was performed before and 5, 20, 40, and 60 minutes after intravenous administration of 12.3 μmol/kg EP-3600. Contrast was administered during stress induced by an infusion of 250 μmol·kg⁻¹·min⁻¹ adenosine. Yb-DTPA was administered simultaneously for comparison of myocardium-to-plasma ratios. Images were assessed subjectively by 2 investigators, and signal-to-noise and contrast-to-noise ratios over time were calculated. Normal myocardium showed a significant signal-to-noise ratio increase during the entire examination time. In all animals (n=5), the perfusion defect in the left anterior descending artery territory could be visualized with a high contrast-to-noise ratio for at least 20 minutes after contrast injection. A significantly higher myocardium-to-plasma ratio was found for EP-3600 compared with the control agent Yb-DTPA (0.85±0.26 versus 0.22±0.08, respectively; P<0.01).

Conclusion—EP-3600 is a new molecular MR imaging contrast agent that binds to the myocardium and enables prolonged, high-contrast, high-spatial-resolution visualization of myocardial perfusion defects. (Circulation. 2009;119:1768-1775.)

Key Words: contrast media ■ coronary disease ■ ischemia ■ magnetic resonance imaging ■ perfusion

Over the last decade, cardiac magnetic resonance (MR) imaging (MRI) during rest and stress has been used to assess the significance of coronary artery disease. Typically, a fast cardiac-triggered gradient echo sequence is performed during the first pass of an extracellular contrast agent during stress and subsequently repeated during rest to detect mismatches representing stress-induced ischemia.¹² Because this agent extravasates rapidly, the perfusion defect is visible only briefly during the first pass, and contrast is limited. Furthermore, the high temporal resolution required (acquiring MRI during each cardiac cycle) is at odds with complete coverage of the heart (≈6 slices). The inversion or saturation preparation prepulses needed to increase T1 weighting limit the remaining time period for data sampling during each R-R interval.³ As a result, initial myocardial MR perfusion studies using the first pass were limited to a single-slice approach.¹ Subsequently, with improved hardware and software (ie, improved gradient performance, echo-planar imaging, interleaved slice scanning, and accelerated imaging strategies),⁴⁻⁶ multislice approaches have been implemented with 3 to 4 imaging planes to increase spatial and temporal resolution. However, these techniques are limited by the need for repeated imaging during stress and subsequently during rest to detect perfusion defects. Further, technical limitations of current fast imaging techniques, such as the use of saturation or inversion prepulses to increase T1 weighting, have hampered the use of extracellular contrast agents, which are limited by spatial and temporal resolution achievable and by artifacts seen in ultrafast MR imaging.⁷ Thus, the use of molecular MR imaging with a target-specific contrast agent with perfusion-dependent binding to myocardium may enable prolonged visualization of perfusion defects and thus may help to overcome limitations of currently used first-pass extracellular MR imaging. EP-3600 is a new gadolinium-containing molecular contrast agent that binds reversibly to myocardial collagen.
images per heart beat. However, spatial resolution is still limited to \( \approx 3 \times 3 \, \text{mm}^2 \) in plane, and only \( \approx 3 \) thick image slices (8 to 10 mm) can be acquired, resulting in partial volume effects and limited heart coverage. Furthermore, the current lower-spatial-resolution imaging approaches suffer from “ringing” artifacts, especially at the subendocardial border of the septum, which can be difficult to differentiate from subendocardial perfusion defects.\(^7\) Because the accuracy of the visual assessment of perfusion defects can be limited, a time-consuming quantitative analysis of the signal kinetics is often added; preferentially, this should be done in all the segments for both the subendocardial and subepicardial layers.\(^8,9\) Because perfusion defects can be visualized only during the first pass, stress has to be performed inside the MR scanner, requiring drug-induced hyperemia by use of adenosine or dobutamine infusion (exercise stress is typically not possible inside the MR gantry). Medical supervision may be limited inside the MR gantry.

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A molecular MRI approach may overcome thesees limitations. The use of a dedicated contrast agent that binds sufficiently (and reversibly) to the myocardium, resulting in a high T1 difference that is dependent on the perfusion, would enable signal difference between the ischemic (hypoperfused) and normal (hyperperfused) areas. The binding to the myocardium would potentially “store” these signal differences, enabling a much longer potential imaging window after contrast. Imaging time constraints imposed by first-pass MRI would be absent, which would allow higher-spatial-resolution imaging and complete coverage of the ventricle, including multiple views.

Consequently, the aim of this study was to investigate the potential of a new molecular MR contrast agent that binds reversibly to myocardium (EP-3600, EPIX Pharmaceuticals, Lexington, Mass) for delayed and high-spatial-resolution visualization of myocardial perfusion defects. To allow comparison of this new MR approach with the currently used settings in humans, a large-animal model (domestic swine) was used.

### Methods

**Subjects**

Noninvasive molecular MRI of myocardial perfusion defects was performed in 7 healthy domestic swine (48±4 kg body weight; range, 45 to 55 kg) as approved by the government committee on animal affairs (Cologne, Germany). After premedication with 0.1 mL atropine IM, 0.2 mL azaperone IM/kg body weight, and 0.1 mL ketamine/kg body weight, a perfusion of propofol (10 mg/1 mL) was administered intravenously via an ear vein as needed. The animals were intubated, and mechanical ventilation was maintained throughout the entire experiment. A 9F sheath (Cordis, Roden, the Netherlands) was placed surgically in the right carotid artery.

**Molecular MR Contrast Agent EP-3600 and Negative Control Yb-DTPA**

EP-3600 (Figure 1) is a small-peptide–gadolinium hybrid (4182 Da). It consists of a 15–amino acid peptide that is functionalized with 3 Gd-DOTA–type chelates on the N terminus and is structurally similar to the compound EP-3533 recently reported.\(^10\) The major difference between the 2 compounds is that EP-3600 uses the more stable DOTA chelator,\(^11\) whereas EP-3533 had 3 DTPA chelators for binding Gd. Given recent concerns about Gd-induced nephrogenic systemic fibrosis, we switched to a more stable chelator for these studies with an eye toward clinical development.\(^12\) Similar to EP-3533, EP-3600 exhibits low micromolar affinity for both porcine and human type I collagen (\(K_a=1.8 \, \mu\text{mol/L}\)) and a high stoichiometry of binding.\(^10\) Minimal nonspecific protein binding exists.\(^10\)

Yb-DTPA is an analog of Gd-DTPA except that the Gd\(^{3+}\) ion is replaced by Yb\(^{3+}\). This substitution does not alter the pharmacokinetics of the compound, but it does render the molecule inactive as an MRI contrast agent because Yb\(^{3+}\) is a very poor relaxation agent. Yb-DTPA was used as an internal negative control. Yb-DTPA was synthesized as disodium salt and formulated at a concentration of 0.5 mol/L in sterilized water.

Injection of EP-3600 was performed systemically at a dose of 12.3 \(\mu\text{mol/kg (36.9 \, \mu\text{mol/kg Gd) over 1 minute during adenosine stress via an ear vein. Yb-DTPA (50 \, \mu\text{mol/kg}) was administered at the same time as EP-3600.**

**Tissue Analyses**

Plasma was separated from blood samples, and the plasma was analyzed by inductively coupled plasma mass spectrometry (ICP-MS) for Gd and Yb content with added Tb as an internal standard. Plasma samples also were analyzed by reverse-phase high-performance liquid chromatography with an ICP-MS detector to determine stability of EP-3600 after injection. Plasma relaxation rates were determined at 1.4 T and 37°C with a Bruker mq60 spectrometer. The myocardial segments (17-segment model\(^13\)) were divided into 2 portions and weighed. One portion was dissolved in concentrated nitric acid and then assayed for Gd and Yb by ICP-MS. The other portion was analyzed by neutron activation for microsphere content.

**MR Scanner**

All in vivo studies were performed on a dedicated interventional 1.5-T whole-body MR scanner (X-MR) combining a 1.5-T Achieva platform with a Pulsera x-ray C arm (both Philips Medical Systems, Best, the Netherlands). The MR scanner and x-ray angiography system are connected by a floating table, which allows easy transfer of the subject between both systems while maintaining identical table position in the MR gantry for subsequent MRI. All swine were examined in the supine position with belts and tape used to reduce any potential bulk motion caused by the transfer between the MR unit and the x-ray unit.

**MR Sequence for Molecular MR Perfusion Imaging**

For molecular MRI of myocardial perfusion, a radiofrequency-spoiled segmented k-space gradient-echo sequence as currently used for first-pass perfusion scanning was modified as follows: The spatial resolution was increased to an in-plane reconstructed voxel size of 1.05×1.05 mm\(^2\). Other parameters include a repetition time of 4.6 ms, an echo time of 1.4 ms, and a flip angle of 12°. To maintain strong T1 weighting, the same saturation prepulse (103 ms for each...
slice) used for first-pass imaging was applied, giving basically similar contrast properties as in first-pass perfusion scanning. Compared with the currently used first-pass approach, data were acquired over multiple heartbeats with a segmented $k$-space scheme combined with a brief (49 ms) acquisition window per slice and R-R interval. With half-Fourier scanning and parallel imaging with an acceleration factor of 2.1, the total imaging time for five 10-mm-thick slices was 21 seconds at a heart rate of 80 bpm, which allowed breath-hold scanning.

Look-Locker Sequence for T1 Measurements

A cardiac triggered Look-Locker sequence was used to calculate the apparent T1 of myocardium, blood, and the perfusion defect and was performed at the level of the perfusion defect. Sixteen phases (with a consequent 16 increasing inversion times) were performed with a reconstructed in-plane resolution of $1.2 \times 1.2$ mm$^2$ and an 8-mm slice thickness. Repetition time was 40 ms; echo time was 4.9 ms; and the flip angle was 15°. Echo-planar imaging with an echo-planar imaging factor of 9 and 2 data acquisition averages resulted in a 22-sec measurement time at a heart rate of 80 bpm, which allowed breath-hold imaging.

Closed-Chest Interventional Model for Coronary Stenosis

Instrumentation with an acute stenosis of the coronary artery is a well-established model in large animals to study myocardial perfusion.14,15 Because open-chest surgical access can result in potential movement or clotting. An additional microsphere injection (10 mL; 25 million microspheres) was injected directly into the left ventricle (∼25 million microspheres). Subsequently, the swine was transferred back into the MR unit (identical position in the magnet), and the molecular MR perfusion and Look-Locker sequences were performed with the angulations maintained from the previous scout scanning. If needed, the position of both the scans was adapted with the midslice of the molecular perfusion scan and the Look-Locker scan localized at the level of the estimated perfusion defect. Then, adenosine stress was induced at a dose of 250 μg·kg$^{-1}$·min$^{-1}$ adenosine over 15 minutes. With this approach, sufficient time was available for microsphere injection and contrast media administration. After 8 minutes of stress, gold-labeled 15-μm microspheres (10 mL; 25 million microspheres) were injected into the left ventricle. After 10 minutes of adenosine stress, the administration of EP-3600 over 1 minute was started at a dose of 12.3 μmol/kg body weight. Yb-DTPA (50 μmol/kg) was simultaneously infused. Stress was maintained an additional 5 minutes to ensure that binding during subsequent passes of the agent also represents flow reduction. Then, the adenosine infusion was stopped, and the molecular perfusion sequence and Look-Locker sequence were repeated. Both sequences also were repeated 20, 40, and 60 minutes after contrast media administration. In between, at 30 minutes, and finally after the 60-minute scans, the swine was again transferred to the x-ray unit to confirm consistent position of the inflated balloon with remaining blood flow surrounding the balloon and to exclude vessel occlusion or thrombosis resulting from balloon movement or clotting. An additional microsphere injection (10 mL; 25 million lutetium-labeled microspheres) into the left ventricle was done after the x-ray angiogram at ~35 minutes after contrast administration. Finally, the animal was killed by an overdose of pentobarbital, and the heart was removed (70 minutes after contrast administration). The heart was cut into 17 defined segments based on the American Heart Association nomenclature.13 From each slice, a thin layer was cut off for triphenyltetrazolium chloride (TTC) staining to demonstrate or to exclude any myocardial infarction at the level of the perfusion defects. Each segment was then divided into 2 portions and weighed; 1 portion was assayed for Gd and Yb concentration by ICP-MS, and the other portion was analyzed by neutron activation for microsphere concentration (BioPAL). All microsphere perfusion data are reported relative to perfusion in the remote myocardium at the same time point. First, the mean of the microsphere counts per gram of tissue in the 6 basal segments located proximally to the stenosis (remote myocardium) was calculated. Regional perfusion data of the other segments were assessed by calculation of the ratio of microsphere counts per gram of tissue in each of the other segments to the mean of the remote myocardium. Regional perfusion was calculated relative to remote myocardium before, during, and after adenosine stress.

Blood samples also were taken before and 1, 3, 5, 10, 15, and 30 minutes after contrast administration and immediately before the animal was killed. These samples were analyzed for Gd and Yb concentrations and for EP-3600 stability. To assess binding of the mid LAD. The swine was heparinized (50 000 IU) to avoid any clotting. A 10-mL suspension of lanthanum-labeled 15-μm microspheres (SteriSpheres, BioPAL, Worcester, Mass) was injected directly into the left ventricle (∼25 million microspheres). Subsequently, the swine was transferred back into the MR unit (identical position in the magnet), and the molecular MR perfusion and Look-Locker sequences were performed with the angulations maintained from the previous scout scanning. If needed, the position of both the scans was adapted with the midslice of the molecular perfusion scan and the Look-Locker scan localized at the level of the estimated perfusion defect. Then, adenosine stress was induced at a dose of 250 μg·kg$^{-1}$·min$^{-1}$ adenosine over 15 minutes. With this approach, sufficient time was available for microsphere injection and contrast media administration. After 8 minutes of stress, gold-labeled 15-μm microspheres (10 mL; 25 million microspheres) were injected into the left ventricle. After 10 minutes of adenosine stress, the administration of EP-3600 over 1 minute was started at a dose of 12.3 μmol/kg body weight. Yb-DTPA (50 μmol/kg) was simultaneously infused. Stress was maintained an additional 5 minutes to ensure that binding during subsequent passes of the agent also represents flow reduction. Then, the adenosine infusion was stopped, and the molecular perfusion sequence and Look-Locker sequence were repeated. Both sequences also were repeated 20, 40, and 60 minutes after contrast media administration. In between, at 30 minutes, and finally after the 60-minute scans, the swine was again transferred to the x-ray unit to confirm consistent position of the inflated balloon with remaining blood flow surrounding the balloon and to exclude vessel occlusion or thrombosis resulting from balloon movement or clotting. An additional microsphere injection (10 mL; 25 million lutetium-labeled microspheres) into the left ventricle was done after the x-ray angiogram at ~35 minutes after contrast administration. Finally, the animal was killed by an overdose of pentobarbital, and the heart was removed (70 minutes after contrast administration). The heart was cut into 17 defined segments based on the American Heart Association nomenclature.13 From each slice, a thin layer was cut off for triphenyltetrazolium chloride (TTC) staining to demonstrate or to exclude any myocardial infarction at the level of the perfusion defects. Each segment was then divided into 2 portions and weighed; 1 portion was assayed for Gd and Yb concentration by ICP-MS, and the other portion was analyzed by neutron activation for microsphere concentration (BioPAL). All microsphere perfusion data are reported relative to perfusion in the remote myocardium at the same time point. First, the mean of the microsphere counts per gram of tissue in the 6 basal segments located proximally to the stenosis (remote myocardium) was calculated. Regional perfusion data of the other segments were assessed by calculation of the ratio of microsphere counts per gram of tissue in each of the other segments to the mean of the remote myocardium. Regional perfusion was calculated relative to remote myocardium before, during, and after adenosine stress.

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EP-3600 to normal myocardium, the mean ratio of gadolinium and mean ytterbium myocardium to plasma was determined. The time schedule for the experiments is shown in Figure 2.

MR data were analyzed on a consensus basis by 2 investigators in terms of readily visible perfusion defects. The investigators were aware of the interventional procedure, but images were interpreted immediately during the MR session and then before any postmortem analyses. The investigators were asked to rank the perfusion defect as good contrast between the perfusion defect and remote myocardium, only moderate contrast but still assessable perfusion defect, and no visual assessable contrast. Objective signal measurements included signal-to-noise measurements of the normal myocardium, the perfusion defect, and skeletal chest muscle whereby noise was defined as the standard deviation of air. All regions of interest (ROIs) were placed in the same slice out of the data set with the best visible perfusion defect. The ROI in air was placed to the closest region of air outside the chest; the ROI of normally perfused remote myocardium, next to the perfusion defect; and the ROI of skeletal muscle, in the thoracic muscle between the perfusion defect and the ROI of air. With this approach, all ROIs were located as close as possible to each other. Identical ROIs were used for precontrast and all the postcontrast images, and signal-to-noise ratio (SNR) changes over time were calculated. This avoided bias of SNR measurements in accelerated MRI. Contrast-to-noise ratio (CNR) determination was calculated between the myocardium (myo) and the perfusion defect (perfd) and between the normal myocardium and the blood pool (blood) as follows: CNR\(_{(\text{myo/perfd})}\) = signal\(_{\text{myo}}\) - signal\(_{\text{perfd}}\)/SD\(_{\text{air}}\) and CNR\(_{(\text{myo/blood})}\) = signal\(_{\text{myo}}\) - signal\(_{\text{blood}}\)/SD\(_{\text{air}}\).

Apparent T1 measurements were calculated from the Look-Locker sequence on the basis of curve fitting in the postcontrast images with reduced T1 values. For assessment of T1 changes over time, data were normalized to the T1 values of the first postcontrast scan 5 minutes after contrast administration. Values are expressed as mean ± SD. Quantitative measurements (Gd/Yb ratios and SNR measurements) were analyzed with paired Student t tests adjusted for multiple comparisons. Perfusion data were analyzed statistically with mixed-effects regression models, treating each animal as a random intercept. Two sets of hypotheses were tested. First, we compared each of segments 7 to 17 during stress separately with the average of the 6 basal segments, adjusting for multiple comparisons. Second, we compared perfusion in the segments that had significantly reduced perfusion during stress for before-during, after-during, and before-after perfusion differences. CNR data for normal myocardium to perfusion defect were also analyzed statistically with similar regression models. Times of 5, 20, 40, and 60 minutes were compared with CNR measured before stress. A value of P < 0.05 was considered significant.

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

Results

Two swine died during the intervention and before contrast administration as a result of arrhythmia. In the remaining 5 swine, the intervention and the MR protocol were successfully completed. In all cases, a nonocclusive coronary stenosis in the LAD could be modeled, which was consistent during the complete experiment. An example is shown in Movie I of the online-only Data Supplement. In none of the animals was any balloon movement, clotting, or vessel occlusion observed during the entire experiment. No artifacts caused by the catheter material were observed on any of the MRIs.

On precontrast images, the myocardium and skeletal muscle signals were similar, whereas the blood pool was hypointense. After intravenous administration of EP-3600, a continuous signal enhancement of the normal myocardium was seen, with a hyperintense signal compared with the skeletal muscle (Figures 3 and 4). Even the thinner right heart myocardium could be seen with bright signal as a result of high-spatial-resolution imaging (Figure 3). The SNR of remote myocardium was significantly increased over all the postcontrast images (P < 0.01 for all time points). In distinction to the myocardium, the blood pool showed a continuous signal decrease over time, resulting in an increased contrast between the myocardium and the blood pool on more delayed imaging, with a slightly hyperintense signal of the myocardium compared with the blood pool (positive CNR) on the images at 40 and 60 minutes after contrast injection (Figure 5). These subjective findings were in good agreement with the objective T1 calculations demonstrating a prolonged T1 shortening in normal myocardium and a fast T1 recovery in normal myocardium to perfusion defect with EP-3600. The perfusion defect in the LAD territory is clearly seen on the MRIs up to 60 minutes after administration of EP-3600 with hypointense signal (arrows), whereas the normal myocardium is seen with a bright signal. The corresponding Look-Locker sequences are shown in Movies II through VI of the online-only Data Supplement.
the blood pool (Figure 5A and Movies II through VI in the online-only Data Supplement).

In all 5 swine, the perfusion defect in the LAD territory could be seen nicely as a hypointense area and with good contrast to the remote myocardium up to 20 minutes after injection (the Table). The area affected varied slightly, depending on individual anatomy and localization of stenosis. In 2 of the 5 swine, the defect could also be seen with good contrast on the 40- and 60-minute scans (Figure 4). In 1 case, on the 40- and 60-minute scans, a small area of hyperintense signal in the initially larger hypointense perfusion defect was seen, suggesting late enhancement in an infarcted area (Figure 6). This was proven by TTC staining, as shown in Figure 6B (small infarction with <5-mm diameter). TTC staining showed an additional very small (<3-mm diameter) infarcted lesion in another swine; this area also was located within the perfusion defect. All other animals were free of any infarction.

Microsphere measurements indicated that with the interventional model used, regional perfusion compared with the remote myocardium (basal segments) was only significantly reduced in the LAD territory (anteroseptal segments 8, 13, and 14), and a significant reduction (P<0.0025) was seen only during adenosine stress (see supplemental Table I). These same territories were hypointense on the MRIs. Mean perfusion in these segments at stress ranged from 42% to 54% compared with the normally perfused (remote) myocardium. Before and after stress in these anteroseptal segments, the flow reduction ranged from 65% to 73% and 63% to 86% of the flow in the remote myocardium at these same time points, respectively. Although flow was lower in the anteroseptal segments during stress compared with before stress, these differences were not significant (P=0.07), likely because of the small sample size in this study.

Binding of EP-3600 to remote myocardium was shown by comparing the ratio of myocardium to plasma for Gd and Yb (postmortem analysis). A significantly higher ratio in the basal segments was observed for Gd (EP-3600) compared with Yb-DTPA (0.85±0.26 versus 0.22±0.08; P<0.01), indicating that EP-3600 was not distributed just in the extracellular matrix like the extracellular tracer Yb-DTPA. The plasma samples also were analyzed by high-performance liquid chromatography–ICP-MS to determine whether EP-3600 was being metabolized significantly. EP-3600 appeared quite stable in vivo. Among all 5 animals, EP-3600 accounted for >80% of the total Gd in the plasma at any time point. The rate of clearance of EP-3600 from plasma was similar to that of Yb-DTPA.

T1 and T2 of the plasma samples were determined at 37°C and 1.4 T. These measurements, combined with the ICP-MS measurements, allowed the determination of the relaxivity of EP-3600 in pig plasma. The T1 relaxivity, \( r_1 \), was 63.8±5.6 mmol·L\(^{-1} \)·s\(^{-1} \) (\( r_1 \mathrm{Gd}=21.3 \) mmol·L\(^{-1} \)·s\(^{-1} \) per Gd) and T2 relaxivity, \( r_2 \), was 115.6±10.7 mmol·L\(^{-1} \)·s\(^{-1} \) (\( r_2 \mathrm{Gd}=38.5 \) mmol·L\(^{-1} \)·s\(^{-1} \) per Gd).

**Discussion**

Cardiac MRI is well established in clinical routine. However, a limitation of cardiac MRI for widespread use is the easy and robust assessment of the hemodynamic significance of coronary artery disease. Thus, all currently used MR perfusion imaging techniques are limited to class II indication or less for clinical use.17

Here, we demonstrate that the new collagen-specific contrast agent EP-3600 addresses the limitations of currently used first-pass MRI after bolus injection of an extracellular contrast agent like Gd-DTPA. EP-3600 binds reversibly to myocardial collagen, enabling the differences in perfusion at stress to be stored by means of differential T1 shortening of the myocardium and thus different signal enhancement patterns on subsequent MRI. Our data suggest that the time
constraints on imaging are significantly relaxed with this agent. Binding of the agent to myocardial collagen enabled visualization of the perfusion defects up to at least 20 minutes after contrast administration. This allows steady-state, higher-spatial-resolution MR perfusion imaging with improved image quality. This approach potentially may allow stress induction and contrast administration outside the MR room, enabling the use of exercise stress (instead of drug-induced hyperemia as used for first-pass MR perfusion imaging).

The use of a molecular targeted agent that binds reversibly to myocardium is similar to the mechanism of nuclear medicine examinations like single-photon emission computed tomography and positron emission tomography, which enable visualization of myocardial perfusion at the cellular level. However, the main limitations of these techniques are low spatial resolution and attenuation artifacts compared with this new molecular MRI approach. Furthermore, some radiation exposure is required.

EP-3600 binds to myocardial collagen, the major constituent of the myocardial extracellular matrix. The relatively small size of the compound (4182 Da) allows rapid extravasation into the interstitial space. The relaxivity of EP-3600 at 1.4 T determined in pig plasma is $110^2$ times higher than standard extracellular Gd contrast. Collagen binding stores the compound in the myocardium relative to flow, and the high relaxivity allows the compound distribution to be detected by MRI. In vivo myocardial collagen binding is strongly suggested by the imaging data. Figure 5B demonstrates that the signal intensity in the normal myocardium was increased after injection of EP-3600 and that this increase was maintained over the course of the study (5 to 60 minutes). Although the normal myocardium remains bright, the compound is steadily being cleared from the blood, as demonstrated in Figure 5A. Quantitative measures of Gd and Yb from blood draws over the course of the experiment indicate that EP-3600 clears from the blood pool at a rate similar to Yb-DTPA. Myocardial retention of EP-3600 also is indicated by quantitative analysis of Gd and Yb levels in heart segments at autopsy, which showed a ratio of myocardium to plasma of $0.85 \pm 0.26$ for EP-3600 compared with $0.22 \pm 0.08$ for Yb-DTPA. The ratio for the extracellular tracer Yb-DTPA is a value for nonspecific distribution. The 4-fold-higher ratio for EP-3600 indicates myocardial binding, likely to collagen. This is consistent with in vitro and ex vivo studies.

Similar to nuclear medicine studies, redistribution of such a specific agent over longer times may need to be taken into account. In our series, we found very good contrast between the ischemic myocardium and the remote myocardium up to 20 minutes after contrast administration. However, in 2 swine, only a moderate contrast was seen in the 40-minute scans, whereas in 1 swine, the ischemic defect disappeared at 60 minutes after injection. This may be associated with gradual fill-in from the remaining EP-3600 in the blood pool after the stress period and possibly redistribution of EP-3600 from the normal myocardium back to the bloodstream and then to the formerly ischemic myocardium. The persistence

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<th>Swine</th>
<th>Before Contrast</th>
<th>At 5 min</th>
<th>At 20 min</th>
<th>At 40 min</th>
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<td>Good</td>
<td>Moderate</td>
<td>Not visible</td>
<td>&lt;3-mm infarct</td>
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<tr>
<td>3</td>
<td>Not visible</td>
<td>Good</td>
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LE indicates small era of late enhancement in the center of the larger perfusion defect. See text for details.
of the hypoenhanced ischemic territory may correlate with the degree of stenosis.

In 1 additional swine, we found a small lesion of late enhancement on the scans acquired 40 and 60 minutes after injection that was suggestive of myocardial infarction, which was confirmed by TTC staining (Figure 6) and is seen infrequently in closed-chest animal models.14,15 In addition to redistribution into the formerly ischemic area, necrosis is known to accumulate extracellular contrast agents because of cell damage with resultant higher contrast concentrations.22 However, in infarction, the amount of assessable collagen may be different and hence may influence the signal with the use of EP-3600. Further investigations are needed to understand the impact of EP-3600 on the imaging of acute and chronic infarction and the interference with perfusion imaging. Ultimately, the potential of molecular MR perfusion imaging remains to be shown in patients with acquired coronary artery disease.

The current use of first-pass MR perfusion imaging with paramagnetic extracellular contrast media is still limited to only a small imaging window during the first pass and has not yet been widely used in clinical routine. Other agents such as blood-pool agents,14,23,24 dendrimer-based agents,25 and manganese-containing agents26 or recently developed approaches using the blood oxygen level–dependent effect27 have also been used to assess myocardial perfusion. When intravasal agents like MS-325 are used, prolonged enhancement of the blood pool is enabled, but the visualization of the perfusion defect also is limited (to \(\approx 40\) seconds in a swine model14) because, after switching of hyperemia, the contrast agent in the blood pool distributes homogeneously into the now less ischemic myocardium.

**Study Limitations**

This first feasibility study has several limitations that have to be addressed. First, direct flow measurement in the coronary lumen during stress was not possible inside the MR gantry. We used a closed-chest model that modeled an acute coronary stenosis. Microsphere distribution at stress confirmed significant flow reduction. However, in a clinical setting, MR perfusion imaging is performed to assess the significance of a more chronic stenosis. The amount of agent available for this first large-animal study was limited to 5 swine. Larger animal studies including comparison of rest and stress in the same model and using this new agent are needed to demonstrate that the signal differences seen are based on the reduced flow during stress. Dose-finding studies and comparison studies to standard extracellular contrast agents/first-pass techniques and other contrast agents (ie, with protein binding) are also needed. The protocol for drug-induced hyperemia used in our study varies from current clinical settings and remains to be optimized for a molecular MRI approach.

**Clinical Implications**

Because of the various limitations, all currently used MR perfusion imaging techniques are limited to class II indication or less for clinical use. Although MRI is the method of choice for cardiac morphology, function, wall motion abnormalities, and viability (class I indication), the assessment of myocardial perfusion remains the main challenge for complete assessment of the myocardium in the field of cardiac MRI, particularly for the assessment of the significance of coronary artery disease. We believe that with this new molecular MRI approach incorporating a Gd-containing, myocardium-targeting contrast agent, a new paradigm has been established that solves the limitations of currently used first-pass MRI techniques. The major benefit for clinical use may include the potential to perform stress outside the MR room (including exercise stress) and subsequent improved high-quality, high-resolution MRI.

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**Disclosures**

Drs Wiethoff and Caravan are former employees of EPIX Pharmaceuticals. Dr Jacques and M.T. Greenfield are employees of EPIX Pharmaceuticals. The other authors report no conflicts.

**References**

13. Cerqueira MD, Weissman NJ, Dilisizian V, Jacobs AK, Kaul S, Laskey WK, Pennell DJ, Rumberger JA, Ryan T, Verani MS. Standardized myocardial segmentation and nomenclature for tomographic imaging of
CLINICAL PERSPECTIVE

Cardiac magnetic resonance (MR) perfusion imaging during the first pass after intravenous administration of extracellular contrast agents is hampered by the spatial and temporal resolution achievable and by the artifacts seen in ultrafast MR imaging. Furthermore, time-consuming quantitative data analysis is often needed. The use of molecular MR imaging with a target-specific contrast agent with perfusion-dependent binding to myocardium may enable prolonged visualization of perfusion defects and thus may help to overcome limitations of currently used first-pass extracellular MR imaging.

EP-3600 is a new gadolinium-containing small-peptide molecular contrast agent that binds reversibly to myocardial collagen. EP-3600 was investigated for myocardial perfusion imaging in a large-animal (swine) model of nonocclusive coronary stenosis. Molecular MR imaging was performed on a clinically used 1.5-T whole-body scanner with a sequence that can be used in humans during daily routine. It could be shown that remote myocardium demonstrates a significant signal increase over the entire examination time (60 minutes), whereas the perfusion defect was visible as a hypointense area for at least 20 minutes after intravenous contrast administration. Thus, EP-3600 enables prolonged, high-contrast, high-spatial-resolution visualization of myocardial perfusion defects in a human-size animal model and may lead to a paradigm shift in clinical cardiac perfusion MR imaging. This new approach could also enable the use of exercise stress outside the MR room instead of currently used drug-induced hyperemia inside the MR gantry.
Molecular Magnetic Resonance Imaging of Myocardial Perfusion With EP-3600, a Collagen-Specific Contrast Agent: Initial Feasibility Study in a Swine Model
Elmar Spuentrup, Karl M. Ruhl, Rene M. Botnar, Andrea J. Wiethoff, Alexandra Buhl, Vincent Jacques, Matthew T. Greenfield, Gabriele A. Krombach, Rolf W. Günther, Mark G. Vangel and Peter Caravan

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