Molecular Magnetic Resonance Imaging of Coronary Thrombosis and Pulmonary Emboli With a Novel Fibrin-Targeted Contrast Agent

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Background—The differential diagnosis of acute chest pain is challenging, especially in patients with normal ECG findings, and may include coronary thrombosis or pulmonary emboli. The aim of this study was to investigate the novel fibrin-specific contrast agent EP-2104R for molecular targeted MR imaging of coronary thrombosis and pulmonary emboli.

Methods and Results—Fresh clots were engineered ex vivo from human blood and delivered in the lungs and coronary arteries of 7 swine. Subsequent molecular MR imaging was performed with a navigator-gated free-breathing and cardiac-triggered 3D inversion-recovery black-blood gradient-echo sequence before and after systemic administration of 7.5 μmol/kg EP-2104R. Two swine served as the control group. MR images were analyzed by 2 investigators, and contrast-to-noise ratio and gadolinium concentration in the clots were assessed. Before contrast media application, no thrombi were visible. After contrast administration, all 32 pulmonary emboli, 3 emboli in the right heart, and 5 coronary thrombi were selectively visualized as white spots with a mean contrast-to-noise ratio of 32 ± 19. The average gadolinium concentration from all 3 types of thrombi was 144 ± 79 μmol/L.


Key Words: contrast media ■ coronary vessels ■ fibrin ■ magnetic resonance imaging ■ thrombosis

Patients with chest pain are frequently sent to the emergency room for diagnostic evaluation and treatment.1,2 In ∼30% of these patients, the primary cause is related to cardiac disease, and in only ∼15% is myocardial infarction with typical ECG changes seen. Hence, further differential diagnoses such as acute coronary syndromes without myocardial necrosis (unstable angina), non–ST-elevation infarction, or pulmonary embolism also have to be taken into account.1,2 These diseases may also be life threatening because of potential subsequent myocardial infarction or circulatory collapse.

Unstable angina and non–ST-elevation myocardial infarction are believed to be caused by plaque rupture and subsequent coronary thrombus formation with potential risk of vessel occlusion.3–5 Currently, coronary thrombosis can be visualized only invasively by x-ray angiography, intravascular ultrasound, or angiography. The decision to use these invasive procedures must depend on the patient-specific total risk, including knowledge of the ischemic cause of the symptoms.6

Pulmonary embolism may also be a differential diagnosis in patients with chest pain (with or without dyspnea). However, clinical findings are often unhelpful.7–9 Furthermore, serum troponin (a marker for myocardial necrosis) can be elevated in patients with pulmonary embolism.10 Thus, a noninvasive imaging modality that allows direct visualization of coronary thrombosis and pulmonary emboli would be of great value.

In the present study, selective and noninvasive molecular MR imaging of coronary thrombi and pulmonary emboli is demonstrated by means of a fibrin-targeted MR contrast agent (EP-2104R, EPIX Pharmaceuticals).

Methods

Subjects

Noninvasive molecular MR imaging of coronary thrombi and pulmonary emboli was performed in 7 healthy domestic swine (48 to 52
Coronary MR Imaging

For coronary MR imaging, a transverse 3D steady-state free-precession scout scan covering the entire heart was performed first for planning of the subsequent targeted double-oblique coronary MR angiography and coronary thrombus imaging. Coronary bright-blood MR angiography was performed with a previously described navigator-gated free-breathing and cardiac-triggered T2-prepared 3D steady-state free-precession sequence allowing visualization of the anatomy of the coronary artery lumen. MR thrombus imaging was also performed by use of a navigator-gated free-breathing and cardiac-triggered inversion-recovery and fat-suppressed 3D black-blood gradient-echo sequence (Figure 1). Spatial resolution of the coronary thrombus scan was increased to 0.6 × 0.6 × 1.5 mm while all other parameters were maintained. To account for more extensive motion of the coronary arteries, the end-diastolic acquisition window was reduced to 12 excitations per R-R interval (56-ms end-diastolic acquisition window), allowing further reduction of intrinsic cardiac motion artifacts. Both white-blood coronary MRA and black-blood coronary thrombus imaging were performed in the same double-oblique imaging planes covering twenty-four 1.5-mm-thick slices.

Two-Dimensional Selective Navigator

For free-breathing data acquisition, all sequences were equipped with a right hemi-diaphragmatic prospective real-time navigator for respiratory movement-artifact suppression. A gating window of 5 mm was used. Because the inversion pulse in the inversion recovery black-blood sequences may reduce navigator performance, the excitation angle of the navigator beam was increased to 45°, and the navigator diameter was set to 50 mm. This allowed high navigator performance, with navigator efficiency always >50%, while maintaining correct navigator position detection. The navigator restore pulse was switched off because this pulse may result in a “spin labeling” of the pulmonary blood, potentially reducing the effectiveness of nulling the blood signal in the lungs.

Fibrin-Targeted Contrast Agent

The novel fibrin-specific MR contrast agent EP-2104R is composed of a small peptide with 4 gadolinium (Gd)-chelate moieties. EP-1873, a similar compound, has been shown to bind to fibrin without binding to circulating fibrinogen. EP-2104R (15 mmol/L in saline) was infused over 3 minutes for a dose of 7.5 μmol/kg.

Pulmonary Multidetector CT Angiography

Multidetector CT (MDCT) was performed for comparison for its demonstrated ability to detect pulmonary embolism in a swine model. CT is currently used clinically in patients with suspected pulmonary embolism and is a tomographic imaging modality that allows a more direct comparison to MR imaging compared with projection techniques like x-ray pulmonary angiography. Furthermore, it is very fast to perform, avoiding any major time delay between contrast media injection and autopsy. Sixteen-row MDCT scanning of the lung was performed with 16 × 0.75-mm collimation (Somatom Sensation, Siemens) with 120-kV tube voltage, 300-mm reconstruction field of view, and 15-mm table feed per rotation after bolus application of 90 mL nonionic contrast material (Ultravist 370, Schering) at a flow rate of 3.5 mL/s. Axial images with 2-mm reconstruction increment and coronal multiplanar reconstructions from 1.0/0.6-mm reconstructions were used as described in literature.

Description of Experiments

For pulmonary embolism, 4 to 8 thrombi per swine were placed in a 12F catheter, and the emboli were delivered via the 16F sheath to the iliac vein by pushing with saline. Coronary thrombi were delivered via a 9F guiding catheter into the left anterior descending (n = 3), right coronary (n = 1), and left circumflex (n = 1) arteries under x-ray guidance. In one control experiment, 7 pulmonary emboli were delivered and imaged without application of extrinsic contrast medium. MR imaging in this pig was repeated after 4 and 6 hours to exclude any signal changes of the clot itself during the experiments.
In a second control swine (8 pulmonary emboli), MR imaging was performed after administration of standard extracellular contrast with a clinical dose (0.1 mmol/kg Gd-DTPA, Magnevist, Schering). All pigs were given heparin to avoid additional clotting.

After clot delivery, the pigs were transferred to the MR unit. Identical MR thrombus imaging sequences of the lungs (coronal slice orientation) and coronary arteries (double-oblique slice orientation along the main axis of the coronary arteries) were performed before contrast media application and repeated ~2 hours after systemic administration of 7.5 μmol EP2104R/kg via an ear vein. Because scanning time varies, depending on heart rate and navigator gating, the time delay between contrast application and imaging varied slightly between 100 and 135 minutes after contrast media injection. After MR imaging, MDCT was performed; immediately afterward, animals were overdosed with heparin and killed, and clots were removed for assessment of Gd concentration.

**Data Analyses**

MR images were analyzed by 2 radiologists by consensus before MDCT and autopsy. Number and localization of readily visible clots were recorded. These data were compared with findings on MDCT and autopsy. For assessment of interobserver variance, images were again reviewed by a second group of 2 investigators blinded to data of the MDCT and autopsy.

Signal analyses included determination of contrast-to-noise ratio (CNR) between the clot and surrounding blood pool. The regions of interest were placed manually in the precontrast images and then copied into the postcontrast images. CNR was calculated as follows: CNR equals the clot signal minus the blood signal divided by the SD of noise, which was determined in a region of air outside the chest.

Gd concentrations were measured by inductively coupled plasma-mass spectrometry. Thrombi were digested overnight with concentrated nitric acid. Digested samples were then diluted with 5% nitric acid containing Tb as an internal standard. The Gd concentration was determined against a calibration curve of the Gd to internal standard ratio versus concentration.

**Statistical Analysis**

CNR values of precontrast and postcontrast images were compared by use of a paired 2-tailed Student t test. A value of P<0.05 was considered significant. Interobserver agreement was assessed through the use of κ statistics.

**Results**

In 7 pigs (n=7), clot delivery and coronary and pulmonary MR imaging sequences were successfully completed. One additional pig not included in this study died during coronary artery thrombus delivery.

Before contrast media injection, neither pulmonary emboli nor coronary thrombi were observed (Figures 2 through 4). Two hours after systemic administration of EP2104R, pulmonary emboli (n=32; Figures 2 and 3) and coronary thrombi (n=5; Figure 4) were seen as white spots with a high contrast to the surrounding tissue, independent of the localization of the clots. In addition to the pulmonary emboli, 3 emboli in the right heart (2 in the right chamber, 1 in the right atrium) were observed with a bright signal (Figure 3, example IV). A very high agreement between both groups of radiologists was found (κ=0.92).

Coronary thrombi and pulmonary emboli were confirmed by x-ray angiography, MDCT, and autopsy. However, 2 pulmonary emboli located adjacent to the aorta or heart and 2 emboli in the right heart were missed on MDCT, probably because of aggravated motion artifacts on CT images in these regions. However, the existence of these emboli was proven macroscopically.

**Figure 2.** Example of molecular MR imaging of pulmonary embolus. A, Three adjacent coronal slices of navigator-gated free-breathing cardiac-triggered inversion-recovery black-blood molecular thrombus MR imaging sequence before (top) and 2 hours after (bottom) administration of EP-2104R. Pulmonary embolus in right lower lobe is seen selectively as white spot on postcontrast images (arrow in A); signal from surrounding blood pool and lung parenchyma is signal suppressed. B, MDCT (transverse slice and magnification) showing corresponding filling defect in vessel lumen (arrow) and surrounding dystelectasis (arrowhead).

CNR measurements yielded a highly significant increase in CNR after contrast media application for lung, right heart, and coronary clots (2±2/4±1/2±1 versus 36±11/56±14/17±6, respectively; P<0.05). The average Gd concentration for lung, chamber, and coronary clots (n=33) was 144±79 μmol/L. The weight and concentration ranges for each group of thrombi are shown in the Table. In the pig that did not receive any contrast medium, there was no Gd in any of the thrombi (n=8). In the pig that received standard extracellular contrast medium (Gd-DTPA), the Gd concentration was 22±16 μmol/L (n=7 clots).

**Figure 3.** Examples of pulmonary emboli (I through III, different pigs) and embolus in right atrium (IV). Coronal single slices before (top) and 2 hours after (bottom) administration of EP-2104R. Pulmonary embolus (arrows in I through III) and atrial clot (arrow in IV) are seen only on postcontrast images as white spots.
In both control pigs (after administration of standard extracellular contrast medium also up to 6 hours after clot delivery without any contrast media application), no thrombi could be delineated. Hence, CNR measurements could be performed only in the swine that received the fibrin-targeted contrast agent EP-2104R. Over the past decade, central and lobar pulmonary emboli have been successfully visualized by MR imaging during the first pass of extracellular contrast medium after bolus contrast media injection (pulmonary MR angiography). With such a technique, the vessel lumen appears bright, whereas emboli can be depicted as filling defects.\(^{20,21}\) However, this method often fails to visualize more distal (subsegmental) emboli\(^ {22}\) because data acquisition has to be performed within a single breathhold, resulting in a limited spatial resolution. In addition, because in these conventional MR angiography techniques the embolus and the lung tissue are signal suppressed, the detection of more distal filling defects or the lack of blood signal in smaller branching vessels as a result of occlusive emboli may be challenging. Motion errors from incomplete breathholding and intrinsic cardiac motion further reduce image quality.\(^ {8,20,21}\) In contrast, with the present approach, direct and positive visualization of the emboli with high contrast to the blood pool and the surrounding tissue was established. Furthermore, navigator technology and cardiac triggering was used, allowing motion-artifact–free chest imaging with high spatial resolution. Patients with chest pain and dyspnea (ie, patients with pulmonary embolism or cardiac diseases) may benefit from such a free-breathing MR imaging method because in these patients reduced image quality is seen more often in standard contrast-enhanced breathhold MR angiography.\(^ {20,21}\) Finally, molecular MR imaging can also be easily combined with MR venography\(^ {21,23}\) for detection of deep vein thrombosis without further contrast media application.

Other modalities for noninvasive imaging of pulmonary embolism may include spiral CT, which gives results similar to those of contrast-enhanced MRA. Spiral CT in conjunction with multidetector arrays is highly accurate for the detection of pulmonary emboli in animals (like pigs) and can be used as an alternative modality in these models.\(^ {16,18}\) However, in patients, several pitfalls have been reported, and visualization of subsegmental thrombi may be limited.\(^ {7,17,18,21}\) Furthermore, CT requires x-ray exposure and administration of potentially nephrotoxic contrast agent. There is still no single noninvasive imaging modality available with sufficient high negative predictive value to rule out pulmonary embolism in all patients, especially because isolated subsegmental embolism may occur in up to 30%.\(^ {7}\)

Over the last decade, coronary MRA using a navigator-gated free-breathing cardiac-triggered T2-prepared 3D...
gradient-echo sequence has been implemented, allowing visualization of the vessel lumen with a bright signal while stenoses can be seen as lumen narrowing. However, with this technique, only the coronary lumen can be delineated; the composition of the stenoses (like soft plaque or thrombus formation) cannot be differentiated. Thrombus formation after rupture of a vulnerable plaque is known to be the main cause of myocardial infarction but is also seen in acute coronary syndromes like unstable angina or non–ST-elevation myocardial infarctions. In non–ST-elevated infarction, fibrin-rich or mixed thrombus layers are seen, and transmural infarction is typically associated with complete vessel occlusion by more red thrombus formation. Selective (targeted) MR imaging of coronary thrombosis may be an ideal noninvasive imaging modality for the visualization of all thrombi, including those associated with plaque rupture. In patients with acute chest pain and suspected myocardial ischemia without ECG changes (the benefit of invasive angiography), direct molecular imaging of coronary thrombosis would be advantageous. In this study, a positive imaging test for noninvasive visualization of coronary thrombi was demonstrated through the use of molecular, fibrin-targeted MR imaging. Because of the high impact of thrombus formation in the progression of coronary artery disease and because thrombosis is a known cause of acute coronary syndromes, the present molecular thrombus MR imaging approach may have a very high clinical potential in patients with chest pain and suspected myocardial ischemia or with non–ST-elevation myocardial infarction. Furthermore, in these patients, less invasive imaging may be especially helpful and predictive because recent studies have suggested a substantial time delay between first thrombus formation and subsequent occlusion. Thus, early detection of nonocclusive coronary thrombus may lead to earlier intervention and prevention of acute myocardial infarction. In addition, other life-threatening thrombosis-related diseases in patients with acute chest pain such as pulmonary embolism may be depicted concurrently with coronary artery thrombosis. Both diseases are important differential diagnoses in patients with acute chest pain. Other indications for specific targeting of fibrin in MR imaging with high clinical impact may include deep vein thrombosis detection, clot visualization in patients with stroke, characterization of intraplaque hemorrhage, and cardiac thrombus visualization (ie, thrombus in the left atrial appendage). Also, molecular MR imaging of thrombus may enable a new tool for more detailed understanding of the impact of thrombus formation in the development of arteriosclerosis.

Multilayer thrombi, consisting of platelet-rich thrombus and red thrombus, are often indicative of intermittent, clinically asymptomatic thrombus formation. These portions are likely related to distinct pathogenic mechanisms. Because each subsequent thrombus layer has the risk of causing myocardial infarction, old layers may be visualized by fibrin-specific MR imaging, which may allow risk stratification. However, because the amount of fibrin may vary in different thrombus layers, further investigations are needed to fully understand the impact of fibrin-binding molecular MR imaging in the field of arteriosclerosis.

A small number of other thrombus-targeting agents such as nanoparticles and ultrasmall iron oxide particles have been developed. However, sufficient local concentration and successful chest MR imaging in a human size animal and after systemic (intra venous) contrast media administration have not been reported. Alternative MR imaging techniques of thrombus visualization may include direct clot imaging, which uses the T1 shortening of methemoglobin. However, such signal enhancement cannot be used in the first hours after clot formation, as is shown in our control swine in which clots could not be seen in the first 6 hours after clot delivery.

Limitations of the present study include the fact that only 1 dosage of the fibrin-targeted contrast EP-2104R was investigated. Furthermore, the optimal time point of MR imaging and the time curve of the Gd concentration in clots remain to be investigated. In this first study, only a model of acute clots was used. Old thrombi are also fibrin rich, but the efficacy of the new compound for detection of clots with different ages remains to be studied. Patient studies are needed to fully understand the potential of molecular thrombus imaging in clinical routine and to evaluate its clinical relevance in patients with acute chest pain.

In conclusion, molecular MR imaging using the fibrin-targeted contrast-agent EP-2104R allows selective visualization of acute coronary, cardiac, and pulmonary thrombi.

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References


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