In Vivo Magnetic Resonance Imaging of Coronary Thrombosis Using a Fibrin-Binding Molecular Magnetic Resonance Contrast Agent

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Background—The advent of fibrin-binding molecular magnetic resonance (MR) contrast agents and advances in coronary MRI techniques offers the potential for direct imaging of coronary thrombosis. We tested the feasibility of this approach using a gadolinium (Gd)-based fibrin-binding contrast agent, EP-2104R (EPIX Medical Inc), in a swine model of coronary thrombus and in-stent thrombosis.

Methods and Results—Ex vivo and in vivo sensitivity of coronary MR thrombus imaging was tested by use of intracoronarily delivered Gd-DTPA–labeled fibrinogen thrombi (n=6). After successful demonstration, in-stent coronary thrombosis was induced by x-ray–guided placement of thrombogenic-coated, MR-lucent stents (n=5). After stent placement, 60 μmol of EP-2104R was injected via the left main coronary artery. Free-breathing, navigator-gated 3D coronary MR angiography and thrombus imaging were performed (1) before and after stent placement and (2) before and after EP-2104R. Thrombi were confirmed by x-ray angiography and autopsy. Fibrinogen thrombi: 5 of 6 intracoronarily delivered Gd-labeled fibrinogen clots (~250 μmol/L Gd) were visible on MRI and subsequently confirmed by x-ray angiography. In-stent thrombi: in-stent thrombosis was observed in all stents after EP-2104R. Four of 5 thrombi were confirmed by x-ray angiography. Chemical analysis of 2 thrombi demonstrated 99 to 147 μmol/L Gd.

Conclusions—We demonstrate the feasibility of MRI of coronary thrombus and in-stent thrombosis using a novel fibrin-binding molecular MR contrast agent. Potential applications include detection of coronary in-stent thrombosis or thrombus burden in patients with acute coronary syndromes. (Circulation. 2004;110:1463-1466.)

Key Words: thrombosis ■ coronary disease ■ magnetic resonance imaging

The majority of acute coronary syndromes (ACS) are believed to result from local thrombosis at sites of ruptured atherosclerotic plaques.1 Although less common, acute or subacute coronary thrombosis is also a serious complication of coronary artery stenting.2 Direct thrombus imaging therefore may be beneficial for both diagnosis and guidance of therapy in patients with these conditions.

Magnetic resonance (MR) thrombus imaging is a promising noninvasive technique that has been demonstrated in large, quasi-static vessels with regard to presence,3 age,4 and thrombus composition.5,6 However, early thrombus formation in ACS or during acute in-stent thrombosis is predominantly the result of platelet/fibrin-rich “white” thrombi. Thus, early detection of white thrombus may be beneficial for both diagnosis and treatment of patients who present with signs of acute in-stent thrombosis or non–ST-segment–elevation ACS.

Recent advances in molecular MR contrast agent technology have led to the development of both fibrin-binding nanoparticles7,8 and peptides.9 By use of these novel compounds, direct thrombus imaging has been demonstrated in vitro and in the aorta and carotid artery of experimental animal models.7,9 The simultaneous development of MR-lucent stents10 and advances in coronary MR angiography (MRA)11 offers the potential for direct imaging of coronary thrombus and coronary in-stent thrombosis, which we examined in a swine model.

Methods

Subjects

Free-breathing coronary MRA and thrombus imaging were performed in female domestic swine (70 to 80 kg; n=6) while in the supine position by use of an interventional 1.5-T Philips Gyroscan ACS-NT short-bore MRI scanner (Philips Medical Systems). The
study was approved by the German government’s committee on animal investigations.

Animal Protocol
After intramuscular premedication with 0.5 mL atropine and 0.2 mL azaperone/kg body wt, an aqueous solution of pentobarbital (1:3) was administered intravenously as needed. The animals were intubated, and mechanical ventilation was maintained throughout the entire experiment.

MRI of Thrombus
Ex Vivo and In Vivo Imaging of Gd-DTPA–Labeled Fibrinogen Thrombi
Four thrombi were engineered in 1-mL syringes by use of Gd-DTPA covalently bonded to human fibrinogen (≈250 μmol/L Gd), 10 NIH units thrombin, 25 mmol/L CaCl₂, and fresh swine blood. One of the Gd-DTPA–labeled thrombi was placed together with a native unlabeled thrombus in a water bath and served as control. The remaining 3 Gd-DTPA–labeled fibrinogen clots were delivered under x-ray guidance into the left coronary artery of 3 swine by use of a 9F guiding catheter and subsequently broke up into 6 thrombi as shown by x-ray angiography (XRA). Free-breathing bright-blood steady-state free precession (bTFE)¹¹ (=coronary MRA) and black-blood inversion-recovery (IR) TFE¹² (=MR thrombus imaging) 3D coronary artery imaging of the left anterior descending coronary artery (LAD) or left circumflex coronary artery (LCx) were performed before and after Gd-DTPA–labeled thrombus delivery.

In Vivo Imaging of Coronary In-Stent Thrombosis
In vivo coronary in-stent thrombosis was induced by x-ray–guided placement of internally glue-coated (Pritt glue, Henkel) (thrombogenic) MR-translucent stents.¹⁰ Five stents (3 LAD, 2 LCx) were placed in 3 swine. After stent placement, 60 μmol EP-2104R (EPIX Medical, Inc)⁹,¹³ diluted in 20 mL saline was delivered via the left main artery by use of an x-ray catheter over =3 minutes, followed by a =5-mL saline flush over =30 seconds. Analogous to the Gd-DTPA–labeled clot experiment, free-breathing bright-blood coronary MRA and black-blood MR thrombus imaging of the LAD or LCx was performed (1) before and after stent placement and (2) before and immediately (=10 minutes) after injection of EP-2104R.

X-Ray Correlation and Autopsy
After completion of MRI, the presence or absence of intracoronary thrombus was confirmed during coronary XRA. In addition, in-stent thrombosis was confirmed by autopsy, and 2 in-stent thrombi were submitted for determination of thrombus Gd concentration.

Imaging Protocols
Except for the first localizer, all data were acquired in mid-diastole, with the navigator placed on the dome of the diaphragm, by use of a 5-mm gating window.

Coronary MRA
The LAD and LCx were imaged in double oblique planes¹⁴ by use of a 3D bTFE coronary MRA sequence.¹¹ Imaging parameters included 1.25×1.25×3-mm voxel size, TR/TE=3.8 ms/1.9 ms, flip angle=75°, and number of slices=12 to 15.

In Vivo Coronary MR Thrombus Imaging
In vivo thrombus imaging was performed in the same imaging plane and with the same voxel size as that used for the coronary MRA. Imaging parameters included TR/TE=4.7 ms/1.4 ms, flip angle=30°, inversion time=285 ms (at 90 bpm), and number of slices=12 to 15.¹² Imaging time was ~6 to 8 minutes.

Image Analysis
Signal-to-noise ratio (SNR) of thrombus was determined by manually segmenting the visually apparent thrombus area (in 3 adjacent slices) and calculating the mean signal (S). Noise (N) was determined as the SD within a region of interest drawn outside of the animal. Contrast-to-noise ratio (CNR) was measured between thrombus and aortic blood and thrombus and adjacent muscle, respectively (eg, CNR=[Sthromus–Sblood]/N). Data are expressed as mean±SD.

Results
Six Gd-labeled fibrinogen clots and 5 MR-lucent stents were successfully delivered/placed under x-ray guidance in the left coronary system.

![Figure 1](https://example.com/figure1.png)

Figure 1. In vivo MRI of Gd-labeled fibrinogen clots. A and D, Coronary MRA before (A) and after (D) thrombus delivery. On both scans, no apparent thrombus is visible (circle). B and E, Black-blood inversion recovery TFE scans before (B) and after (E) clot delivery (same view as A and D). After thrombus delivery (E), 3 bright areas are readily visible (arrows and circle), consistent with location of thrombus. No apparent thrombus was visible on prethrombus (B) images (arrow and circle). C, X-ray angiogram confirming MR finding of thrombus in mid-LAD (circle). F, Magnified view of C. LM indicates left main.
Ex Vivo Imaging of Gd-DTPA–Labeled Fibrinogen Clots

Ex vivo Gd-labeled thrombi (≈250 μmol/L Gd) appeared as bright “hot spots” on the otherwise hypointense IR-TFE images (Gd-labeled thrombus: CNR = 551, SNR = 577, versus native nonlabeled thrombus: CNR = 8, SNR = 18) and had intermediate CNR and SNR on bTFE images (Gd-labeled thrombus: CNR = 23, SNR = 112, versus nonlabeled thrombus: CNR = 58, SNR = 31).

In Vivo Imaging of Gd-DTPA–Labeled Fibrinogen Clots

Five of the 6 intracoronarily delivered Gd-labeled thrombi were readily visible on the IR-TFE MR images (Figure 1E) and were subsequently confirmed by XRA (Figure 1, C and F) (1 x-ray–confirmed thrombus was not visible on MR because the thrombus was outside of the targeted imaging volume). Mean CNR values between Gd-DTPA–labeled thrombi (≈250 μmol/L Gd) and immediately surrounding tissues were 21±8 (SNR_{clot} = 24±9). Consistent with in vitro data, bright-blood bTFE images (Figure 1, A and D) provided minimal information with respect to presence and location of the Gd-labeled fibrinogen clots.

In Vivo Imaging of Coronary Stent Thrombosis

In-stent thrombi were observed in all 5 stents after injection of EP-2104R (Figure 2, E and H), with an average thrombus SNR and CNR of 11±2 and 9±2 with IR TFE imaging. Four of these thrombi were subsequently confirmed by XRA (Figure 2, C, F, and I). One of the MR-detected thrombi was visible on the initial post–EP-2104R IR-TFE data set and was absent on subsequent IR-TFE scans. Consistent with interim thrombus migration, no thrombus was seen on the subsequent XRA. Bright-blood coronary MRA provided minimal information with regard to presence and location of in-stent thrombus (Figure 2, A, D, and G). No contrast uptake was observed in surrounding tissues or in the coronary lumen or ventricular blood pool. Chemical analysis of 2 thrombi indicated Gd concentrations of 99 μmol/L (≈38 mg thrombus) and 147 μmol/L (≈51 mg thrombus).
Discussion
In this study, we demonstrated the feasibility of MR thrombus imaging using a novel fibrin-binding molecular MR contrast agent and an MR-lucent stent.

Fibrin-Specific Contrast Agent
Only a relatively small concentration (≈100 μmol/L) of Gd was necessary for detection of in-stent thrombus. Intracoronary delivery of EP-2104R (60 μmol) over about a 3-minute period was sufficient for the fibrin-specific peptide to bind to intracoronary thrombi and to create sufficient signal for immediate detection.

Coronary MRA and Thrombus Imaging
The use of a flow-independent IR sequence together with a T1 shortening contrast agent allowed for imaging of Gd-labeled fibrinogen clots and coronary in-stent thrombosis with excellent delineation of thrombus from surrounding myocardium and blood. In contrast, bright-blood coronary MRA provided only minimal information with respect to the presence and location of intracoronary thrombus. Furthermore, because of the high contrast, a relatively coarse spatial resolution (1.25×1.25×3 mm) was sufficient for good depiction of in-stent thrombosis, thereby overcoming the inherent resolution limits of MRI (≈1.5 T).

Related Work
Recently, MRI of intraplaque hemorrhage/red cell–rich thrombus has been demonstrated in complex carotid plaques.6 Image contrast relied primarily on the intrinsic T1 shortening effect of methemoglobin.6 Intraplaque hemorrhage and red cell–rich thrombus consequently appeared bright on the otherwise isointense images.

Direct coronary thrombus detection has also been demonstrated with angiography. However, this approach is invasive and has a low sensitivity for the detection of white fibrin-rich thrombus.15 By use of angiography, white thrombus can be differentiated from complex plaque in only ≈50% of cases.15

Clinical Relevance
In the early stages of ACS or during acute in-stent thrombosis, thrombi are predominantly platelet- and fibrin-rich. Furthermore, patients with ACS may present without ECG changes in the emergency room. Thus, early detection of these thrombi may be beneficial both for diagnoses and early treatment of patients presenting with chest pain.

Limitations
Although in practice, intravenous injection of the thrombus-avid agent would be expected, intra-arterial delivery of EP-2104R was performed in this study because of the limited supply of EP-2104R. The total dose was chosen so that intra-arterial injection mimicked the arterial concentration from an intravenous injection of 2 μmol/kg EP-2104R.

In future studies, intravenous administration of EP-2104R, with pulmonic bed passage, should allow us the full advantage of the noninvasive nature of this novel test.

Conclusions
We demonstrated the feasibility of navigator-gated MRI of in-stent coronary thrombosis using a novel fibrin-binding molecular MR contrast agent and an MR-lucent stent. Potential applications include detection of coronary in-stent thrombosis and for patients presenting with possible ACS.

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References
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