Molecular Magnetic Resonance Imaging of Atrial Clots in a Swine Model

Elmar Spuentrup, MD; Bernd Fausten, MD; Sylvia Kinzel, VD; Andrea J. Wiethoven, PhD; Rene M. Botnar, PhD; Philip B. Graham, PhD; Stephan Haller, MD; Marcus Katoh, MD; Edward C. Parsons, Jr, PhD; Warren J. Manning, MD; Thomas Busch, MD; Rolf W. Günther, MD; Arno Buecker, MD

Background—The detection and differentiation of intracardiac masses is still challenging and may include neoplasms and thrombi. The aim of this study was the investigation of a targeted, fibrin-specific contrast agent (EP-2104R) for molecular targeted magnetic resonance imaging (MRI) of left atrial clots.

Methods and Results—Chronic human thrombi were surgically implanted in the left atrial appendage of 5 swine. Molecular MRI was performed with a navigator-gated, free-breathing, cardiac-triggered 3D inversion-recovery, black-blood, gradient-echo sequence before and after systemic administration of 4 μmol/kg EP-2104R. MR images were analyzed by 2 investigators, and the contrast-to-noise ratio was calculated. Location of clots was confirmed by autopsy, and the gadolinium concentration in the clots was assessed. Before contrast agent administration, thrombi were not visible on black-blood MR images. After contrast administration, all atrial clots (n=5) were selectively visualized as white spots with a high contrast-to-noise ratio (clot/blood, 29.7±8.0). The gadolinium concentration in the clots averaged 74±45 μmol/L.

Conclusions—The fibrin-specific MR contrast agent EP-2104R allows for selective and high-contrast visualization of left atrial clots by means of molecular targeted MRI. (Circulation. 2005;112:396-399.)

Key Words: arrhythmia • atrium • imaging • fibrin • thrombus

Transesophageal echocardiography is well established for visualization of intracardiac masses. However, exclusion or detection of left atrial thrombi can be hampered because of dense, spontaneous echo contrast.1-2 Accurate detection or exclusion of atrial clots is needed in patients with a history of thromboembolic stroke and before cardioversion.1-3 Magnetic resonance imaging (MRI) has been shown to be superior for noninvasive imaging of intracardiac and paracardiac masses.4-6 Typically, the mass (eg, tumor or clot) can be delineated from the blood pool by different signal properties on standard cardiac-triggered spin-echo, gradient-echo, or steady-state free-precession sequences. However, the signal of the mass can be similar to that of myocardium,7,8 which may complicate detection of a thin thrombus layer or small thrombi in the left atrial appendage with its trabecular structures.1 Hence, more selective MRI of cardiac thrombi would be very valuable. In the present study, we investigated the potential of a novel, targeted, fibrin-specific MR contrast agent (EP-2104R, EPIX Pharmaceuticals, Cambridge, Mass)9 for molecular MRI of human atrial clots in a swine model.

Methods

Animal Model

Noninvasive molecular MRI of atrial clots was performed in 5 healthy, domestic swine (48 to 52 kg) according to a protocol approved by the German government committee on animal affairs. After premedication with 0.5 mL atropine IM, 0.2 mL azaperone IM per kilogram of body weight, and 0.1 mL ketamine IM per kilogram of body weight, an aqueous solution of pentobarbital (1:3, vol/vol) was administered into an ear vein as needed. The animals were intubated, and mechanical ventilation was maintained throughout the entire experiment. Before surgery, 0.3 mg buprenorphine IM was given, and an infusion of 0.1 mg fentanyl/h was started.

Open-chest surgery on the beating heart was performed after left lateral thoracotomy (with the animal in the right lateral position). After pericardiotomy to expose the left atrium, the left atrial appendage was clamped, allowing for an ≈10-mm-long atriotomy. Fragments of human chronic clots (243±133 mg with ages between 4 weeks and 1.5 years) previously removed from patients during aortic aneurysm repair (n=2) or extraction from a vena cava thrombus (n=3) were then placed in the left atrial appendage and secured at one end to the left atrial wall by a suture (Prolene, Ethicon Inc). Subsequently, the incision was closed and heparin was given to avoid additional clotting.

Received December 17, 2004; revision received March 2, 2005; accepted March 8, 2005.

From the Departments of Diagnostic Radiology (E.S., S.H., M.K., R.W.G., A.B.), Thoracic and Cardiovascular Surgery (B.F., T.B.), and Laboratory Animal Science (S.K.), Aachen Technical University, Aachen, Germany; EPIX Pharmaceuticals (A.J.W., P.B.G., E.C.P.), Cambridge, Mass; and the Departments of Medicine, Cardiovascular Division (R.M.B., W.J.M.), and Radiology (W.J.M.), Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Mass.

Guest Editor for this article was Robert O. Bonow, MD.

Reprint requests to Elmar Spuentrup, MD, Department of Diagnostic Radiology, University Hospital, Aachen Technical University, Pauwelsstrasse 30, 52057 Aachen, Germany. E-mail: spuentrup@rad.rwth-aachen.de

© 2005 American Heart Association, Inc.

Circulation is available at http://www.circulationaha.org

DOI: 10.1161/CIRCULATIONAHA.104.529941

396
Magnetic Resonance Imaging
All studies were performed with a 1.5-T Gyroscan Intera whole-body MR system (Philips Medical Systems). A 4-element, wrap-around, phased-array coil was used for signal reception. All animals were examined in the supine position.

Molecular MRI Sequence
MRI of the left atrium consisted of a navigator-gated, free-breathing, cardiac-triggered, inversion-recovery and fat-suppressed 3D black-blood, gradient-echo sequence. Sequence parameters include a repetition time of 4.6 ms, echo time of 1.4 ms, flip angle of 30°, field of view of 320×320 mm, and a 256×256 matrix reconstructed to 0.6×0.6 mm in-plane resolution. For optimized contrast enhancement between the thrombus and surrounding tissue and blood pool, heart rate–specific inversion times were used. Suppression of intrinsic cardiac motion artifacts was obtained with use of a brief (56 ms, 12 excitations per R-R interval) data acquisition window during late diastole. Thirty-two slices with two 2-mm thicknesses (including zero filling in the z direction) were acquired with a transverse and a double-oblique (along the left atrial appendage) imaging plane. Positioning of the 3D imaging volume was obtained from a previously described 3D steady-state free-precession black-blood scout scan.

For respiratory motion artifact reduction during free breathing, prospective real-time, navigator gating was used with the navigator beam positioned on the right hemidiaphragm. A gating window of 5 mm was applied. Because the inversion pulse in the inversion-recovery black-blood sequences may reduce navigator performance, a local navigator restore pulse was added. This allowed for high navigator performance, with navigator efficiency always >50% while maintaining correct navigator position detection.

Fibrin-Specific Contrast Agent
The fibrin-specific MR contrast agent EP-2104R (EPIX) is composed of a small peptide with 4 Gd-chelate moieties. Similar to EP-1873, a former generation of this compound, it binds selectively to fibrin without binding to circulating fibrinogen. EP-2104R (15 mmol/L in saline) was infused over 3 minutes for a dose of 4 mol/kg.

Description of Experiments and Data Analyses
After surgical implantation of the chronic clots into the left atrium, the swine were transferred to the MR unit. The molecular MRI sequence (both orientations) was performed before and 1.5 hours after contrast agent injection. Because the time slot for the experiment after surgery had to be kept as short as possible, no further sequences (eg, cine sequences) could be performed before contrast agent administration.

MR images were analyzed by 2 radiologists in consensus. Localization of the readily visible clot was recorded and compared with subsequent findings at autopsy. Signal analyses included determination of contrast-to-noise ratio (CNR) between the clot and the surrounding blood pool/myocardium. The regions of interest were placed manually on the postcontrast images and then copied into the precontrast images. CNR was calculated as CNR(clot/blood pool) = [signal(clot) − signal(blood pool)]/SD(air) and CNR(clot/myocardium) = [signal(clot) − signal(myocardium)]/SD(air). The SD of the signal in air [SD(air)] was determined in a region of interest outside the chest. CNR values of precontrast and postcontrast images were compared with a paired, 2-tailed Student t test. A probability value of <0.05 was considered significant. After MRI, the animals were euthanized, and clots were removed to allow assessment of Gd concentration. Gd concentrations were measured by inductively coupled plasma–mass spectrometry.

Results
In all pigs (n=5), clots were successfully implanted by surgery into the left atrium, and molecular MRI was performed before and after intravenous contrast agent administration.

Discussion
In the present study, selective molecular MRI of implanted chronic left atrial clots in vivo was established with the use of systemic (intravenous) administration of a fibrin-specific contrast agent, EP-2104R. With this contrast agent, a high local Gd concentration in the clots was achieved, allowing for selective visualization of the clots with a high signal (white spots) on MRI. With the current technique, a new molecular contrast agent for direct visualization of thrombus formation was enabled, extending former MRI techniques that have been used for thrombus detection in vivo.

Before contrast agent injection, clots could not be observed on the black-blood MR images (Figure 1). After injection of EP-2104R, all 5 clots were seen as high-signal-intensity spots, whereas the signal from surrounding tissue was suppressed (Figures 1 and 2). In 4 of 5 cases, clots were located next to the atrial wall, and in the remaining case, the clot was located more in the lumen of the left atrium because of the longer suture that had been used for fixing the clot. Respective localization of clots matched the findings seen at autopsy.

CNR measurements yielded a highly significant increase in CNR after contrast agent application (CNR clot/blood pool, 1.5±6.3 versus 27.9±8.0; CNR clot/myocardium, 2.9±5.1 versus 27.7±9.4; p<0.05). The average Gd concentration found in the clots (n=5) was 74±45 μmol/L.

![Figure 1. Two different examples of molecular targeted MRI of left atrial (LA) clots. Transverse (I) and double-oblique (II) single slices from 3D data set before (left) and after (right) administration of 4.0 μmol/kg EP-2104R. In both cases, clot located adjacent to left atrial wall is seen only on postcontrast images as white spots (arrows). LV indicates left ventricle.](http://circ.ahajournals.org/doi/abs/10.1161/CIRCULATIONAHA.116.026461)
used the (lower) intrinsic contrast between the clot, myocardium, and the blood pool.\textsuperscript{1,4,6,8}

The detection of left cardiac masses and especially thrombi is important in patients with thrombembolic diseases such as stroke. Because even small clots may be the origin of emboli, highly sensitive detection of clots is mandatory. Furthermore, accurate exclusion of any clot is required before cardioversion.\textsuperscript{3} Transesophageal echocardiography has been shown to be superior when compared with transthoracic echocardiography to detect thrombi. However, dense, spontaneous echo contrasts may reduce image quality.\textsuperscript{2} Therefore, MRI has been used for improved 3D visualization of cardiac masses. Typically, the intrinsic contrast between the mass or clot and the blood pool is used.\textsuperscript{4} However, signal properties of cardiac masses may be similar to those of myocardium on conventional sequences,\textsuperscript{1,7} which may hamper detection of smaller clots, especially in the trabecular-rich, left atrial appendage.\textsuperscript{1} Currently, there are no data available that clearly show that transesophageal echocardiography or MRI confidently rule out all clots, including such small thrombus layers.

With the present molecular MRI technique, a novel (selective) high-contrast visualization of left atrial clots was enabled. Both the blood pool and the atrial wall are signal suppressed. Such contrast may be very helpful especially in the detection of smaller clots in the left atrial appendage. Furthermore, such contrast may support the differentiation of clot, neoplasm, or appositional thrombus on masses when compared with results obtained with conventional MRI techniques.\textsuperscript{1,6,8} In addition to examination of patients before cardioversion or with thrombembolic stroke with an embolus of unknown origin, further potential indications for molecular MRI of clots may include selective visualization of the embolus itself (ie, embolic occlusion of the cerebral artery in patients with stroke) or the detection of other potential origins of embolism, such as a ruptured (carotid) plaque\textsuperscript{13,14} or thrombi from coronary plaque rupture.\textsuperscript{14}

In our model, we implanted chronic human clots that had been removed from patients with aortic aneurysm or vena cava thrombosis. The use of human clots more closely resembles routine clinical use, because binding of the targeted contrast agent was optimized to human fibrin. Furthermore, fresh clots are difficult to fix in the left atrial appendage by surgery. Because clots in the left atrium may be of different ages and more recent apposition of the thrombus may carry a higher risk for an embolic event, molecular targeted MRI of clots should allow detection of all of them, independent of age. Because both recent and chronic clots have a high fibrin content,\textsuperscript{15} fibrin-specific EP-2104R is expected to bind to clots of different ages. The potential of EP-2104R for selective, targeted MRI of fresh, human clots has been recently demonstrated,\textsuperscript{10} whereas in the present study, selective visualization of chronic human clots was shown. The potential of EP-2104R for selective MRI of clots independent of age is a major advantage compared with other MRI sequences, which use the intermittent appearance of hemoglobin breakdown products for contrast.\textsuperscript{8,16,17}

During the past decade, a small number of other thrombus-targeting agents have been developed, such as nanoparticles and ultrasmall iron oxide particles.\textsuperscript{18,19} However, sufficient local concentration and successful chest MRI in a human-size animal have not, to our knowledge, previously been reported. Furthermore, those agents may be less likely to come into clinical use because of safety issues.\textsuperscript{20} Although the study compound rapidly clears from the blood pool, carefully performed safety studies in humans are needed because prolonged binding to fibrin has been shown.\textsuperscript{13} Furthermore, although not seen in our animal studies, binding to other surfaces may need to be considered.

With fibrin-targeted EP-2104R, the clot is selectively visualized while the signal of the surrounding tissue (blood and myocardium) is suppressed. With such a selective and high-contrast (“white spot”) imaging approach, the required spatial resolution for detection of smaller clots is less critical.\textsuperscript{9} For example, in the lungs, clots as small as 15 $\mu$g could be detected.\textsuperscript{10} However, the potential of this new approach for the detection of very small atrial clots in patients remains to be investigated and must be compared with conventional (lower spatial resolution) MRI sequences, like spin-echo and cine sequences or those used for myocardial late enhancement imaging. The latter is performed after administration of a standard contrast medium and allows visualization of thrombi as an avascular area.\textsuperscript{7,21}

**Limitations**

In the present study, only a small number of animals were investigated. Furthermore, molecular MRI was performed $\approx$ 1.5 hours after contrast agent injection to allow its wash-out from the blood pool. Earlier scanning may be compromised by suboptimal signal suppression of the blood pool and would
require careful adjustment of inversion time. However, prolonged delay between contrast medium administration and MRI may complicate clinical routine. The optimal dosage and time of imaging in patients remain to be investigated. Furthermore, the superiority of this new approach compared with other imaging techniques must be shown in patients in a clinical setting.

Conclusion
The fibrin-specific MR contrast agent EP-2104R allows selective and high-contrast visualization of chronic human clots in the left atrium by means of molecular targeted MRI.

Acknowledgments
The study was funded in part by EPIX Pharmaceuticals, Cambridge, Mass. The authors thank Richard Looby, Kirsten Overoye-Chan, and Steven Witkowski for the synthesis and characterization of EP-2104R as well as Charles Chesna for assistance with the inductively coupled plasma samples.

Disclosure
Drs Wiethoff, Graham, and Parsons are employed by and have ownership interests in EPIX Pharmaceuticals, which is developing EP-2104R for clinical indications. Dr Botnar has received a research grant and has stock ownership interests in EPIX Pharmaceuticals, which is developing EP-2104R for clinical indications. Drs Wiethoff, Graham, and Parsons are employed by and have ownership interests in EPIX Pharmaceuticals, which is developing EP-2104R as well as Charles Chesna for assistance with the inductively coupled plasma samples.

References
Molecular Magnetic Resonance Imaging of Atrial Clots in a Swine Model
Elmar Spuentrup, Bernd Fausten, Sylvia Kinzel, Andrea J. Wiethoff, Rene M. Botnar, Philip B. Graham, Stephan Haller, Marcus Katoh, Edward C. Parsons, Jr, Warren J. Manning, Thomas Busch, Rolf W. Günther and Arno Buecker

Circulation. 2005;112:396-399; originally published online July 11, 2005; doi: 10.1161/CIRCULATIONAHA.104.529941

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/112/3/396

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

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

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