Noninvasive In Vivo Magnetic Resonance Imaging of Experimental Coronary Artery Lesions in a Porcine Model

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Background—The ability to characterize and quantify coronary artery atherosclerotic lesions accurately, reproducibly, and noninvasively may allow the stratification of risk for future acute coronary syndromes and help direct therapeutic management. MRI has been shown to accurately characterize and quantify atherosclerosis; however, because of the combination of cardiac and respiratory motion artifacts, nonlinear course, and relatively small size of the coronary arteries, these techniques have not been able to be translated to the coronary system in vivo.

Methods and Results—Coronary lesions were induced in Yorkshire albino swine (n=6) with balloon angioplasty, and 4 weeks later MRI of the coronary artery lesions was performed. High-resolution in vivo images of the coronary artery wall and lesions were obtained with a double-inversion-recovery fast-spin-echo sequence in a 1.5-T MR system. There was good agreement between measurements of vessel wall thickness and area from MR images of the coronary arteries and the matched histopathology sections (n=43). The mean difference (MRI minus histopathology ± SD) for mean wall thickness was 0.26±0.18 mm, and for vessel wall area, 5.65±3.51 mm². MRI was also able to visualize intraluminal hematoma (sensitivity 82%, specificity 84%).

Conclusions—Using a clinical MR system, we were able to image coronary artery lesions in vivo in an experimental porcine model. Further studies are needed to assess the ability of MRI to characterize coronary atherosclerotic lesions in vivo. (Circulation. 2000;101:2956-2961.)

Key Words: magnetic resonance imaging • arteries

Despite advances in our understanding of the cellular and molecular mechanisms in the pathogenesis of atherosclerosis, coronary artery disease remains the single largest cause of mortality in Western society.1,2 The composition of the atherosclerotic plaque, rather than the degree of vessel stenosis, is known to modulate both the risk of rupture and subsequent thrombogenicity.3–5 MRI has been shown to accurately characterize and quantify atherosclerotic lesions in various arterial systems.6–10 The critical limitation of MRI has been the inability to translate these techniques to the coronary artery system in vivo, because of the combination of cardiac and respiratory motion artifacts, nonlinear course, and relatively small size of the coronary arteries. Using a double-inversion-recovery fast-spin-echo MRI sequence in a clinical whole-body 1.5-T MR system, we were able to visualize coronary lesions in vivo in an experimental porcine model. The ability to noninvasively characterize and quantify coronary artery atherosclerotic lesions may allow stratification of risk for future acute coronary syndromes. Moreover, it could permit tailoring of therapeutic approaches on the basis of atherosclerotic plaque characteristics and sequential assessment of their efficacy.

The process of atherogenesis is often unpredictable, because it is well documented that mild coronary lesions may be associated with significant progression to severe stenosis or total occlusion.3,4,11,12 Plaque disruption with subsequent thrombosis appears to be the main cause of this nonlinear and episodic progression, accounting for the processes of both intermittent plaque growth and acute occlusive coronary syndromes.3,4 Postmortem studies have greatly improved our understanding of which coronary atherosclerotic lesions are associated with these complications of plaque disruption.12,13 In many patients who die of coronary artery disease, the culprit lesion is a plaque occupying <50% of the vessel lumen associated with thrombosis.12,13 A large necrotic lipid core and a thin fibrous cap characterize these culprit or vulnerable plaques.3,4 Disruption of the fibrous cap exposes the highly thrombogenic lipid core to flowing blood, thus promoting thrombus formation.5 Therefore, the ability to identify patients with vulnerable plaques within the coronary arteries could provide a useful tool for stratification of risk for cardiovascular events.

The imaging of vulnerable plaques needs to provide information about composition as well as degree of encroach-
ment on the vessel lumen by the atherosclerotic plaque. The ideal imaging modality would be safe, noninvasive, accurate, and reproducible, thus allowing longitudinal studies in the same patient.\textsuperscript{14} Many currently available imaging techniques for the assessment of coronary artery disease are invasive (ie, coronary angiography and intravascular ultrasound). Coronary angiography provides information about the residual lumen and no information about plaque composition. Apart from invasive intravascular probes, B-mode ultrasound is limited to superficial arteries. Furthermore, it has yet to be proven that ultrasound can accurately and reproducibly distinguish between lipid-rich and fibrous regions.\textsuperscript{15} Ultrafast electron beam CT, although potentially able to provide angiographic data,\textsuperscript{16} has been used mainly to supply information about the calcium composition of atherosclerotic lesions.\textsuperscript{17} MRI is unique in that it is the only potentially noninvasive imaging modality currently available that is able to identify all components of complex atherosclerotic lesions, including lipid-rich, fibrous, calcified, and hemorrhagic components.\textsuperscript{7,8}

The porcine model has been extensively used for the purposes of atherosclerosis research. The pig coronary anatomy closely resembles that of humans, and the size of the coronary arteries is similar.\textsuperscript{18} Consequently, the MRI sequences and resolution required to accurately image coronary artery lesions would be comparable for pigs and humans. Using a double-inversion-recovery fast-spin-echo MRI sequence in a clinical MR system, we were able to perform high-resolution in vivo MRI of coronary artery lesions.

**Methods**

**Animal Species and Interventions**

Yorkshire albino swine ($n=6$, weight 30 to 35 kg) were selected for this study. Coronary lesions were induced in all 3 major epicardial coronary arteries (left anterior descending [LAD], left circumflex [LCx], and right coronary artery [RCA]) by balloon angioplasty. The Mount Sinai School of Medicine animal management program, under accreditation from the American Association for the Accreditation of Laboratory Animal Care, approved all intervention procedures.

Anesthetic and surgical preparation for the coronary angioplasty was performed as previously described.\textsuperscript{18} Coronary angioplasty was performed in the proximal segments of the 3 major epicardial coronary arteries by 5 inflations of a 4.5×20-mm angioplasty balloon (Titan, Cordis Corp) to 14 atm. Each inflation lasted 15 seconds, separated by a 60-second interval. Postprocedure management was as previously reported.\textsuperscript{18}

**In Vivo MRI**

Four weeks after the coronary interventions, the pigs were again anesthetized with ketamine (15 mg/kg IM), and anesthesia was induced with propofol (Zeneca Pharmaceuticals) (10 mg/kg IV). The pigs were then intubated and mechanically ventilated with an MR-compatible ventilator (pneuPAC). Anesthesia was maintained with a continuous infusion of propofol (10 to 15 mg · kg$^{-1}$· h$^{-1}$ IV) and intermittent boluses of doxazosin (Catalytica Pharmaceuticals) (150 µg/kg IV). The animals were placed supine in the magnet, MR-compatible ECG leads were positioned, and a cardiac phased-array surface coil was applied to the anterior chest wall.

MRI was performed in a Sigma clinical 1.5-T magnet (GE Medical Systems). After initial gradient-echo series to localize the heart, all subsequent imaging used the double-inversion-recovery fast-spin-echo sequence. Nonselective and selective preparatory inversion pulses,\textsuperscript{18} long echo train imaging, and short radiofrequency pulses, maximizing blood flow suppression and minimizing vessel motion artifacts, characterize this sequence. This allows for proton density-weighted (PDW) and T2-weighted (T2W) imaging through direct manipulation of the echo time (TE), using a constant repetition time (TR) of twice the R–R interval (2RR) while maintaining cardiac gating of the sequence to end diastole. The double-inversion-recovery fast-spin-echo sequence permits the acquisition of single images within a time period (<30 seconds) that makes breath-hold imaging possible and thus feasible in humans. Image slices were obtained perpendicular to the long axis of the coronary artery.

The inversion time was determined close to the null point of the blood signal and is based on the longitudinal relaxation value of the blood and the TR interval. A TE of 42 ms was chosen for T2W images on the basis of previous work estimating the T2 values for various atherosclerotic lesion components.\textsuperscript{8} Even though this was not essential for our model, which does not have lipid-rich lesions, it showed that this technique could be transferred to humans. Thus, the parameters determining the contrast-to-noise ratio were predetermined on the basis of previously published work. MRI of 3 normal pigs was performed before the study was begun to assist the selection of MR parameters. However, a formal study for sequence optimization was not performed. The following imaging parameters were used: T2W; TR/TE, 2RR′/42 ms; PDW; TR/TE, 2×RR′/17 ms; receiver bandwidth ±62.5 Hz; echo train length 32 ms; echo spacing 4.4 ms; field of view 10×10 cm (or 12×12 cm for fat-suppressed images); matrix 256×256; slice thickness 5 mm; 2 signal averages. A saturation pulse was used to eliminate the epicardial fat signal and thus enhance the definition of the outer boundary of the vessel in some images. The in-plane resolution obtained was therefore 390 to 470 × 390 to 470 µm.

**Euthanasia and Specimen Fixation**

The animals were recovered after MRI. The following day, euthanasia and subsequent coronary artery fixation were performed as previously reported.\textsuperscript{18}

**Ex Vivo MRI**

On the evening before imaging, the specimens were removed from the fixative bath and washed overnight with water. The following morning, the samples were placed in resealable plastic bags, allowing direct application of a conventional 7.6-cm-diameter surface coil to the specimen. MR images were obtained with the same parameters as in vivo imaging. The TR was fixed at 2000 ms (equivalent to an in vivo heart rate of 60 bpm) for both the PDW and T2W images.

**Histopathology**

Serial sections of the coronary arteries were cut at 5-mm intervals matching corresponding MR images. Coregistration was carefully performed by use of ≥1 landmark structures external to the coronary arteries, including arterial branches. Surrounding epicardial fat and myocardium were included in the section for arterial support during fixation and to enhance coregistration through the use of fiducial markers. Coronary specimens were first embedded in paraffin, and thereafter, sections 3 µm thick were cut and stained with a combined Masson’s trichrome elastin stain.

**Image and Data Analysis**

The MR images were transferred to a Macintosh computer for analysis. The histopathological sections were digitized to the same computer from a camera (Sony, 3CCD Video Camera) attached to a Zeiss Axioskop light microscope. The MR images were then matched with corresponding histopathological sections for the coronary specimens ($n=43$).

Cross-sectional areas of the lumen and outer boundary of the vessels were determined for both MR images and histopathology by manual tracing with ImagePro Plus (Media Cybernetics). For the in vivo MR images, the outer vessel boundary was defined as the vessel wall–epicardial fat interface; for histopathology, the outer boundary was defined as the dense adventitia–epicardial fat interface. From these measurements, mean wall thickness and vessel wall area were...
calculated for all sections. Separate investigators, blinded to the results of others, performed each analysis. These data were then analyzed as described by Bland and Altman. To define intraobserver and interobserver variability, a random subset of coronary segment MR images (n=12) and corresponding histopathology sections were reanalyzed and the intraclass correlation coefficients determined.

All MR images obtained were analyzed for the presence or absence of vessel wall hematoma. On the basis of previous work on atherosclerotic plaque characterization, we defined the presence of low-signal (dark) regions within the otherwise high-signal (bright) vessel wall as indicating vessel wall hematoma. With the presence of vessel wall hematoma as identified by histopathology as the gold standard, the sensitivity and specificity of MRI to detect vessel wall hematoma in this study were determined.

**Results**

High-resolution in vivo MR images of all 3 coronary arteries were obtained, with an in-plane resolution of 390 to 470 × 390 to 470 μm, including noninjured and injured segments, with consistent suppression of potential motion artifacts. Cardiac motion was minimized by gating the imaging acquisition to short periods during diastole, when myocardial motion is least. The use of propofol to maintain anesthesia with mechanical ventilation provided stable anesthesia of the pigs, maintaining the heart rate between 60 and 90 bpm, and we believe this assisted in obtaining good cardiac gating.

Ex vivo MR images of the same coronary segments were obtained from the intact heart, and there was good correlation with the corresponding in vivo MR images (Figure 1), confirming the effectiveness of the motion suppression techniques used for the in vivo imaging.

Image slices were obtained perpendicular to the long axis of the coronary artery to be imaged, providing cross-sectional images of the coronary arteries despite their nonlinear course (Figures 2, 3, and 4). Histological validation of the in vivo MR images from the coronary arteries was performed by careful matching of the MR images with the corresponding histopathology sections (n=43). This included sections from the LAD (n=18), LCx (n=13), and RCA (n=12). There was good agreement between MRI and histopathological analysis for estimation of mean wall thickness and vessel wall area using Bland-Altman analyses, and this is summarized in Figure 5. The mean difference (MRI minus histopathology ± SD) for mean wall thickness was 0.26±0.18 mm (Figure 5A) and for vessel wall area, 5.65±3.51 mm² (Figure 5B). Thus, there was a tendency for measurements by MRI to be slightly larger than by histopathology. However, the reasonable SDs for paired measurements of both mean wall thickness and vessel wall area confirm agreement between MRI and histopathology.

T2W MRI was often able to identify intralesion hematoma/thrombus. A total of 11 of the 43 coronary segments analyzed had vessel wall hematoma/thrombus on histopathology. By the criteria previously defined for MR detection of hematoma in the vessel wall, MRI correctly identified 9 of the 11 coronary segments with vessel wall hematoma (specificity 82%) and correctly identified its absence in 27 of the 32 coronary segments without vessel wall hematoma (specificity 84%) (Figure 4). Fibrocellular components of the coronary lesions were noted to appear as bright (high-signal) structures in images in which the epicardial fat signal was suppressed but less bright than the surrounding epicardial fat in non–fat suppressed images (Figure 3). In this study, however, we were not able to test the ability of MRI to characterize atherosclerotic components in vivo.

Intraobserver and interobserver variability assessment by intraclass correlation for both MRI and histopathology showed good reproducibility, with the intraclass correlation coefficients ranging from 0.96 to 0.99 (Table).

**Discussion**

In vivo noninvasive MR is able to provide high-resolution images of coronary artery lesions in this porcine model. This study documents the feasibility of MRI for coronary lesion imaging in vivo, but further studies are necessary to confirm the ability of MRI to accurately quantify and characterize atherosclerotic lesions. Given that the size and anatomy of coronary arteries in the pig are comparable to those in
humans, it may be possible to extrapolate this technique to humans.

We and others have recently demonstrated in vivo MRI of noncoronary atherosclerotic lesions in animals and humans. However, it is acknowledged that substantial improvements in the signal-to-noise ratio and reductions in the significant motion artifacts would be necessary before coronary atherosclerosis imaging could be performed. We have achieved this using cardiac gating, limited breath-holding, and a double-inversion-recovery fast-spin-echo imaging sequence that is nevertheless compatible with currently available clinical 1.5-T MRI systems.

The small but consistent overestimation of mean wall thickness and vessel wall area by MRI in comparison to histopathology may relate to partial-volume effects by MRI as well as shrinkage of the histopathology specimens as a consequence of their preparation. The Bland-Altman analyses show that in general, there is good agreement between MR and histopathological measurements, although further work is needed to improve the accuracy of MRI for the quantification of coronary lesions.

This experimental porcine model produces coronary artery lesions that contain fibrocellular and hemorrhagic regions without lipid deposition. Thus, we are unable to test the

Figure 2. Detection of coronary artery lesions with MRI. Unmagnified axial MR images showing short-axis views through heart from animals without (a) and with (b) balloon angioplasty. Magnifications of a and b (c and d, respectively) showing thin wall of uninjured LAD (white arrow, c) in comparison with eccentric thickening of LAD wall after balloon angioplasty (white arrow, d). Abbreviations as in Figure 1.

Figure 3. MRI and gross pathology from injured LAD. Magnified MR images transverse to LAD after balloon injury are taken without (a) and with (b) fat suppression. Thus, we can appreciate that suppression of perivascular fat allows clearer definition of outer vessel wall. Good agreement between MR images and corresponding gross pathological specimen (c) can be seen, as indicated by eccentric coronary plaque, which is mainly fibrocellular (white arrows in a and b, black arrow in c).
ability of in vivo MRI to characterize coronary atherosclerotic lesions. MRI was able to correctly identify intralesion hematoma/thrombus in $\approx 4$ of every 5 cases. This was confounded by the difficulty in distinguishing the low-signal (dark) regions due to thrombus from the lumen and low-signal adventitial structures. Indeed, the resolution used both in-plane (390 to 470 $\mu$m) and through-plane (5 mm) limits the ability to discern lesion composition. Thus, further improvements will be necessary to accurately characterize and quantify atherosclerotic lesions in human coronary arteries. Although caution should be used before extrapolating experimental studies to humans, given the previously mentioned

Figure 4. Identification of hematoma/thrombus. a, Unmagnified MR image (T2W) transverse to long axis of LCx. b, Magnification of a, showing region of decreased signal intensity (arrow) that appears to correlate with area of hematoma/thrombus on corresponding histopathology (arrow, c). However, it may be difficult to discern region of lower intensity in wall of LCx from artery lumen and arterial adventitia, which also appears dark. Clearly, improvements in resolution and more than a single image weighting (ie, T2W, T1W, and/or PDW) may be needed to accurately characterize coronary artery lesions in vivo. LV indicates left ventricle; LA, left atrium.

Figure 5. Bland-Altman analyses comparing (A) mean wall thickness (MWT) and (B) vessel wall area (VWA) by MRI and histopathology.
anatomic characteristics of this model, this imaging technique could be translated to human coronary arteries.

The ability to perform noninvasive coronary artery imaging in humans could lead to the monitoring of potential future management options for patients both at risk of and with coronary artery disease. The noninvasive nature of this imaging could permit the longitudinal analysis of a given coronary atherosclerotic lesion in patients, and thus the potential exists for monitoring lesions over time before and during therapies such as lipid lowering.

Lipid-lowering therapies have been shown to reduce cardiovascular mortality by 30% to 35%. Modification or stabilization of vulnerable plaques in the coronary arteries by strengthening the fibrous cap and decreasing the lipid core has been proposed to be the mechanism responsible for the observed beneficial clinical effect of these lipid-lowering therapies. Thus, in the future, one might be able to sequentially image and monitor such compositional atherosclerotic plaque changes with MRI.

We are reporting the feasibility of MR for the noninvasive imaging of coronary artery lesions. Continued improvements in MRI techniques and further studies are necessary, however, to confirm the ability of MRI to noninvasively quantify and characterize coronary artery atherosclerosis in vivo.

Acknowledgments

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