MRI of Atherothrombosis Associated With Plaque Rupture

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Objective—MRI would be a valuable noninvasive diagnostic tool to study plaque-associated thrombi. We defined the imaging characteristics of these thrombi, composed primarily of platelets and fibrin, and distinguished them clearly from the vessel lumen and underlying atherosclerotic plaque in an animal model of plaque rupture.

Methods and Results—After triggering plaque rupture in New Zealand White male rabbits, segments of infrarenal aorta containing either red or white thrombi were fixed in formalin. Compared with postmortem red cell–rich thrombi, atherothrombi yielded complex magnetic resonance images with intermediate signal intensity in standard T1- and T2-weighted imaging sequences and were often difficult to distinguish from the aortic wall. Diffusion-weighted imaging sequences revealed restricted diffusion of the atherothrombus relative to the vessel wall and provided excellent contrast. The apparent diffusion coefficient of the thrombus is $1.0 \times 10^{-3}$ mm$^2$/s, compared with $1.5 \times 10^{-3}$ mm$^2$/s in tissue. Similar results were obtained using purified aggregated platelets.

Conclusions—We present the first detailed description of the MRI appearance of plaque rupture–associated thrombosis in histologically validated platelet-rich thrombi. Diffusion-weighted imaging provided the best distinction between thrombus and vessel wall and has potential application for the noninvasive in vivo detection of atherothrombosis.


Key Words: magnetic resonance imaging ■ thrombosis ■ atherosclerosis

Thrombosis in the setting of atherosclerotic disease (atherothrombosis) is the pathophysiological mechanism responsible for the majority of myocardial infarctions and strokes.1-6 However, clinical evaluation of plaque-associated thrombus has proven elusive. The ability to detect such thrombi could identify patients at imminent risk for acute ischemic syndromes and improve treatment. MRI is an attractive imaging modality for this purpose. It is noninvasive, can reveal chemical and morphological features of plaque lesions in situ, and has the potential to provide a detailed characterization of thrombi.

Molecular mechanisms underlying the initiation of atherothrombosis are likely distinct from mechanisms initiating thrombosis within the venous system. Thrombi that form in regions of stasis or low flow are composed of entrapped red blood cells with interspersed fibrin and a smaller component of aggregated platelets. These thrombi are colored darkly by abundant hemoglobin and are often referred to as “red” thrombi. Conversely, the thrombi occurring in the context of atherosclerotic arteries are platelet-rich.7 These thrombi are off-white to gray in appearance and often called “white” thrombi.

Most MRI studies of thrombosis to date have focused on the appearance of either hematomas8-12 or venous thrombi.13-18 Both occur during blood stasis; thus, they contain a large mass of red blood cells and appear as red thrombi.7 The hemoglobin products of red blood cells have a dramatic effect on water magnetic relaxation and are therefore the main determinants of the MRI appearance.9,19 In contrast, platelet-rich thrombi that form on arterial walls after plaque rupture initially contain few red blood cells, and therefore heme products do not determine their image characteristics. The MRI appearance of these thrombi has been noted in a clinical setting but is not well described.6,20,22

Our interest in plaque lipids and plaque rupture has led us to investigate a model of atherosclerosis in rabbits. We have detected thrombosis in vivo, using this model with MRI at 1.5 T.22 Thrombus was poorly distinguished from the aortic wall at the low field and with the pulse sequences used. In continuation of this work, we investigated plaque rupture and thrombosis at high field (11.7 T) in excised aortas from this rabbit model. We found that a combination of higher field and a pulse sequence based on diffusion weighting permit better visualization of the thrombus and better differentiation of the thrombus and the vessel wall.

Methods

Rabbit Housing and Diet
Adult male New Zealand White rabbits weighing 3 kg (Millbrook Immunoserv, Amherst, Mass) were continuously housed at the...
institution’s animal care facilities. All studies were performed under the approval of the Institutional Animal Care and Use Committee on Animal Investigations.

Eight animals were used for this study. The study protocol is described in detail elsewhere. Briefly, animals were fed a 1% high-cholesterol diet incorporated into the rabbit chow (Purina modified 1% cholesterol diet 5735C-G) beginning immediately after aorta balloon injury. During balloon-induced arterial wall injury of the aorta, rabbits were anesthetized with ketamine (35 mg/kg IM), xylazine (2.5 mg/kg IM), and acepromazine (0.75 mg/kg IM). Anesthesia was maintained during the procedure with isoflurane inhalation through mask.

After endothelial denudation and 8 weeks of high-cholesterol diet, plaque disruption was triggered by injection of Russell’s viper venom (0.15 mg/kg IP, Sigma Chemical Co), followed 30 minutes later by histamine (0.02 mg/kg IV) at 48 and at 24 hours before euthanasia (ketamine [35 mg/kg IM] and xylazine [2.5 mg/kg IM], followed by a bolus injection of sodium pentobarbital [100 mg/kg IV]). Heparin was infused to inhibit postmortem coagulation. The aorta, from the aortic valve to the femoral bifurcation, was removed, sectioned, and catalogued. The samples were fixed with formalin, embedded in paraffin, and then processed for general histological staining with hematoxylin-eosin or hematoxylin-phloxine-saffron.

### Immunohistology

**Antigen Retrieval**

Paraffin sections were deparaffinized, rehydrated, and incubated in 10 mM citrate buffer (pH 6.0) for 30 minutes in a microwave oven for antigen retrieval. The sections were then blocked for 20 minutes in 10% normal goat serum (NGS) in phosphate-buffered saline (PBS) at room temperature. Mouse monoclonal antibodies were used for the primary antibodies, and these were incubated at 4°C overnight.

**Secondary Antibodies**

The sections were then incubated with biotinylated secondary antibody (rabbit anti-goat IgG) for 30 minutes at room temperature. This was followed by incubation in ABC-AP reagent, Vector Red alkaline phosphate (AP) substrate for 30 minutes, and then counterstained with hematoxylin. Samples were dehydrated, cleared, and mounted.

**Counterstaining**

Sections were counterstained with hematoxylin (1% in 70% ethanol) for 15 seconds, rinsed in water, and then mounted.

### Platelet Aggregates

Platelet aggregates were collected from whole blood from a single donor as previously described. Separated platelets were divided into 2 aliquots at a concentration of ~1×10^7/mL and were allowed to aggregate in the presence of 25 μmol/L thrombin receptor agonist peptide. Aggregated platelets were gently stirred to induce settling. One aliquot was immediately imaged; the other was treated with 10% formalin for several days before imaging.

### Fibrin Polymerization

Fibrinogen and thrombin were purchased from Sigma-Aldrich Co and used without further purification. Fibrinogen (5 weight percent) was dissolved in PBS pH 7.5, activated with 10 U thrombin, and allowed to polymerize into a gel for 5 minutes at 37°C.

### Immunohistology

Goat anti–β3 integrin antibody was purchased from Santa Cruz Biotechnology Inc. Briefly, sections were incubated with the primary antibody diluted in 1% BSA in PBS. After washing, sections were incubated with biotinylated secondary antibody (rabbit anti-goat IgG) diluted in 1% BSA in PBS. They were then successively incubated in ABC-AP reagent, Vector Red alkaline phosphate substrate, and then counterstained with hematoxylin. Gram-Weigert staining was performed according to standard techniques.

### Analysis by Ex Vivo MRI

MRI was performed on a Bruker Avance 500 wide bore spectrometer (11.7 T; 500 MHz for proton) fitted with a gradient amplifier for imaging. Data were processed with Paravision software provided by the vendor. Arteries were suspended in formalin buffer and imaged within 7 days of euthanization. A small amount of shrinkage of the plaque occurs with formalin fixation, but both the MR images and the histology were obtained with the fixed tissue. Registration between MRI and histology slices was done by measurement from the distal end of the segment. Purified platelets and fibrin were similarly imaged. Initial studies showed no difference in appearance of rabbit arterial samples or platelet aggregates imaged at room temperature or 37°C. All experiments are at room temperature. A 20-mm birdcage transmitter/receiver coil was used. A slice thickness of 0.3 mm was used for all experiments. Spin echo parameters were echo time (TE)=5 ms, repetition time (TR)=160 ms, number of excitations (NEX)=100, matrix=128, and field of view (FOV)=12 mm for T1-weighted (T1W) images and TE=30 ms, TR=3000 ms, NEX=64, matrix=256, and FOV=12 mm for T2-weighted (T2W) images. Gradient-recalled echo (GRE) parameters used were TE=8 ms, TR=8 ms, flip angle=30°, NEX=1, and matrix=128. T2 relaxation times were determined using a Carr-Purcell-Meiboom-Gill sequence with TE=8 ms. Diffusion-weighted images were obtained using 1 slice, 1.5 mm thick, for each sample. Other parameters for diffusion imaging were TE=43 ms, TR=500 ms, NEX=8, matrix=128, and FOV=18 mm. Diffusion gradient parameters were Δ=20 ms and gradient duration=10 ms. The apparent diffusion coefficient (ADC) was calculated from 4 experiments, with total diffusion b values of 225, 350, 500, and 700 s/mm^2. Average intensities from regions of interest (eg, thrombus, wall) were used to calculate T2 and ADC. Least squares analysis fit the data very well, with coefficients of determination (R^2)>0.99. However, within each region of interest, different pixels had a distribution of T2 or ADC values. The standard deviation of values within a region was used to estimate uncertainty.

### Results

#### Visual Inspection and Histology Confirms Atherosclerosis

Before imaging experiments, excised arterial segments (0.5 to 1 cm in length) were inspected visually. All samples were diseased with thickened intimae. Plaque components, especially yellow-shaded areas, could be detected within the wall without staining. Gross anatomic components of the atherosclerotic lesion, as well as adherent thrombi, were readily identified. Histological correlates to these visual assessments are described in detail below.

#### Red-Appearing Thrombi and White-Appearing Thrombi Have Different Imaging Characteristics

We studied a total of 8 rabbits whose arteries were found to have 6 red cell–poor and 7 red cell–rich thrombi. The presence of both red- and white-appearing thrombi in our samples permitted comparison of their image characteristics. All thrombi of each type had identical imaging characteristics. We illustrate their appearance here with 4 examples. Figure 1A illustrates a cross-sectional T2W image of an artery with a red-appearing thrombus. A histological slice from the same region (Figure 1B) demonstrates thickening of the intima and a thrombus composed of red blood cells, which formed from postmortem coagulation.

As seen in both the MR image and histological slices, the intima of the artery is severely thickened (5 to 10× thicker than normal) and heterogeneous in appearance, reflecting advanced atherosclerosis.

We explored the imaging characteristics of this red blood cell–rich thrombus with 3 different imaging sequences (T1W, T2W, and GRE). Figure 1C through 1E illustrates longitudinal sections. In the T2W image (Figure 1C), the thrombus is uniformly dark and easily distinguished from the vessel wall. Conversely, in the T1W image (Figure 1D), parts of the thrombus appear bright, and the image is not as uniform as the T2W image. There are regions of gray intensity as well. Finally, in the GRE image (Figure 1E) thrombus image intensity is heterogeneous.

An artery with an occlusive thrombus that has a markedly thicker than normal)
vessel wall and the thrombus is limited when compared with the red blood cell–rich specimen. Figure 2D illustrates a cross-sectional T2W image of the same artery demonstrating intimal thickening and an occlusive thrombus, as validated by a corresponding histological slice (Figure 2E).

MR images and corresponding histology of a thrombus containing both red cell–rich and red cell–poor regions are shown in Figure 3. In each of the MR image sequences (Figure 3A through 3C), a central region of intermediate intensity is seen. An area that appears dark on T2W and GRE images, but bright in the T1W image, surrounds this region. These imaging findings are consistent with a thrombus composed of a red cell–poor core surrounded by a red cell–rich region. The corresponding histological section (Figure 3D and 3E) confirms the MRI findings.

MR images of isolated blood constituents (Figure 4) were obtained to provide additional validation of the correlation of MR images and the thrombus constituents illustrated in Figures 1 through 3. The images of isolated aggregated platelets (Figure 4, top) are remarkably similar to those of the white-appearing or red cell–poor thrombus. As with preceding images of red cell–poor regions of thrombi, the T1W image is uniformly bright, and the T2W and GRE images are inhomogeneous with regions of differing contrast. There were some subtle differences between the ex vivo and in vitro images: there is a small amount of red cells in the thrombi studied, usually as tiny isolated spots. This can be appreciated in Figure 2D. The isolated platelet sample was entirely free of red cells. Images of aggregated red blood cells (Figure 4, bottom) were similar to images of the histologically verified red cell–rich thrombus; however, the GRE image of isolated red cells was uniformly dark. Fibrin, polymerized at a concentration 10-fold that in plasma, produced images that were similar to buffer alone (data not shown).

The differences in T2W images of red cell–rich and red cell–poor thrombi were further validated by calculation of their respective transverse (T2) relaxation times. The average T2 measured in the regions of red cell–poor thrombi was 22±3 ms, comparable to the T2 measured in the aortic wall/atherosclerotic plaque (18±3 ms). In comparison, the average T2 measured in red cell–rich thrombi was much shorter (7.7±1 ms). This observation is consistent with the paramagnetic effect of hemoglobin metabolites, which shorten T2 relaxation.

**White-Appearing Thrombi Are Composed of Platelets and Fibrin**

The composition of white-appearing thrombus regions was examined in greater detail by immunohistochemistry and specific stains. Figure 5A demonstrates avid staining for the platelet antigen β3 integrin. Figure 5B demonstrates nonspecific staining without addition of the primary antibody. Moreover, in Figure 5C, a Gram-Weigert stain of the thrombus identifies abundant fibrin. The corresponding T1W MR
image demonstrates similar imaging characteristics of white-appearing thrombus (Figure 6A).

**Diffusion-Weighted Imaging Resolves Thrombus From Vessel Wall Components**

Although differentiation of red cell–rich and platelet/fibrin-rich thrombus regions is feasible with standard MR imaging protocols, the white-appearing thrombus remains inadequately distinguished from the vessel wall or underlying atherosclerotic plaque even with the more ideal conditions of ex vivo imaging at high field (eg, Figure 2). Based on the observations of others,\textsuperscript{24} we considered that the mobilities/diffusion of water protons in the constituents of the red cell–poor thrombus may differ from that of the underlying atherosclerotic plaque and vessel wall. We therefore tested diffusion-weighted imaging (DWI) as a method to enhance contrast between the thrombus and vessel wall. Figure 6A illustrates a T1W image from the vessel for which histology was shown in Figure 5. The DWI (Figure 6B) is bright, surrounded by a darker region clearly demarcating the thrombus from the vessel wall/atherosclerotic plaque. To further confirm that the bright central region represents restricted diffusion compared with the arterial wall, the ADC image was calculated (see Methods). As expected, the ADC image (Figure 6C) is the inverse of the diffusion-weighted image, with the darker region representing slower diffusion. The thrombus is now well differentiated as a dark circular area surrounded by a brighter region. Similar imaging characteristics were obtained using the model system of isolated platelets in vitro (Figure 6D through 6E). The ADCs calculated from the data were $1.5\pm0.2\times10^{-3}$ mm$^2$/s in the vessel wall and $1.0\pm0.2\times10^{-3}$ mm$^2$/s in the thrombus. Similarly, a coefficient of $1.0\pm0.2\times10^{-3}$ mm$^2$/s was calculated in platelet aggregates in vitro.

**Discussion**

This study demonstrates the high-field MRI characteristics of atherothrombosis in situ. Thrombosis of an atherosclerotic plaque is fundamentally different in composition than venous thrombosis or hematoma formation. Although considerable data exists regarding the MR imaging characteristics of red blood cell–rich thrombi, MRI has poorly defined the atherothrombi, which mainly contain platelets and fibrin. Using high-field MRI at 11.7 T, we present ex vivo imaging characteristics of atherothrombi using standard imaging sequences. Further, we propose the application of DWI to achieve improved contrast between the thrombus and under-
lying plaque, a limiting problem associated with both lower field-strength and standard imaging sequences.

**High-Field MRI Is Able to Differentiate Thrombi of Different Histological Types**

The MR imaging characteristics of red blood cell–rich thrombi in this study are consistent with expected appearance of subacute to chronic red cell–rich thrombi, based on previous reports. We found low signal intensity in T2W and GRE images and high signal intensity in T1W images. These results were reproduced by similar experiments on isolated coagulated red blood cells. Signal intensities are strongly affected by the hemoglobin metabolites deoxyhemoglobin and methemoglobin because these paramagnetic molecules enhance both transverse (T2) and longitudinal (T1) relaxation.

Although other investigators have published MRI studies of thrombosis in the context of atherosclerosis, thrombus was identified in those studies by the presence of red blood cells or hemoglobin metabolites. Red cells can accumulate in the necrotic core of the atheroma and the vasa vasonum of the adventitia. However, the more relevant form of thrombus for acute cardiovascular syndromes is the platelet/fibrin-rich thrombus that develops initially at a site of plaque rupture and has low content of red cells. Our study specifically addresses this process. White-appearing thrombi in excised rabbit aortic specimens (1) had a much different visual appearance than red-appearing thrombi, (2) were histologically distinct, and (3) yielded different MR images. The relative paucity of red blood cells and the abundance of platelets and fibrin in these white-appearing thrombi were clearly confirmed by histology. As these thrombi are composed mainly of non–hemoglobin-containing cells and connective tissue, the overall appearance of MR images might be expected to be similar to the arterial wall, itself rich in connective tissue, consistent with our observations.

Furthermore, it has been suggested previously that neither clumped platelets nor fibrin polymers have a strong effect on water proton relaxation. Formalin fixation has been shown to alter water proton relaxation times in atherosclerotic vascular samples. We did note an effect of fixation on relaxation values in the in vitro experiments, but these quantitative differences did not affect our qualitative conclusions.

**DWI Differentiates Thrombus From Vessel Wall**

An important objective of these experiments was to enhance the discrimination of a thrombus associated with plaque rupture from the underlying atherosclerotic plaque. Our previous images of rabbit aortas at low field in vivo revealed inadequate resolution to make such a distinction, consistent with earlier results from human aortic hematomas. In this study, we obtained better distinction between thrombus and plaque using both T1W and T2W sequences, likely caused by the improved resolution at a higher magnetic field. However, these sequences will probably not provide adequate contrast at lower field strength.

To enhance contrast between the thrombus and the underlying atherosclerotic plaque, we exploited expected differences in mobilities of protons contained in these 2 components of atherothrombosis. We demonstrated that DWI and the calculated ADC greatly enhanced image contrast and allowed better differentiation of the thrombus from the vessel wall. With this improved contrast, thrombi associated with plaque rupture may be distinguished at the lower fields used in human clinical studies. In our study, we used a lower maximal b value (700 s/mm²) than has been used in previous studies (1700 s/mm² or more). We chose this b value because (1) T2 is shorter at higher fields, (2) 700 s/mm² is comparable to the b value used clinically (generally 800 to 1000 s/mm²), and (3) this b value provided excellent contrast.

Although it has been suggested that decreased diffusion within a thrombus is caused by fibrin crosslinking, we did not find evidence for this idea. Aggregated platelets alone exhibited decreased diffusion and can account for the DWI appearance of the native thrombi, whereas polymerized fibrin did not show decreased diffusion (data not shown). It is possible that thrombus retraction affects diffusion within thrombi, and factors known to cause retraction (such as factor XIII) are being investigated in our laboratory. We are also studying alternative sequences to highlight the protein (fibrin) composition of thrombi.

**Advantages of the Experimental Model**

We have previously identified atherothrombosis by MRI in vivo using this rabbit model of plaque rupture. However, the in vivo studies were performed at low field (1.5 T), and the thrombus was not differentiated from the vessel wall, except with injection of a contrast reagent. The ex vivo studies presented here exploit significant advantages not available in our prior in vivo work. These include (1) high-field strength that affords greater signal and contrast to noise, (2) the lack of motion artifacts, and (3) the lack of time constraints for evaluation of innovative pulse sequences.

**Application and Clinical Relevance**

In this study, we demonstrate the MR imaging characteristics of atherothrombosis composed predominantly of platelets and fi-
brin as distinct from the red blood cell–rich thrombi typical of the venous system. Using this information, as well as the novel application of DWI to accentuate contrast between thrombus and plaque, we propose that MRI may be used to detect atherothrombosis in vivo. We have also recently demonstrated, in this same rabbit model, the utility of a fibrin-specific contrast agent for the identification of atherothrombosis in vivo. The images in this study show the potential to image atherothrombosis reliably in vivo without contrast reagents and the necessity for baseline images, which will not generally be available for patients. The details of these high-field observations will not be obtained at lower field strengths, but DWI images together with the calculated ADC images will very likely provide an improvement over standard T1W and T2W imaging protocols. Although the noninvasive identification of atherothrombosis remains an unrealized clinical goal, the MRI techniques proposed in this study represent a significant step forward and have potential application to the treatment of patients with acute ischemic syndromes.

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References

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