MRI of Rabbit Atherosclerosis in Response to Dietary Cholesterol Lowering

Michael V. McConnell, Masanori Aikawa, Stephan E. Maier, Peter Ganz, Peter Libby, Richard T. Lee

Abstract—Direct imaging of the atherosclerotic plaque, rather than the angiographic lumen, may provide greater insight into the response of atherosclerosis to cholesterol-lowering therapy. Aortic plaque was studied in vivo by MRI in rabbits undergoing dietary cholesterol intervention. Thirty-one rabbits underwent aortic balloon injury and high-cholesterol diet for 4 months and then were assigned to low-cholesterol versus continued high-cholesterol diet for up to an additional 16 months. High-resolution (310 μm) fast spin-echo MRI of the abdominal aorta was performed at 4, 12, and 20 months and compared with histology. MRI demonstrated a significant reduction in % area stenosis in rabbits placed on low-cholesterol diet (44.6±2.1% at 20 months versus 55.8±1.5% at 4 months, P=0.0002). In contrast, % area stenosis increased in rabbits maintained on high-cholesterol diet (69.8±3.8% at 20 months versus 55.8±1.5% at 4 months, P=0.001). Similarly, plaque thickness decreased significantly in the low-cholesterol group (0.60±0.05 mm at 20 months versus 0.85±0.06 mm at 4 months, P=0.006), with a trend toward increase in the high-cholesterol group (1.02±0.08 mm at 20 months versus 0.85±0.06 mm at 4 months, P=0.1). Thus, in rabbits undergoing dietary cholesterol lowering, MRI detected regression of aortic atherosclerotic plaque in vivo. Plaque progression was seen with maintenance of high-cholesterol diet. MRI is a promising noninvasive technology for directly imaging atherosclerosis and its response to therapeutic interventions. (Arterioscler Thromb Vasc Biol. 1999;19:1956-1959.)

Key Words: atherosclerosis ■ MRI ■ cholesterol

Atherosclerosis remains the leading cause of death in industrialized nations and is projected to increase globally.1 Clinical care of atherosclerosis has traditionally focused on flow-limiting luminal stenoses. However, recent basic and clinical research findings have challenged this emphasis and shifted the focus away from the vessel lumen to the underlying atherosclerotic plaque.2,3 Indeed, plaque rupture and acute myocardial infarction have been shown to occur more often in mild-to-moderate, rather than severe, stenoses.4–6 Furthermore, cholesterol-lowering therapy causes minimal improvement in coronary stenosis severity, and yet has demonstrated major reductions in clinical cardiovascular events.7–9 Thus, studying the atherosclerotic plaque directly may offer insights into the mechanisms of plaque regression and progression, and thereby aid in the development and evaluation of therapeutic strategies.

An important challenge has been the development of technologies that directly image the atheroma itself, rather than simply the angiographic lumen.10,11 MRI is a promising technology in that it can provide noninvasive imaging with sub-millimeter resolution and high tissue contrast. This has been applied to the imaging and spectroscopy of ex vivo and in vivo atherosclerotic plaque, in both animals and humans.12–20 Although plaque progression by MRI has been shown in animals,15,16 the use of MRI to study atherosclerosis regression and the effects of cholesterol lowering remains untested, to our knowledge. In this study, MRI was used to image rabbit aortic atherosclerotic plaque in vivo in response to dietary cholesterol lowering.

Methods

Animal Protocol

Aortic atherosclerosis was induced in 31 male New Zealand White rabbits (Millbrook Farms, Amherst, MA) by high-cholesterol diet and balloon injury (Figure 1). Specifically, all rabbits were fed a high-cholesterol-inducing diet (purified rabbit chow supplemented with 0.3% cholesterol and 4.7% coconut oil) for 4 months. One week into the diet, balloon injury (4F Fogarty) of the aorta from the arch to the left iliac artery was performed under anesthesia (intramuscular ketamine, 35 mg/kg, and xylazine, 7 mg/kg). After the initial 4 months of high-cholesterol diet, rabbits were randomly assigned to a low-cholesterol diet (chow with no added cholesterol or fat) or continuation of the high-cholesterol diet, for up to a total of 20 months from balloon injury. Cholesterol supplementation in the high-cholesterol group was adjusted (0.05% to 0.2%) to maintain a serum cholesterol level of approximately 1000 mg/dL. Animals underwent MRI at 4 months (baseline, n=9), 12 months (n=17), and 20 months (n=13) after balloon injury. Animals were euthanized at these multiple time points for validation studies (described below) and for a separate analysis of immunohistochemistry endpoints.21 Therefore, true serial data could not be obtained. The protocol was...
Figure 1. Rabbit experimental protocol in which aortic atherosclerosis was induced with balloon injury and 4 months of high-cholesterol diet, followed by high- versus low-cholesterol diet out to 20 months. MRI was performed at 4, 12, and 20 months.

Approved by the Harvard Medical Area Standing Committee on Animals.

MRI
Rabbits were sedated with ketamine/xylazine (as above) and imaged supine in a 1.5 Tesla MRI system (SIGNA, General Electric). A high-strength (30 mT/m) insert gradient system (Bruker Instruments) was used with a cylindrical 17-cm diameter radiofrequency coil. Gradient-echo scout images were used to identify the abdominal aorta and its bifurcation. Then, 13 axial slices (2-mm thick with a 1-mm gap) of the aorta from the level of the bifurcation were obtained using a T2-weighted fast spin-echo sequence with an in-plane resolution of 310×310 μm (FOV=8cm, TE=45 ms, TR=2300 ms, echo-train length=5, NEX=8). The TE was chosen to provide a T2-weighting intermediate to the reported T2 measurements of fibrous versus lipid plaque components. Superior and inferior saturation slabs were used to null signal from blood. Electrocardiographic gating was not used (vessel motion artifacts were not seen). Fat suppression was used to null signal from peri-adventitial fat, which can obscure the vessel wall due to chemical shift. In contrast to peri-adventitial fat, the relatively immobile lipid protons in plaque have been shown to contribute only 10% of the signal and thus fat suppression has a negligible effect on the plaque itself.

MRI Analysis
MRI images were transferred to a workstation (Sun Microsystems) where a custom-designed MRI image analysis program was used to quantitate plaque size. The MRI images were centered on the aorta and magnified 4-fold. The aortic lumen and outer wall were traced manually using a mouse device by an observer blinded to dietary therapy. Plaque thickness and area stenosis ([outer wall area−lumen area]/[outer wall area]) were calculated for each slice as for intravascular ultrasound data.

Histology
Rabbits were euthanized within 48 hours after MRI by intravenous injection of sodium pentobarbital (120 mg/kg), as well as heparin (1000 U/kg) to prevent blood clotting. The abdominal aorta was marked at 3 mm intervals from the bifurcation (corresponding to the MRI slice positions) before excision. Six rabbits at the 12-month time point underwent pressure-perfusion fixation in 2% paraformaldehyde for validation of MRI measurements with histomorphometry (immunohistochemistry studies; precluded pressure-perfused fixation for all rabbits). Aortas from all other rabbits were snap frozen using isopentane chilled with liquid nitrogen and stored at −80°C. Specimens were later embedded in paraffin, sectioned in 3 μm slices, and stained with hematoxylin and eosin.

Histology Analysis
The pressure-perfused histology specimens from the validation subset were photographed and then manually traced using a mouse device by a second observer blinded to dietary therapy and the MRI data. Plaque thickness and % area stenosis were calculated as described above.

Statistical Analysis
Linear regression was used to correlate the individual MRI and histomorphometry measurements from the validation subset. The Student’s t test was used to compare the mean MRI plaque measurements between dietary interventions at the 3 time points. All probability values are 2-sided, with significance at the 0.05 level.

Results

Cholesterol Levels
The baseline serum cholesterol after 4 months on the high-cholesterol diet was 1883±208 mg/dL (mean±SEM). For the low-cholesterol group, serum cholesterol levels dropped to the normal range at the 12-month (72±12 mg/dL) and 20-month (19±2 mg/dL) time points. In the high-cholesterol group, serum cholesterol levels remained elevated at 12 months (1081±115 mg/dL) and 20 months (1108±158 mg/dL).

Validation Measurements
In the subset of 6 animals that underwent pressure-perfused fixation at the 12-month time point, MRI measurements of vessel wall area (r=0.86) and lumen area (r=0.82) correlated closely with histomorphometric measurements (both P<0.0001) (Figure 2).

Diet Effects on Plaque Size
In the rabbits subjected to dietary cholesterol lowering, MRI detected a significant reduction of % area stenosis (44.6±2.1% at 20 months versus 55.8±1.5% at baseline, P=0.0002) (Table 1, Figures 3 and 4). Similarly, plaque thickness decreased significantly in this low-cholesterol group (0.60±0.05 mm at 20 months versus 0.85±0.06 mm at baseline, P=0.006) (Table 2). In contrast, there was a significant increase in % area stenosis in the rabbits maintained on high-cholesterol diet (69.8±3.8% at 20 months

Table 1. Change in % Area Stenosis over Time Based on Cholesterol Diet

<table>
<thead>
<tr>
<th>Diet</th>
<th>Baseline</th>
<th>12 Months</th>
<th>20 Months</th>
<th>(20 Months vs Baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low cholesterol</td>
<td>55.8±1.5%</td>
<td>46.7±2.2%</td>
<td>44.6±2.1%</td>
<td>0.0002</td>
</tr>
<tr>
<td>High cholesterol</td>
<td>58.8±3.8%</td>
<td>69.8±3.8%</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>P (High vs low)</td>
<td>0.01</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM.
versus 55.8±1.5% at baseline, \( P=0.001 \), with a trend toward increase in plaque thickness (1.02±0.08 mm at 20 months versus 0.85±0.06 mm at baseline, \( P=0.1 \)).

Comparing low- and high-cholesterol groups, the decrease in % area stenosis with cholesterol lowering was evident by 12 months (46.7±2.2% low versus 58.8±3.8% high, \( P=0.01 \)) and greater by 20 months (44.6±2.1% low versus 69.8±3.8%, \( P=0.0001 \)) (Table 1, Figure 3). The difference in plaque thickness between low- and high-cholesterol groups was also significant by 12 months (0.63±0.05 mm low versus 0.87±0.08 mm high, \( P=0.02 \)) and at 20 months (0.60±0.05 mm low versus 1.02±0.08 mm high, \( P=0.001 \)) (Table 2).

**Discussion**

In atherosclerotic rabbits undergoing dietary cholesterol lowering, MRI demonstrated significant regression of aortic plaque in vivo. This contrasted with progression of disease with continued hypercholesterolemia. Thus, by directly imaging the atherosclerotic lesion, MRI can noninvasively quantitate plaque burden and detect the effects of a therapeutic intervention.

MRI of atherosclerotic plaque was initially demonstrated ex vivo with the use of both imaging and spectroscopic methods.12,13,18 In vivo plaque imaging14–17,19 has been more challenging, given the presence of biological motion and time constraints. In vivo MRI of atherosclerosis has been demonstrated in several animal models, including rabbits,14,15 rats,16 and mice.17 MRI of plaque progression was shown by Skinner et al15 in 6 balloon-injured rabbits placed on a high-cholesterol diet for up to 16 months. Summers et al16 demonstrated the development of carotid thickening up to 2 weeks after balloon injury in the rat. In vivo MRI of human atherosclerosis was demonstrated by Tousaint et al,19 who imaged advanced carotid plaques in 6 patients undergoing carotid endarterectomy. With T2-weighted imaging, they found relative signal loss within the lipid regions of the plaque compared with the fibrous regions, as identified on histology.

The primary goal of the study was to detect changes in plaque size in response to low- and high-cholesterol diets. Plaque characterization was limited, as areas of signal loss within the plaque (corresponding to the lipid-rich regions on histology) were seen only in the animal with very advanced plaque thickening (Figure 4D and 4E). Plaque components were not generally detected likely due to (1) spatial resolution, (2) suboptimal tissue contrast, and/or (3) differences between human and rabbit plaque. A resolution of 300 \( \mu \)m still only provides 3 pixels to discriminate plaque structure in a typical 1-mm thick plaque. The optimal T2-weighting may differ between human and atherosclerotic rabbit plaque and potentially may relate to the particular diet used. Differences in lipid composition and MRI appearance between human and atherosclerotic rabbit plaque have been documented.20 Other MRI contrast mechanisms (eg, diffusion,24

**TABLE 2. Change in Plaque Thickness (mm) over Time Based on Cholesterol Diet**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Baseline</th>
<th>12 Months</th>
<th>20 Months</th>
<th>( P ) (20 Months vs Baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low cholesterol</td>
<td>0.85±0.06</td>
<td>0.63±0.05</td>
<td>0.60±0.05</td>
<td>0.006</td>
</tr>
<tr>
<td>High cholesterol</td>
<td>0.87±0.08</td>
<td>1.02±0.08</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>( P ) (High vs low)</td>
<td>0.02</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM.
magnetization transfer,25 and chemical-shift imaging13) offer additional approaches to plaque characterization and warrant further investigation.

There are limitations to comparing in vivo MRI data with histomorphometric measurements. Pressure-perfused fixation is typically used to minimize shrinkage. However, there can be further shrinkage with histologic staining,26 as well as vessel shape changes due to sectioning.12 In addition, the slice thickness of the MRI image (2 mm) greatly exceeds that of histology (3 μm), a concern raised by previous authors.12,26 This volume averaging on MRI, which is exacerbated if there is any angulation of the aorta to the imaging plane, can contribute to an overestimation of plaque size and an underestimation of lumen size by MRI. An additional limitation is that sacrificing animals at multiple time points for histologic validation and immunohistochemistry studies precluded serial observations and limited the use of pressure-perfused fixation.

The balloon-injury model was used, rather than hypercholesterolemia alone, as it generates larger more uniform plaques with more human-like fibrous regions overlying lipid-rich regions.21 Clearly, these rabbit atheromata develop over months, compared with decades for humans. Thus, it is not surprising that significant regression can be induced with dietary intervention, despite the lack of evidence for substantial regression in human trials. The immunohistochemistry data in rabbits show a reduction in lipid content and cellular infiltrate with cholesterol lowering.21 A major difference in the human studies is that the angiographic lumen rather than the actual plaque was measured, making it possible that human plaque regression occurs but is not detected as lumen size is maintained.

The ability of MRI to study the atheroma directly and noninvasively has the potential for greater understanding of both the mechanisms of plaque progression and the effects of therapy on plaque size and structure. Further advances in MRI, such as higher-field magnets, high-strength gradient systems,14 and phased-array,15 implanted,16 or intravascular26,27 radiofrequency coils, will contribute to the improvement of in vivo plaque characterization. Thus, MRI is a promising noninvasive technology for studying the atherosclerotic plaque and its response to therapeutic interventions.

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References

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