Visualization of Fibrous Cap Thickness and Rupture in Human Atherosclerotic Carotid Plaque In Vivo With High-Resolution Magnetic Resonance Imaging

Thomas S. Hatsukami, MD; Russell Ross, PhD†; Nayak L. Polissar, PhD; Chun Yuan, PhD

Background—The results of studies of advanced lesions of atherosclerosis suggest that the thickness of the fibrous cap that overlies the necrotic core distinguishes the stable lesion from one that is at high risk for rupture and thromboembolic events. We have developed a high-resolution MRI technique that can identify the fine structure of the lesion, including the fibrous cap, in vivo. The aim of the present study was to determine the agreement between in vivo MRI and lesion architecture as seen on histology and gross tissue examination to identify fibrous cap thickness and rupture.

Methods and Results—Twenty-two subjects who were scheduled for carotid endarterectomy underwent MRI with a 3-dimensional multiple overlapping thin slab angiography protocol. The appearance of the fibrous cap was categorized as (1) an intact, thick, (2) an intact, thin, or (3) a ruptured fibrous cap on MRI, gross, and histological sections. Thirty-six sites were available for comparison between MRI and histology. There was a high level of agreement between MRI and histological findings: 89% agreement, \( \kappa \) (95% CI) = 0.83 (0.67 to 1.0), weighted \( \kappa = 0.87 \). Spearman’s correlation coefficient was 0.88 (significant to the 0.01 level).

Conclusions—These findings indicate that high-resolution MRI with a 3-dimensional multiple overlapping thin slab angiography protocol is capable of distinguishing intact, thick fibrous caps from intact thin and disrupted caps in atherosclerotic human carotid arteries in vivo. This noninvasive technique has the potential to permit studies that examine the relationship between fibrous cap changes and clinical outcome and to permit trials that evaluate therapy intended to “stabilize” the fibrous cap. (Circulation. 2000;102:959-964.)

Key Words: atherosclerosis ■ magnetic resonance imaging ■ imaging

Rupture of the fibrous cap, with the resultant exposure of thrombogenic subendothelial plaque constituents, is believed to be the critical event that leads to thromboembolic complications in atherosclerotic coronary and carotid artery disease. Falk1 noted that the majority of coronary thrombotic events are precipitated by fibrous cap rupture, and Carr et al2 found that the prevalence of plaque rupture was significantly higher among patients with a past history of an ischemic neurological event.

The mechanisms that lead to fibrous cap disruption are not well defined, in part because of a lack of animal models that mimic advanced atherosclerotic disease with plaque rupture. Therefore, much of what is known about fibrous cap rupture is based on histological studies of plaques excised at the time of surgery or during postmortem examination. A shortcomings of histological studies is that conclusions regarding the relationship between plaque features and clinical events must be based on histological findings from a single point in time. A prospective, serial examination of the lesion is needed to better understand the processes involved in the development of the high-risk atherosclerotic plaque. To accomplish this goal in vivo, an accurate, reproducible, and preferably noninvasive imaging tool that can characterize the fibrous cap is required.

MRI is ideal for serial studies of lesions of atherosclerosis over time because it is noninvasive and is superior to other imaging modalities in distinguishing soft tissue contrast. A number of studies have demonstrated that MRI can be used to identify morphological and compositional features of atherosclerotic plaque both in vitro and in vivo.3–13 Soila et al14 and Maynor et al8 published early reports that lipid components of atherosclerotic plaque can be distinguished with MRI. Tousaint et al13 noted that other plaque constituents, such as calcification, fibrous intimal tissue, and hemorrhage, could be identified in vivo in a series of 7 patients.

This study represents the first report in the literature of a noninvasive, high-resolution imaging technique that is capable of identifying the fibrous cap in atherosclerotic carotid arteries in vivo. A comparison with the examination of excised carotid endarterectomy specimens demonstrates a
TABLE 1. MRI Criteria That Identify Thick, Thin, and Disrupted Fibrous Caps

<table>
<thead>
<tr>
<th>Fibrous Cap Characteristics</th>
<th>Characteristics on 3D MOTSA Images</th>
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<tbody>
<tr>
<td>Thick fibrous cap</td>
<td>Dark band between white lumen and gray wall</td>
</tr>
<tr>
<td>Thin fibrous cap</td>
<td>No band between white lumen and gray wall</td>
</tr>
<tr>
<td>Recent cap rupture</td>
<td>No band between white lumen and gray wall</td>
</tr>
<tr>
<td></td>
<td>Bright gray region near lumen With or without irregular lumen surface depending on extent of cap rupture</td>
</tr>
</tbody>
</table>

high level of agreement among MRI, histology, and gross examinations of the specimen.

Methods

Study Population

Between June 1997 and May 1998, 22 consecutive patients scheduled to undergo carotid endarterectomy at the University of Washington Medical Center or the VA Puget Sound Health Care System were recruited for the study after informed consent was obtained. The consent forms and protocol were approved by the institutional review board of each facility. Each patient underwent an MRI study of the extracranial carotid arteries within 1 week before surgery.

MRI Protocol

All of the studies were conducted with a 1.5-T whole body scanner (SIGNA, Horizon EchoSpeed, version 5.6/5.7; GE Medical Systems). A custom-made phased-array carotid coil was used to improve the signal-to-noise performance of the scanner. A head holder was used to maintain the subjects in a comfortable and stable position. A 3-dimensional (3D) multiple overlapping thin slab angiography (MOTSA) sequence, originally designed to visualize flowing blood, was used to visualize arterial wall fine structure in this study. The imaging parameters were TR 34 or 22 ms, TE 2.9 to 4.4 ms, slice thickness 1 to 2 mm, field of view 13 to 16 cm, and matrix size 256×160 to 256×256. The number of excitations were 1 for a thickness 1 to 2 mm, field of view 13 to 16 cm, and matrix size of 256×160 to 256×256. The number of excitations were 1 for a thickness 1 to 2 mm, field of view 13 to 16 cm, and matrix size of 256×160 to 256×256.

Histological Processing

The atherosclerotic carotid plaques were excised intact without disruption of the luminal surface of the lesion. This was accomplished by scoring the adventitia and outer media with a scalpel and removing the plaque as an intact tube. The formalin-fixed specimens were decalcified, paraffin embedded, sectioned every 0.5 to 1.0 mm, and stained as previously described. Readers (T.S.H. and R.R.) who were unaware of the MRI findings classified the fibrous cap in each histological section by using the same categories described earlier (Table 1). Caps with a uniform thickness of >0.25 mm were categorized as thick. Plaques that demonstrated any region with a cap thickness of <0.25 mm were classified as thin. Recent fibrous cap rupture was identified by evidence of recent mural thrombus or plaque hemorrhage, as previously defined, adjacent to a disrupted fibrous cap.

Coregistration

To ensure that matching cross sections were compared between MRI and histology examination, the common carotid bifurcation was used as an internal fiducial marker. The carotid bifurcation was defined as the last cross section on which a single common carotid artery lumen was identified. Therefore, by definition, the next sequential cross section contained the 2 lumina of the internal and external carotid arteries. A region that encompassed cross sections 2 mm proximal and 2 mm distal to the bifurcation point was used for comparison. The proximal extent of the plaque into the common carotid artery was also identified for a second comparison between MRI and histology. This region encompassed the proximal 2 mm of the plaque.

Data Analysis

The $\kappa$ values were determined to measure the level of agreement between MRI and histology categorization of the fibrous cap. Unweighted $\kappa$ values, standard error for unweighted $\kappa$, and Spearman’s correlation coefficients were calculated with SPSS for Windows (Version 7.5.1). Weighted $\kappa$ was calculated as described by Fleiss and Cicchetti. Full weight was given to perfect agreement, half-weight was given to disagreement by 1 grade, and zero weight was given to other disagreements.

The association of the histological appearance of the fibrous cap at the 2 measurement points (the proximal end of the plaque in the common carotid artery and in the carotid bifurcation region) was also analyzed with weighted Cohen’s $\kappa$. The weighted $\kappa$ value was 0.03, indicating virtually no dependence or agreement. Agreement between the MRI-rated grade for the appearance of the cap in the common carotid artery and in the carotid bifurcation region was also analyzed with Cohen’s $\kappa$. Weighted $\kappa$ was 0.08. Because it appears that there is no association between histology for the 2 regions or between MRI ratings for the regions, the regions were considered independent. Each region was used as a separate observation without any adjustment for the zero or negligible statistical dependence.

Results

Two subjects were excluded from the analysis because the MRI quality was poor throughout (rating of 1 and 2). One additional subject with a heavily calcified carotid plaque was excluded because the histological sections were not interpretable. Among the remaining 19 patients, data from 2 carotid bifurcation regions were excluded because the MRI quality was poor in 1 patient and the histological section was not interpretable in another. Therefore, of 44 possible cases, 36 observations were available for comparison between MRI and histology examinations.

Examples of thick, thin, and ruptured fibrous caps are demonstrated in Figures 1A to 4. The MRI appearance of an intact, thick fibrous cap is shown in Figure 1A; there is a uniform dark band between the bright lumen and the gray plaque core. The lesion in Figure 1B would be categorized as having an intact, thin fibrous cap, given the absence of the dark band adjacent to the lumen. Figures 2A and 2B demon-
The level of agreement was similarly high for distinguishing plaque with intact, thin fibrous cap, in which dark band adjacent to lumen is absent. The level of agreement was similarly high for distinguishing plaque with intact, thick fibrous cap, in which there is a uniform dark band between bright lumen and gray plaque core. B, MRI appearance of plaque with intact, thin fibrous cap, in which dark band adjacent to lumen is absent.

Strate the MRI and matched histological cross sections of another plaque with a thick fibrous cap. There are areas of dense calcification at the 12- and 3-o’clock positions on the MRI and histological cross sections. In Figure 3, a series of axial common carotid artery images from 1 patient illustrate how the cap appearance is not uniform, even within 1 cross section. Figures 4A to 4C show an example of a plaque with fibrous cap rupture on gross section, histology, and MRI, respectively, and provides an explanation for the nonuniform appearance of the cap in Figure 3. On the gross and histological sections (Figures 4A and 4B), there is an area of cap rupture (arrow 1) next to a region where the fibrous cap is thick (arrow 3). The cap rupture site corresponds to a region where the dark band is absent and a hyperintense, bright region is seen adjacent to the lumen on MRI (Figure 4C). Furthermore, there is a hyperintense region in the plaque core on MRI that corresponds to a region of recent intraplaque hemorrhage on the gross and histological cross sections (arrow 2).

There was a high level of agreement between MRI and histological findings, with a κ (95% CI) value of 0.83 (0.67 to 1.0) and a weighted κ value of 0.87 (Table 2). Spearman’s correlation coefficient was 0.88 (significant to the 0.01 level). The level of agreement was similarly high for distinguishing intact from ruptured fibrous caps (Table 3; κ [95% CI]=0.85 [0.65 to 1.0]).

Discussion
According to the National Center for Health Statistics, cardiovascular disease is the leading cause of death in the United States, and >70% of these deaths are related to atherosclerosis. Atherosclerotic cerebrovascular disease is the third leading cause of death and the leading cause of major disability among adults. Improved methods of diagnosis, treatment, and prevention of coronary and cerebrovascular atherosclerosis would result in significant improvement in quality of life and major savings in health care costs.

Traditionally, the severity of arterial stenosis has been used to identify the high-risk atherosclerotic plaque. However, in 1988, Ambrose et al18 and Little et al19 demonstrated in angiographic studies that mild-to-moderate coronary artery stenoses may lead to acute myocardial infarction and suggested that lumen narrowing was not the sole predictor for thrombotic events. Based on histopathological studies, Davies and Thomas,20 Fuster et al,21 and Falk1 suggested that plaque erosion or disruption was the critical feature in these moderately stenotic, high-risk lesions. Falk1 noted that >75% of major coronary thrombotic events were precipitated by atherosclerotic plaque rupture, resulting in the exposure of thrombogenic subendothelial plaque constituents. In a study that involved 44 carotid endarterectomy specimens (25 from asymptomatic patients and 19 from symptomatic patients), Carr2 noted plaque rupture in 74% of plaques from symptomatic patients compared with 32% of plaques from asymptomatic patients. Fibrous cap thinning was noted in 95% of symptomatic lesions and 48% of asymptomatic lesions.

To improve the identification of these high-risk lesions and to better understand the relationship between fibrous cap rupture and thromboembolic events, an accurate, reproducible imaging method of characterizing the fibrous cap in vivo is required. A number of reports suggest that intravascular ultrasound is capable of identifying cap rupture in coronary arteries.22–26 However, the technique is not optimally suited for serial studies given its invasive nature and the risks associated with catheterization. Optical coherence tomography has great promise for the assessment of the microstructure of the plaque, given its high resolution, which is on the order of 10 to 15 μm.27–29 However, to achieve this resolution, the vessel lumen must be replaced with saline due to attenuation from the presence of blood. Also, the depth penetration with optical coherence tomography is limited to 2 to 3 mm, which would permit only partial interrogation of larger vessels with advanced atherosclerosis.

The present study provides the first evidence that the appearance of the fibrous cap on noninvasive, high-resolution MRI closely agrees with gross and histological findings in...
advanced atherosclerotic carotid plaques. The \( \kappa \) values were 0.83 for distinguishing intact thick fibrous, intact thin caps, and ruptured caps and 0.85 for distinguishing intact and ruptured caps. Kappa values of >0.7 indicate good agreement between 2 tests. In 1 of the 36 comparisons, the fibrous cap was deemed ruptured on histology and was thought to be intact on MRI. Possible explanations for disagreement between histology and MRI include disruption of the cap caused by surgical manipulation or histological sectioning, problems with misregistration of sections in the comparison of histology with MRI, and insufficient image resolution to detect small areas of fibrous cap disruption. Last, although the case numbers are small, the observed sensitivity and specificity for the identification of fibrous cap rupture were promising (89% and 96%, respectively). Although suggestive, more research is needed to establish the sensitivity, specificity, and positive and negative predictive values of the MRI examination, because the 95% CIs for data in the present study are wide.

For this study, a thickness of 0.25 mm was chosen as the level for distinguishing thick and thin fibrous caps. The threshold thickness that poses an increased risk for cap rupture has not been established in a prospective study of carotid arteries. However, in a study that compared morphological features in carotid endarterectomy specimens harvested from asymptomatic and symptomatic patients, Basissiouny et al\(^30\) found that the distance between the necrotic core and lumen was 0.27 mm in symptomatic lesions and 0.5 mm in asymptomatic lesions.

The 3D MOTSA imaging sequence used in this project was originally developed as a MR angiography (MRA) technique to study vessel lumen caliber.\(^{31,32}\) The technique, which is relatively simple to implement, provides enhanced signal from flowing blood and a mixture of T1 and proton density contrast weighting. In typical application, the imaging parameters for MRA are chosen to suppress background signal to increase the contrast between the blood and stationary surrounding soft tissues. However, we have previously shown that this time-of-flight (TOF) technique can be used to visualize not only flowing blood but also structures within the vessel wall.\(^{33}\) Although a number of investigators have shown that spin echo– or fast spin echo– based MR sequences such as T1-, proton density–, and T2-weighted imaging can identify plaque constituents such as the lipid core, intraplaque hemorrhage, and calcification,\(^{3,5,13,34–36}\) to date, there have been no reports in the literature that suggest the fibrous cap can be identified with spin echo– or fast spin echo– based MR sequences.

The appearance of a dark band in TOF images has been incidentally noted in previous reports.\(^{37,38}\) In a study that examined the temporal changes of carotid wall enhancement after the injection of MR contrast material, Aoki et al\(^{38}\) described the presence of a hypointense inner rim on the TOF images and postulated that this represented the intima. Räsänen et al\(^{37}\) noted that fibrous intimal thickening noted on intravascular ultrasound was seen as a hypointense region on 3D TOF MRA, but the study did not have histological verification of these findings.

Von Ingersleben et al\(^{39}\) postulated that the hypointense region may be due to the layered, organized structure seen in collagen-rich, thick fibrous caps. The layered structure seen

### Table 2. Cross-Tabulation of Intact, Thick Fibrous Caps Versus Intact, Thin Caps Versus Ruptured Fibrous Caps on 3D MOTSA and Histology

<table>
<thead>
<tr>
<th>MRI Findings</th>
<th>Intact Thick Cap</th>
<th>Intact Thin Cap</th>
<th>Ruptured Cap</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histology Findings</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact thick cap</td>
<td>15</td>
<td>. .</td>
<td>.</td>
<td>15</td>
</tr>
<tr>
<td>Intact thin cap</td>
<td>2</td>
<td>9</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>Ruptured cap</td>
<td>1</td>
<td></td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>9</td>
<td>9</td>
<td>36</td>
</tr>
</tbody>
</table>

\( \kappa (95\% CI)=0.83 (0.67–1.0), \) weighted \( \kappa=0.87. \)
in this type of fibrous cap would shorten T2 times, thus leading to the dark appearance in the 3D MOTSA image. We would not expect to see a dark band with fibrous caps that have a less-organized structure, such as those that are undergoing degradation by matrix metalloproteinases. Although we did not examine the specimens for matrix metalloproteinase activity in the present study, we are currently testing the hypothesis that the dark band is absent with caps undergoing matrix degradation. If proved correct, these results will lend further support to the favorable prognosis associated with this MRI finding.

Difficulties with MRI interpretation may arise from motion artifacts due to patient movement, swallowing, and artery wall pulsation. We developed a custom-made support device that permits the subject to lie comfortably and to minimize movement. A technique that avoids the acquisition of data during patient swallowing by tracking the motion of the larynx is currently under evaluation, and vessel wall motion artifact from arterial pulsation can be reduced with cardiac gating. Dense calcification, if located adjacent to the lumen, will also appear as a dark region on MRI and may be confused with the appearance of a thick fibrous cap. However, this problem can be reconciled by examining the spin echo images, because calcification will appear dark on T1-, T2-, and proton density–weighted images, whereas the fibrous tissue will appear gray. Last, identification of finer structures within the diseased arterial wall will require higher image resolution. Further improvement in image resolution is possible with unilateral coil design, high matrix size imaging, modification of imaging parameters, and improved, faster hardware.

The eventual goal is to apply this noninvasive technique to the identification of high-risk coronary artery lesions. However, there are significant obstacles that must be addressed, such as cardiac motion and small vessel structure, through shortening of image acquisition times, gating, and improvement in resolution. Future directions include investigation of the biomechanical forces, such as shear and circumferential stress on the fibrous cap, with either ultrasound or MRI. Studies by Mears and Hennerici and Iannuzzi et al suggest that ultrasound is capable of quantifying wall motion in carotid atherosclerosis and that there appears to be a relationship between lesion motion and a prior history of ischemic neurological events. Preliminary studies in our laboratory indicate that it is technically feasible to quantify flow and wall motion with MRI, but this work is in its early stages and needs considerable development and validation.

In summary, the present findings demonstrate a high level of agreement between high-resolution in vivo MRI, gross, and histological findings on the thickness and sites of potential rupture of the fibrous cap in advanced carotid artery atherosclerosis. MRI may have important applications in natural history studies and in clinical trials that examine the process of cap thinning and disruption and may ultimately provide an accurate, noninvasive diagnostic and prognostic tool to treat and prevent the sequelae of this insidious disease.

Acknowledgments
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