Early Change in Ferumoxytol-Enhanced Magnetic Resonance Imaging Signal Suggests Unstable Human Cerebral Aneurysm
A Pilot Study

David Hasan, MD; Nohra Chalouhi, MD; Pascal Jabbour, MD; Aaron S. Dumont, MD; David K. Kung, MD; Vincent A. Magnotta, PhD; William L Young, MD; Tomoki Hashimoto, MD; H. Richard Winn, MD; Donald Heistad, MD

Background and Purpose—The clinical significance of early (ie, within the first 24 hours) uptake of ferumoxytol by macrophages in the wall of human cerebral aneurysms is not clear. The purpose of this study was to determine whether early uptake of ferumoxytol suggests unstable cerebral aneurysm.

Methods—Thirty unruptured aneurysms in 22 patients were imaged with magnetic resonance imaging 24 hours after infusion of ferumoxytol. Eighteen aneurysms were also imaged 72 hours after infusion of ferumoxytol. Aneurysm dome tissue was collected from 4 patients with early magnetic resonance imaging signal changes, 5 patients with late signal changes, and 5 other patients with ruptured aneurysms. The tissue was immunostained for expression of cyclooxygenase-1, cyclooxygenase-2, microsomal prostaglandin E2 synthase-1, and macrophages.

Results—In 23% (7/30) of aneurysms, there was pronounced early uptake of ferumoxytol. Four aneurysms were clipped. The remaining 3 aneurysms were managed conservatively; all 3 ruptured within 6 months. In 53% (16 of 30) of aneurysms, there was pronounced uptake of ferumoxytol at 72 hours. Eight aneurysms were surgically clipped, and 8 were managed conservatively; none ruptured or increased in size after 6 months. Expression of cyclooxygenase-2, microsomal prostaglandin E2 synthase-1, and macrophages was similar in unruptured aneurysms with early uptake of ferumoxytol and ruptured aneurysms. Expression of these inflammatory molecules was significantly higher in aneurysms with early uptake of ferumoxytol versus aneurysms with late uptake.

Conclusions—Uptake of ferumoxytol in aneurysm walls within the first 24 hours strongly suggests aneurysm instability and probability of rupture within 6 months, and may warrant urgent intervention. (Stroke. 2012;43:3258-3265.)

Key Words: aneurysm ■ ferumoxytol ■ macrophages ■ magnetic resonance imaging ■ rupture

Ferumoxytol (AMAG Pharmaceuticals, Lexington, MA), an iron oxide nanoparticle coated by a carbohydrate shell, is a member of the class of nanoparticles known as ultrasmall superparamagnetic particles of iron oxide. The drug was developed as a treatment for iron deficiency anemia in patients with chronic renal failure and was approved by the Food and Drug Administration in 2009.1,2 Ferumoxytol is used in magnetic resonance imaging (MRI) studies for prolonged intravascular imaging. It is also useful as an inflammatory marker when imaging is delayed because it is cleared by macrophages.3,4 Ferumoxytol appears hypointense on MRI T2*-weighted gradient echo sequences and can appear hyperintense on T1-weighted spin echo sequences. The drug can be visualized intravascularly for ≤72 hours but begins to clear within 24 hours; delayed visualization (secondary to macrophage uptake) occurs within 24 hours.

Ultrasmall superparamagnetic particles of iron oxide–enhanced MRI allows detection of phagocytic activity of inflammatory cells, such as macrophages. Several studies in experimental animals and humans have demonstrated that ultrasmall superparamagnetic particles of iron oxide accumulate in atherosclerotic plaques in the abdominal aorta and internal carotid artery.5–11 Thus, the method may allow noninvasive assessment of the inflammatory status of atherosclerotic lesions, and perhaps allow assessment of the effects of antiinflammatory pharmacological interventions on these lesions.5–11

We have demonstrated recently the feasibility of imaging macrophages within the wall of human cerebral aneurysms...
using ferumoxytol-enhanced MRI.\textsuperscript{12} We also reported that imaging with T2* gradient echo MRI at 72 hours after infusion of 2.5 to 5.0 mg/kg of ferumoxytol provides optimal dose and timing parameters for imaging of macrophages within the aneurysm wall. The significance of early uptake (ie, within the first 24 hours) of ferumoxytol however is not clear. We postulated that early uptake of ferumoxytol may indicate active inflammation in aneurysm walls and therefore unstable aneurysm. The current study correlates these imaging findings with the risk of intracranial aneurysm rupture.

Methods

**Study Population**

Patients with known unruptured untreated intracranial aneurysms, presenting to the neurosurgery service at the University of Iowa Hospitals and Clinics, were prospectively enrolled in the study between January 2011 and June 2012. Five patients with ruptured intracranial aneurysms were enrolled for tissue analysis alone and did not undergo the imaging protocol. Exclusion criteria were age <18 years; pregnant women; history of allergy/hypersensitivity to iron, dextran, or iron polysaccharide preparations; requirement for monitored anesthesia or intravenous sedation for MRI; contraindication to MRI; renal insufficiency, hepatic insufficiency, or iron overload; and combination antiretroviral therapy. The study protocol was approved by the University of Iowa Institutional Review Board, and all enrolled patients provided written informed consent to participate in the study.

**MRI Protocol and Analysis**

Ferumoxytol (2.5–5.0 mg/kg at a dilution of 30 mg/mL) was administered as a 1-time dose to all patients with unruptured aneurysms enrolled in the study. A subset of these patients was used previously in establishing the feasibility of imaging macrophages in the wall of human cerebral aneurysms.\textsuperscript{12} Safety data of the agent have been previously published,\textsuperscript{4,5} and the drug is commercially available as a treatment for iron deficiency anemia. Off-label use of the drug in a research protocol was approved by the institutional review boards at the University of Iowa, and patients were monitored for adverse reactions to infusion of ferumoxytol. A Siemens 3T TIM Trio system was used for MRI. Patients completed a baseline MRI consisting of time-of-flight angiography and T2* gradient echo sequences. The time-of-flight angiographic sequence was collected using a 3D multislab technique with the following parameters: TE=3.6 ms, TR=20 ms, field-of-view=200×200×200 mm, matrix=384×384×20, and bandwidth=165 Hz/pixel. Four slabs were collected with a 20% overlap. The T2*-weighted sequence was collected using a 2D gradient echo sequence with the following parameters: TE=20 ms, TR=500 ms, flip angle=20, field-of-view=220×220, matrix=512×384, slice thickness per gap=3.0×0.3 mm, and bandwidth=260 Hz/pixel. The T1-weighted spin echo images were collected using these parameters: TE=2.6 ms, TR=317 ms, flip angle=70°, field-of-view=220×220, matrix=384×307, bandwidth=330 Hz/pixel, averages=2, and slice thickness per gap=3.0×0.3 mm. Imaging was performed at 4 times: before infusion, immediately after injection, and ~24 and 72 hours after infusion of ferumoxytol. Aneurysm size was measured using baseline computed tomography angiogram, magnetic resonance angiogram, or digital subtraction diagnostic angiogram.

**Analysis**

Images obtained before, immediately after, and 24- and 72 hours after infusion of ferumoxytol were compared. A loss of signal intensity (from preinfusion to delayed postinfusion imaging) detected on T2*-weighted sequences (corresponding with extraluminal regions of the imaged lesions) was considered a positive finding. Postinfusion images were coregistered to the baseline images using a rigid transformation and a mutual information similarity metric. Histogram matching was then performed between the 2 data sets before the baseline image was subtracted from the postinfusion image (ie, difference indicates postinfusion minus baseline). The difference image allowed demonstration of a relative signal change from baseline to postinfusion. Two neuroradiologists independently reviewed baseline, postinfusion, and subtracted images from all patients in a blinded fashion and rated the change in signal intensity (considered as consistent or inconsistent with uptake of ferumoxytol). The percentage of agreement and k estimate of agreement were used to calculate interobserver agreement.

**Histology of Aneurysms**

Fourteen patients underwent microsurgical clipping of their aneurysms. Aneurysm dome tissue was analyzed histologically (Figure 1). Four patients had unruptured aneurysms and uptake of ferumoxytol was measured within 24 hours of infusion; 5 patients had unruptured aneurysms and uptake of ferumoxytol was measured at 72 hours; and 5 patients had ruptured aneurysms. As explained earlier, the 5 patients with ruptured aneurysms did not undergo the imaging protocol.

Aneurysm tissues were immunostained with monoclonal antibodies to cyclooxygenase-1 (Epitomics, Burlington CA), cyclooxygenase-2 (COX-2 [Epitomics, Burlington CA]), microsomal prostaglandin E2 synthase-1 (mPGES-1 [Cayman Chemical, Ann Arbor, MI]), M1 macrophages using anti-human leukocyte antigen-DR for M1 (Abcam, Cambridge MA), and M2 macrophages using anti-CD163 for M2 (Abcam).

Semi-quantitative analysis of the slides was performed based on cell count (immunostained positive cells) per high-power field (HPF; 400); grade 0=0 cells per HPF; grade 1=0 to 10 cells per HPF; grade 2=10 to 20 cells per HPF; grade 3=20 cells per HPF. Assessment of slides stained for cyclooxygenase-1, COX-2, mPGES-1, M1, and M2 was made by an observer who was not aware of the source of tissues. Statistical analysis was performed using Kruskal-Wallis test, a nonparametric analysis of variance test. \( P<0.05 \) was considered statistically significant.

**Results**

Twenty-two patients harboring a total of 30 aneurysms completed the imaging study and were included in the imaging analysis protocol (Figure 1). Six patients were male and 16 were female. Eleven aneurysms were <7 mm and 19 were >7 mm. The Table summarizes patient demographics, aneurysm characteristics, and imaging findings. No patient experienced ferumoxytol-related adverse events.

**Aneurysm Imaging**

Thirty aneurysms were imaged and analyzed. There was early uptake of ferumoxytol on MRI in 7 aneurysms (23%); no uptake on both early or late MRI in 7 aneurysms (23%); and only late MRI signal change from uptake of ferumoxytol in 16 (53%) aneurysms (Figure 2). Six of 7 aneurysms with early signal changes were ≥7 mm, and all those that ruptured were ≥15 mm (Figure 3). Ninety-four percent of aneurysms with early signal change were <15 mm. Sixteen aneurysms were observed, and 14 were surgically clipped (4 aneurysms with early MRI signal changes, 8 aneurysms with late MRI signal changes, and 2 with no MRI signal changes). The decision to observe these 16 aneurysms was based on aneurysm size, patient’s age, comorbidities, and preferences.

Of the 16 aneurysms that were observed, 3 aneurysms had early signal changes, 8 aneurysms had only late signal changes, and in 5 there were no signal changes. All 3 aneurysms with early signal changes ruptured within a 6-month observation period. All 3 patients were female, and their aneurysms measured 14×15, 35×30, and 25×15 mm, respectively. Two of
these patients were not operated on because of their age, and the high morbidity and mortality associated with a technically complex surgery. The third patient underwent stent-assisted coiling after aneurysm rupture. Of the 8 aneurysms with late uptake of ferumoxytol that were managed conservatively, none ruptured or increased in size based on follow-up magnetic resonance angiogram or computed tomography angiogram in the follow-up period. Of the 30 aneurysms reviewed by 2 neuroradiologists, there was a lack of agreement about the MRI signal change for only 1 aneurysm. The interobserver agreement was found to be 95% using the percentage of agreement method and 92% using the \( \kappa \) estimate of agreement.

**Histological Findings**

COX-2, mPGES-1, and M1 cells were increased in ruptured aneurysms, and similar in magnitude to unruptured aneurysms with early MRI signal changes (Figures 4 and 5). Expression of COX-2 and mPGES-1, and number of M1 cells were greater in unruptured aneurysms with early MRI signal changes than in unruptured aneurysms with late signal changes (\( P<0.05 \)). Immunostaining of cyclooxygenase-1 and M2 were similar in the groups.

**Discussion**

Substantial evidence suggests that inflammation is a key component in the pathophysiology of cerebral aneurysm formation and rupture.\(^{13}\) Ferumoxytol-enhanced MRI is a noninvasive method for detection of the phagocytic activity of inflammatory cells in the walls of aneurysms using macrophages as a surrogate marker. The current study provides preliminary evidence that aneurysms with early uptake of ferumoxytol on MRI are prone to rupture and thus may warrant early operative intervention.

**Inflammation and Cerebral Aneurysms**

In response to hemodynamic stress, the endothelium undergoes several proinflammatory changes\(^{14}\) including activation of nuclear factor \( \kappa \)B,\(^{15}\) monocyte chemoattractant protein 1,\(^{16,17}\) and vascular cell adhesion molecule 1.\(^{18,19}\) These molecules are highly chemotactic to macrophages and other inflammatory cells.

Macrophages play a crucial role in the pathogenesis of cerebral aneurysms.\(^{13,20}\) Macrophage depletion and knockout of the monocyte chemoattractant protein 1 gene reduce the incidence of cerebral aneurysms in mice.\(^{20}\) Macrophages, which have also been observed in the wall of intracranial aneurysms in rats, secrete extracellular matrix–degrading proteolytic enzymes and induce apoptosis of smooth muscle cells.\(^{13,20}\) Macrophages are an important source of matrix metalloproteinases 2 and 9, which presumably decrease the mechanical strength of aneurysms and contribute to their rupture.\(^{13}\)

COX-2, mPGES-1, and prostaglandin E receptor 2 are induced in endothelial cells of cerebral aneurysms.\(^{21}\)
Suppression of nuclear factor κB–mediated chronic inflammation reduces the incidence of cerebral aneurysm in rats.\textsuperscript{15} We have recently demonstrated that expression of COX-2 and mPGES-1 is increased in the walls of human cerebral aneurysms, and that expression of these molecules is greater in ruptured than in unruptured aneurysms.\textsuperscript{22}

In summary, studies in both humans and experimental animals suggest an important role of macrophages and inflammation in the pathophysiology of intracranial aneurysms.

**MRI Signals Versus Risk of Rupture of Aneurysms**

Aneurysms may show early signal changes (within the first 24 hours), late signal changes (at 72 hours postinfusion), or no signal changes on MRI T2* gradient echo, depending on the uptake of ferumoxytol nanoparticles by macrophages localized in their walls. This study suggests that aneurysms with early MRI changes have a higher risk of rupture, as compared with aneurysms with late or no signal changes. It is interesting to note that the size of aneurysms with early MRI signal changes was variable (6 of 7 aneurysms were ≥7 mm, and aneurysms that ruptured were ≥15 mm), and they did not cluster in a specific location. The sample is too small, however, to draw a strong conclusion that the intensity of the inflammatory response in the aneurysm wall is independent of aneurysm size and location. These 2 factors are major determinants of the risk of aneurysm rupture.\textsuperscript{23}

Early MRI signal changes noted on T2* gradient echo sequence are produced by increased uptake of ferumoxytol nanoparticles by macrophages that are localized within aneurysm walls. Early uptake therefore suggests an active inflammatory process. Indeed, this hypothesis was confirmed histologically, because inflammatory cells and molecules (COX-2, mPGES-1,
Figure 2. A, T2* gradient echo (GE) magnetic resonance imaging (MRI) sequence at baseline and 24 hours postinfusion showing early signal changes in the walls of 3 cerebral aneurysms. (A 1–4, corresponds with patient No. 1 with a right vertebral artery aneurysm; B 1–4, corresponds with patient No. 2 with a left supraclinoid internal carotid artery [ICA] aneurysm; and C 1–4, corresponds with patient No. 3 with a fusiform vertebrobasilar artery aneurysm). Difference images (B and C) demonstrate the relative signal loss after ferumoxytol infusion. All 3 aneurysms ruptured within 6 months. B, T2* GE MRI sequence at baseline and 24 hours postinfusion, and subtraction images showing no early signal changes in 3 aneurysms from patient No. 3 (A 1–3, right ICA terminus aneurysm; B 1–3, anterior communicating artery aneurysm; and C 1–3, right cavernous ICA aneurysm). None of these aneurysms ruptured during the follow-up period. C, T2* GE MRI sequence at 5 different times and magnified subtraction image at 72 hours postinfusion, showing late MRI signal changes in 3 aneurysms. A 1–6, corresponds with patient No. 8 with a right middle cerebral artery aneurysm; B 1–6, corresponds with patient No. 19 with a left middle cerebral artery aneurysm; and C 1–6, corresponds with patient No. 9 with anterior communicating artery and basilar tip aneurysms (the left middle cerebral artery aneurysm was on a different cut). MRI signal changes are best detected in the wall of these aneurysms at 72 hours postinfusion of ferumoxytol. None of these aneurysms ruptured during the follow-up period. ICA indicates internal carotid artery.
and macrophages) were found to be markedly upregulated in aneurysms with early signal changes. It is important to note that expression of these molecules was significantly higher in aneurysms with early MRI changes versus aneurysms with late or no MRI changes. Furthermore, expression of inflammatory molecules and cells was similar in unruptured aneurysms with early signal changes and ruptured aneurysms; this finding strongly suggests that aneurysms with early signal changes are unstable lesions with high risk of rupture when we did not intervene early. The finding that all 3 aneurysms with early signal changes that were managed conservatively progressed to rupture in <6 months further supports this hypothesis.

These observations support the hypothesis that inflammation is an important cause of aneurysm rupture, rather than a consequence of rupture. Previous studies that reported inflammatory changes in the walls of ruptured aneurysms fell short of demonstrating that the inflammatory response was present before rupture of aneurysms, as opposed to a response to aneurysm rupture. We thus provide the first direct evidence that inflammation is a causal factor in progression of cerebral aneurysms in humans to rupture.

If the findings of this preliminary report are validated in a larger study, ferumoxytol-enhanced MRI could be applied in clinical practice as a noninvasive tool to differentiate unstable aneurysms that require early intervention from stable aneurysms in which observation may be safe. Specifically, this technique could prove particularly useful in identifying rupture-prone aneurysms in patients that often pose a therapeutic dilemma, namely elderly patients (>70 years) and patients harboring small aneurysms (<7mm).

Limitations
The relatively small number of patients enrolled and the short follow-up period are limitations of this study. Sufficient data were obtained however to reveal consistent patterns of labeling and to demonstrate the potential clinical usefulness of this method. Longer follow-up periods are necessary to determine the natural history of aneurysms with late signal changes, and the ability of ferumoxytol-enhanced MRI to determine whether there is progression of these stable aneurysms to an unstable phenotype that is prone to rupture.

Summary
Unstable aneurysms, suggested by early uptake of ferumoxytol in aneurysm walls within the first 24 hours and inflammation, are generally large and warrant early intervention. This novel MRI technique holds promise for patients harboring unruptured cerebral aneurysms. In addition, histological findings support the hypothesis that inflammation plays an integral part in progression of cerebral aneurysms.

Figure 3. Distribution of aneurysms with early magnetic resonance imaging (MRI) signal change versus late MRI signal change in relation to aneurysm size. Early MRI signal change was independent of aneurysm size. Late MRI signal change was noted in 50% of aneurysms <7 mm, and 44% in aneurysms 7 to 14 mm.

Figure 4. Immunostaining for 3 different aneurysm categories: aneurysms with late magnetic resonance imaging (MRI) signal changes, ruptured aneurysms, and aneurysms with early MRI signal changes. Immunostaining for cyclooxygenase-1 (COX-1) and M2 cells is similar in the 3 categories of aneurysms. Immunostaining for cyclooxygenase-2 (COX-2), microsomal prostaglandin E2 synthase-1 (mPGES-1), and M1 cells indicates upregulation in aneurysms with early MRI signal changes in a similar manner to ruptured aneurysms.
aneurysms to rupture. Larger clinical studies are needed to validate these observations.

Acknowledgments
We are grateful to Wendy Smoker, MD, and Bruno Policeni, MD (University of Iowa, Carver College of Medicine, Department of Neuroradiology), for reviewing all images for the study. We also thank Christine Hochstedler for helping with the histology slides for the study.

Sources of Funding
This study was supported by National Institutes of Health grant No. R03NS07922 (and the Brain Aneurysm Foundation) to D.H. No funding was provided by AMAG Pharmaceuticals Inc, the manufacturer of ferumoxytol.

Disclosures
None.

References


Early Change in Ferumoxytol-Enhanced Magnetic Resonance Imaging Signal Suggests Unstable Human Cerebral Aneurysm: A Pilot Study

David Hasan, Nohra Chalouhi, Pascal Jabbour, Aaron S. Dumont, David K. Kung, Vincent A. Magnotta, William L. Young, Tomoki Hashimoto, H. Richard Winn and Donald Heistad

Stroke. 2012;43:3258-3265; originally published online November 8, 2012;
doi: 10.1161/STROKEAHA.112.673400
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/43/12/3258

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke 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 Stroke is online at:
http://stroke.ahajournals.org/subscriptions/