Lipid-Rich Atherosclerotic Plaques Detected by Gadofluorine-Enhanced In Vivo Magnetic Resonance Imaging

Marc Siroil, MD; Vitalii V. Itskovich, PhD; Venkatesh Mani, PhD; Juan Gilberto S. Aguinaldo, MD; John T. Fallon, MD, PhD; Bernd Misselwitz, PhD; Hanns-Joachim Weinmann, PhD; Valentin Fuster, MD, PhD; Jean-François Toussaint, MD, PhD; Zahi A. Fayad, PhD

Background—MRI of specific components in atherosclerotic plaque may provide information on plaque stability and its potential to rupture. We evaluated gadofluorine in atherosclerotic rabbits using a new MR sequence that allows plaque detection within 1 hour after injection and assessed enhancement in lipid-rich and non–lipid-rich plaques.

Methods and Results—Twelve rabbits with aortic plaque and 6 controls underwent MRI before and up to 24 hours after gadofluorine injection (50 μmol/kg). Two T1-weighted, segmented gradient-echo sequences (TFL) were compared to enhance vessel wall delineation after injection: (1) an inversion-recovery prepulse (IR-TFL) or (2) a combination of inversion-recovery and diffusion-based flow suppression prepulses (IR-DIFF-TFL). With the use of IR-TFL at 1 hour after injection, the vessel wall was not delineated because of poor flow suppression; at 24 hours after injection, the enhancement was 37% (P<0.01). IR-DIFF-TFL showed significant enhancement after versus before contrast (1 hour: 164% [P<0.005]; 24 hours: 207% [P<0.001]). At 1 hour and 24 hours after injection, the contrast-to-noise ratio was higher with the use of IR-DIFF-TFL than with IR-TFL (1 hour: 13.0±7.7 versus 19.8±10.3 [P<0.001]; 24 hours: 15.2±5.9 versus 11.4±8.9, respectively [P=0.052]). There was no enhancement in the vessel wall after gadofluorine injection in the control group. A strong correlation was found (r²=0.87; P<0.001) between the lipid-rich areas in histological sections and signal intensity in corresponding MR images. This suggests a high affinity of gadofluorine for lipid-rich plaques.

Conclusions—Gadofluorine-enhanced MRI improves atherosclerotic plaque detection. The IR-DIFF-TFL method allows early detection of atherosclerotic plaque within 1 hour after gadofluorine injection. (Circulation. 2004;109:2890-2896.)

Key Words: atherosclerosis ■ contrast media ■ magnetic resonance imaging ■ plaque
(eg, single or multiple inversion-recovery [IR] preparatory pulses).

The goals of our study were to evaluate the use of gadofluorine in atherosclerotic rabbits with the use of a new MR sequence (new black-blood technique) that allows plaque detection within 1 hour after gadofluorine injection and to assess the plaque enhancement after injection (up to 24 hours) in lipid-rich (LR) and non–lipid-rich (non-LR) plaques.

Methods

Animal Protocol

Atherosclerotic aortic lesions with fibrotic and lipidic components were induced in New Zealand White (NZW) rabbits (n=12; aged 3 months; 3.0 to 3.5 kg body wt; Covance, Princeton, NJ) by high-cholesterol diet and balloon injury, as previously described.12,13 Rabbits were fed a high cholesterol–inducing diet (Purina Rabbit Chow, 0.1% cholesterol; Research Diets) for a minimum of 4 months. One week into the diet, aortic denudation from the renal arteries to the iliac bifurcation was performed by balloon injury (embolocatheter catheter 4F) under anesthesia (ketamine 35 mg/kg IM and xylazine 7 mg/kg IM). After 6 months of atherogenic diet, the animals underwent MRI. Six age- and sex-matched NZW rabbits were used as controls; no balloon injury was performed, and no hypercholesterolemic diet was administered. The Mount Sinai Institute of Animal Care and Use Committee approved all experiments.

Contrast Agent

Gadofluorine (Schering AG) is a lipophilic, macroyclic (1528 Da), water-soluble, gadolinium chelate complex (Gd-DOTA derivative) with a perfluorinated side chain. Gadofluorine forms 5-mm-diameter micelles or aggregates in aqueous solution.14,15 Gadofluorine elicits an R1 relaxivity (at 37°C and 1.5 T) of 17.4 L·mmol−1·s−1 in blood. The average gadofluorine plasma half-life in NZW rabbits is approximately 11 hours (B. Missetzlviz, PhD, unpublished data, 2003). The majority of gadofluorine is eliminated by the hepatobiliary (66%) and renal (34%) route within 7 days.16 Because of the higher relaxivity of gadofluorine compared with conventional gadolinium chelates, we injected 50 μmol gadolinium per kilogram body weight. Gadofluorine is at a stage of research and preclinical development. No studies have been performed on humans.

Magnetic Resonance Imaging

Rabbits were sedated with ketamine/xylazine (as above) and imaged supine in a 1.5-T MRI system (Siemens). Sequential transverse images of the abdominal aorta, from the renal arteries to the iliac bifurcation, were obtained with a TI-weighted, 2D, segmented gradient-echo sequence (TFL) (1) with an IR preparatory pulse17 (IR-TFL) and (2) with a combination of IR and diffusion-based flow suppression prepulse18 (IR-DIFF-TFL). TI-weighted imaging was performed before and after administration of gadofluorine (immediately after injection) by intravenous injection of sodium pentobarbital (120 mg/kg). Aortas were excised and perfusion-fixed as previously described.12,13 The adventitia of excised aorta was immediately marked with india ink at the posterior face of the artery to facilitate matching of the histological slides and MR images. Serial sections of the aorta were cut at 3-mm intervals. Coregistration was performed carefully by utilizing the position of the renal arteries and iliac bifurcation.12,13,19 The selected aortic specimens were embedded in paraffin, and 5-μm-thick sections were cut and stained with hematoxylin-eosin (H&E) or with Masson’s trichrome elastin. An independent experienced pathologist blinded to the MR findings performed the histological analyses following the classification from the Committee on Vascular Lesions of the Council of Atherosclerosis, American Heart Association. Tissue component areas were traced manually. Two different areas were distinguished for further analysis: LR and non-LR areas. LR area was defined as the paler pink plaque areas in each quadrant of aortic sections (H&E).

Image and Data Analysis

Images were analyzed with ImagePro Plus (Media Cybernetics). For each time point, a total of 3 slices at different locations were analyzed along the abdominal aorta. Wall and lumen signal intensities (SI) were determined with standard region-of-interest (ROI) measurements on the corresponding MR images (n=108). An ROI containing no motion artifacts was placed outside of the animal to measure the SD of the noise signal. Both enhancement ratio (ER) of the SI (ER=SLpost/SLpre) and normalized CNR (CNR=SLpost−SLnoise/SDnoise) were calculated, where SLpost is signal intensity of the arterial wall after contrast injection and SLpre is signal intensity of the arterial wall before contrast injection. An experienced observer drew all ROIs. Both ER and CNR were calculated at 3 different time points: before gadofluorine injection, 1 hour after injection, and 24 hours after injection.

Histopathology

Rabbits were killed after the last set of MR images (ie, 24 hours after injection) by intravenous injection of sodium pentobarbital (120 mg/kg). Aortas were excised and perfusion-fixed as previously described.12,13 The adventitia of excised aorta was immediately marked with india ink at the posterior face of the artery to facilitate matching of the histological slides and MR images. Serial sections of the aorta were cut at 3-mm intervals. Coregistration was performed carefully by utilizing the position of the renal arteries and iliac bifurcation.12,13,19 The selected aortic specimens were embedded in paraffin, and 5-μm-thick sections were cut and stained with hematoxylin-eosin (H&E) or with Masson’s trichrome elastin. An independent experienced pathologist blinded to the MR findings performed the histological analyses following the classification from the Committee on Vascular Lesions of the Council of Atherosclerosis, American Heart Association. All tissue components (loose fibrous, media, dense fibrous, fibrocellular [cap], lipid/necrotic [core]) present in histopathological specimens were identified.20,21 Tissue component areas were traced manually. Two different areas were distinguished for further analysis: LR and non-LR areas. LR area was defined as the paler pink plaque areas in each quadrant of aortic sections (H&E).

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images and were thereafter classified as LR or non-LR on the basis of histological sections, as explained earlier. The MR-derived SI and CNR were correlated with the lipid content for each image quadrant. Only the corresponding area on MR image was taken when compared with CNR.

**Statistical Analysis**

One-way ANOVA was used to compare derived parameters from IR-TFL and IR-DIFF-TFL images at the same sites in the abdominal aorta for the 3 time points (before injection, 1 hour after injection, 24 hours after injection) with a Tukey test for post hoc comparisons. All probabilities are 2-sided and expressed as mean ± SD. Probability values <0.05 were considered statistically significant.

**Results**

All the MR images (n=108) were interpretable. MRI before gadofluorine, with the use of the conventional method (IR-TFL) and newly developed sequence (IR-DIFF-TFL), showed no enhancement in the abdominal aorta in either atherosclerotic (n=12) or control (n=6) rabbits. CNR in the atherosclerotic group was 8.1±5.1 (conventional) and 6.1±4.7 (new sequence) (P=NS) and was 8.4±7.0 and 6.7±5.5, respectively, in the control group (P=NS).

In the atherosclerotic group (n=12), vessel lumen appeared bright with the conventional method and black with the new sequence immediately after gadofluorine injection (Figure 2).

The intravascular blood signal was suppressed with the new sequence immediately after contrast injection compared with the conventional method, as indicated by CNR: 13.0±7.7 versus −19.8±10.3, respectively (P<0.001). The negative CNR value with the conventional method was due to the high aortic intraluminal SI compared with SI from the adjacent aortic wall. In this group, the enhancement of the aortic wall with the new sequence was 164% at 1 hour after injection compared with precontrast imaging (P<0.005). ER was higher at 1 hour with the new sequence compared with the conventional method (2.64 versus 0.47, respectively; P<0.001). At 24 hours after injection, with the new sequence, the enhancement was 207% compared with before contrast (P<0.001). ER was 3.07 with the new sequence and 1.37 with the conventional method (P=0.052). There was a trend toward higher enhancement of the aortic wall with the new sequence versus the conventional method. The CNR values were 15.2±5.9 (new sequence) and 11.4±8.9 (conventional method). No enhancement was present in the aortic wall in the control group (n=6) at 1 hour or 24 hours after injection.

In the atherosclerotic group, at 24 hours after injection, pronounced enhancement occurred along the abdominal aorta, but regions with different enhancement intensity were
easily discerned. Figure 3 shows the heterogeneous enhancement of the plaque along the aorta. These plaques exhibited various histological features such as LR areas, collagen content, and fibrous cap thickness. At 24 hours after injection, each plaque was analyzed in different quadrants on histopathology sections and on MR images (Figure 4 and Figure 5).

Although the pattern of enhancement was circular in all transverse MR images analyzed (n=108), measurement of SI and CNR was higher in the LR quadrants compared with non-LR quadrants, as shown by histopathology (P<0.05, P<0.001, respectively) (Figure 6). A strong correlation (r²=0.87; P<0.001) was found between CNR at 24 hours...
after injection and LR areas, suggesting a high affinity of gadofluorine for lipid components.

**Discussion**

**Contrast Agent for Plaque Characterization**

Gadofluorine is a macrocyclic gadolinium-based contrast agent with high relaxivity, long plasma half-life, water solubility, and lipophilicity compared with Gd-DTPA. In Watanabe heritable hyperlipidemic rabbits, with the use of a T1-weighted sequence (conventional IR-TFL) at 48 hours after contrast injection, gadofluorine enhanced atherosclerotic plaque and improved plaque detection compared with non-contrast-enhanced MRI. Few studies have been performed with this new class of contrast agent. The exact mechanism of gadofluorine uptake and accumulation into the atherosclerotic plaque is still unknown. It might leak out of the lumen into plaques because of enhanced endothelial permeability in atherosclerotic plaques. It can also penetrate plaque through the adventitia as a result of increased vasa vasorum feeding the plaque neovascularature. Future studies are under way to determine the uptake and specific accumulation.

Conventional contrast-enhanced MR in which gadolinium chelates are used alters the MR proton relaxation of the imaged tissue. These contrast agents do not penetrate phospholipid cellular membranes because of their highly hydrophilic properties. These agents are confined to the extracellular space after intravenous administration, do not bind to plasma proteins, and are eliminated without being metabolized by the kidneys. In atherosclerotic rabbits, after Gd-DTPA had cleared from the blood, no aortic wall segment enhancement could be detected. Gadofluorine forms aggregates or micelles in aqueous solution, and, because of its lipophilic properties, the compound has the ability to pene-
trate and accumulate within the plaque after intravenous injection. In our experience, gadofluorine lasts within the plaque no more than 72 hours after injection, but future studies are needed.

We demonstrate in this study that due to the high T1 relaxivity (R1) of gadofluorine compared with gadolinium chelate contrast agents, the use of conventional T1-weighted imaging (ie, IR-TFL) is not adequate for wall enhancement detection immediately after injection. Atherosclerotic plaque detection was possible with the use of a combination of IR-DIFF-TFL and gadofluorine as early as 1 hour after injection because of the intravascular signal suppression from the diffusion preparation. We also demonstrate that 24 hours after injection, enhancement of plaques was heterogeneous. Although this study was not designed to assess plaque characterization, we found a strong correlation between LR areas in histological sections and the high CNR in matched MR images, suggesting a high affinity of gadofluorine for LR plaques. Furthermore, plaques with low lipid content, such as fibrocellular plaques, had a weaker enhancement compared with the LR areas. Fibrocellular plaques are usually considered less vulnerable plaques than plaques with a LR core and/or with a thin fibrous cap. This affinity to the LR areas may facilitate imaging and characterization of atherosclerotic plaques. More work must be done to confirm these findings, especially to better define the relation between gadofluorine, lipids, and other plaque components.

**MR Technique**

The use of diffusion gradients has been considered an efficient way of suppressing flowing spins. Flow suppression depends on vessel size and is more efficient in large arteries (eg, aorta), where the flow is laminar. The IR pulse was used to suppress the signal from the perivascular tissues (fat and muscle), and the diffusion module was used for suppression of the blood signal by a mechanism independent of longitudinal relaxation. This was needed because of the relatively long gadofluorine plasma half-life, high relaxivity, and short T1 in the lumen during the 24 hours after injection. Fat suppression was used to null signal from periadventitial fat, which can obscure the vessel wall because of chemical shift. In contrast to periadventitial 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. The present study demonstrates that the newly developed T1-weighted IR-DIFF-TFL sequence provides effective and improved atherosclerotic plaque detection after gadofluorine in vivo imaging. As shown here, the use of new high-relaxivity contrast agents will most likely necessitate the utilization of novel MRI methods to take advantage of the contrast agent properties.

The use of gadofluorine-enhanced imaging, as shown in our study, can be combined with high-resolution, black- and bright-blood, stationary and dynamic, multicontrast-weighted (eg, T1-weighted, T2-weighted, proton density–weighted, before and after gadofluorine) imaging for improved plaque detection. More work must be done to confirm these findings, especially to better define the relation between gadofluorine, lipids, and other plaque components.
boundary and component assessments and should be a subject of future studies.

**Conclusion**

Contrast-enhanced MR with the gadofluorine contrast agent facilitates and improves plaque detection in NZW atherosclerotic rabbits compared with conventional contrast-enhanced and non-contrast-enhanced MRI. We developed a new T1-weighted sequence (IR-DIFF-TFL) that allows in vivo detection of atherosclerotic plaque within the first hour after injection of gadofluorine because of its capability of suppressing the intravascular blood signal in a 1.5-T MR clinical system. The strong correlation between LR areas, as shown by histopathology and CNR in matched MR images, suggests a high affinity of gadofluorine for plaque LR regions. Gadofluorine-enhanced MRI may be helpful in detection of atherosclerotic plaque burden and components and assessment of the efficacy of antiatherogenic therapies.

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**References**

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