Molecular MRI of Atherosclerotic Plaque With Targeted Contrast Agents

David E. Sosnovik, MD, and Peter Caravan, PhD

Corresponding author
David E. Sosnovik, MD
Massachusetts General Hospital, 149 13th Street,
Charlestown, MA 02129, USA.
E-mail: sosnovik@nmr.mgh.harvard.edu

Current Cardiovascular Imaging Reports 2009, **2:**87–94
Current Medicine Group LLC ISSN 1941-9066

Molecular MRI of atherosclerosis involves the use of novel contrast agents to image cellular and molecular processes within atherosclerotic plaque. Agents to image plaque lipid content, inflammation, angiogenesis, and thrombosis have been developed and studied extensively in animal models of atherosclerosis and vascular injury. Selected agents have also been studied in humans, with highly promising initial results. In this brief review, recent advances as well as opportunities and challenges in the field are discussed.

Introduction

The characterization of atherosclerotic plaque and its propensity to rupture remains one of the principal challenges in cardiovascular medicine. Although MRI of atheromatous plaques with endogenous contrast mechanisms remains of significant value, additional information regarding plaque composition and metabolic activity is needed to fully characterize a plaque and its propensity to rupture. Molecular MRI provides this additional information by imaging the cellular and molecular characteristics of atheromatous plaque, in a noninvasive, quantifiable, and serial manner. In this brief review we highlight recent advances in molecular MRI of atherosclerotic plaque. The interested reader is referred to several detailed articles for a more comprehensive and longitudinal review of the field [1,2].

Molecular magnetic resonance contrast agents can be broadly divided into two categories: paramagnetic gadolinium (Gd)-based agents and superparamagnetic (iron-oxide) nanoparticles. The ability of these agents to image aspects of plaque biology, including the expression of endothelial adhesion molecules [3••], plaque lipid and macrophage content [4••,5••], angiogenesis [6], and plaque thrombosis [7], has been demonstrated in mice, in large animal models, and more recently in humans [8,9,10••]. Molecular MRI provides high-resolution images of these key molecular processes in atherosclerotic plaque, allowing integrated anatomical, physiological, and molecular imaging to be performed in a single comprehensive and integrated dataset. The recent development of integrated magnetic resonance–positron emission tomography (PET) scanners also raises the possibility of dual modality imaging, exploiting the strengths of both PET- and MR-based molecular imaging.

Gadolinium-Based Agents

There has been considerable effort to use Gd-based probes to target specific plaque components. Gd offers the benefit of positive image contrast on T1-weighted images. The majority of work to date has been preclinical, with the exception of the fibrin-targeted probe EP-2104R, for which limited data in humans have been reported [10••]. Contrast agents targeted to the $\alpha_v\beta_3$ integrin [6], fibrin [7,11,12], myeloperoxidase activity [13], matrix metalloproteinases [14], macrophage scavenger receptor [15,16••,17], oxidized low-density lipoprotein (LDL) [4••,18], and high-density lipoprotein [19] have been described. In addition, compounds with nonspecific protein binding such as gadofluorine-M [20,21], and Gd linked to long-chain fatty acids [15], have also shown plaque enhancement in mouse and rabbit models of atherosclerosis. Even conventional Gd-DTPA has been shown to enhance vessel wall in atherosclerotic lesions under delayed contrast enhancement techniques [22]. It is hoped, however, that the molecular specificity of the targeted agents will inform not only on the presence of the plaque but also its stage and its risk for rupture.

There are basically two platforms for Gd-based plaque agents (Fig. 1). The first is a discrete small- to medium-sized molecule (< 10 kDa) comprising a plaque recognition element and one or more Gd-chelates. The benefits of this approach are 1) typically fast uptake into the plaque because of the small size of the agent; 2) fast blood clearance and renal excretion (this leads to good target:background at early time points as well as better elimination of the potentially toxic Gd metal ion); and 3) the discrete nature of the molecule enables reproducible manufacturing, which is a key feature for translation to the clinic. The major drawback of the small-molecule approach is that many molecular targets of interest are

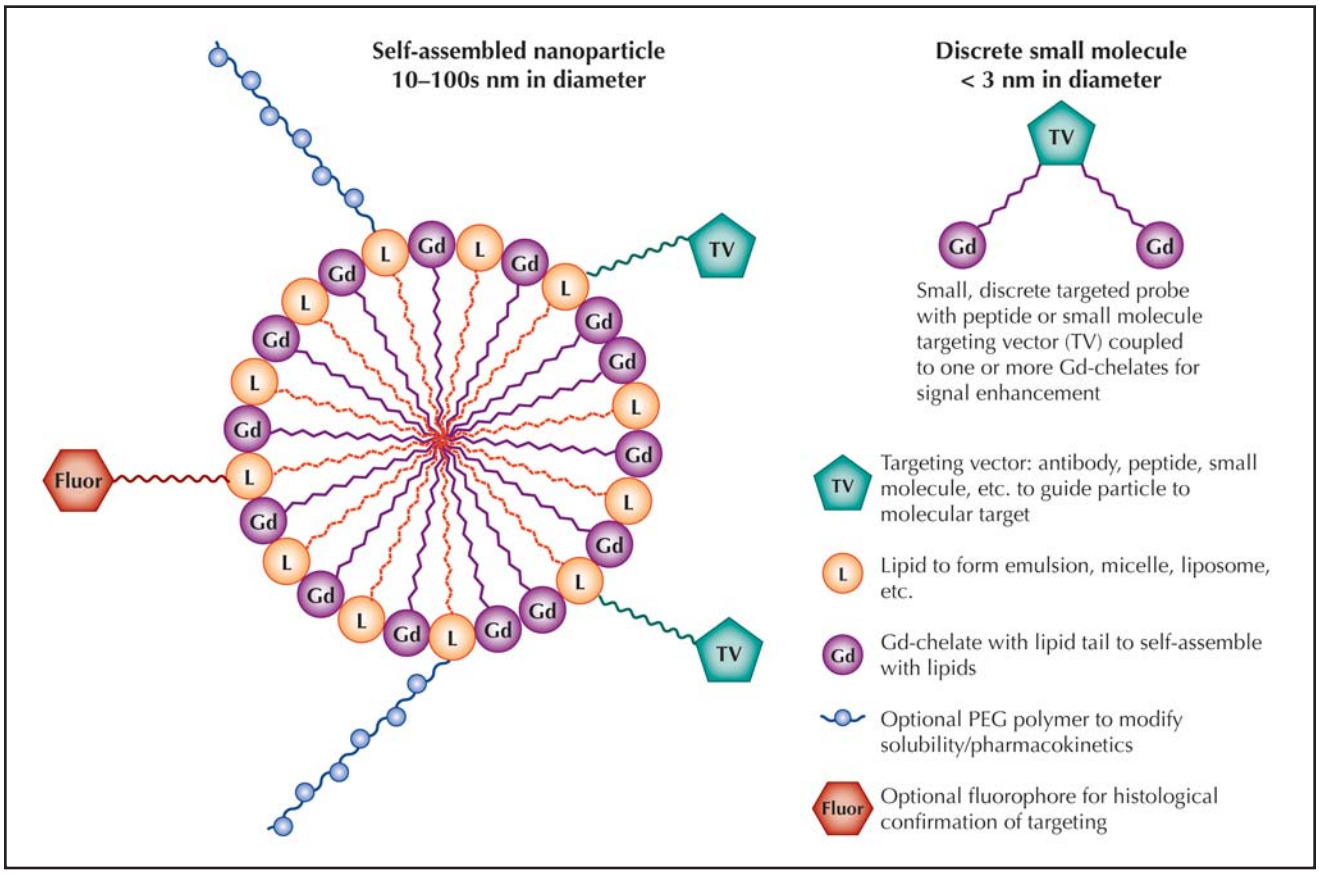

Figure 1. Platforms for gadolinium (Gd)-based plaque agents. One strategy, shown on the right, involves the synthesis of a discrete small- to medium-sized molecule (< 10 kDa) comprising a plaque recognition element and one or more Gd-chelates. The second strategy involves nanoparticle self-assembly, in which 1000s of Gd-chelates provide signal enhancement, and targeting is achieved by incorporation of a recognition element (eg, antibody, peptide) for the plaque component. Additional components can be added for fluorescence detection and/or pharmacokinetic modification. PEG—polyethylene glycol.

present at too low a concentration (submicromolar) in plaque to be measurably enhanced by a few Gd chelates. This has necessitated a nanoparticle self-assembly approach in which 1000s of Gd-chelates provide signal enhancement and targeting is achieved by incorporation of some recognition element (eg, antibody, peptide) for the plaque component (Fig. 1). In general, optimal image contrast may occur at hours to days postinjection because of slower targeting/elimination pharmacokinetics.

Fibrin targeting for advanced plaque

Rupture of an atheromatous plaque resulting in thrombosis is the accepted cause of most acute coronary syndromes and sudden cardiac death. Many atherosclerotic plaques do not limit flow but pose a great risk of an ischemic event, especially in asymptomatic patients. Such plaques may rupture or erode, creating a thrombogenic surface and subsequent thrombosis of the vessel or embolism [23–25]. The presence of fibrin is indicative of stage VI plaque, the most advanced state of disease. Because fibrin is not found in the vasculature outside of thrombi, it represents a useful target for identifying late-stage complex plaque.

Circulating fibrinogen is present in the 5- to 10-μM range. In the clotting cascade, fibrinogen is polymerized to fibrin, resulting in a localized fibrin monomer concentration in the 10s to 100s of μM. This high concentration is compatible with magnetic resonance detection using the targeted small-molecule approach. However, the first targeted fibrin magnetic resonance probe was based on a self-assembled particle. Flacke et al. [11] described a Gd-based nanoparticle targeted to fibrin via an antibody and demonstrated its efficacy in a canine venous thrombus model. Later, Botnar et al. [7] described a fibrin-specific peptide derivatized with four Gd-DTPA units (termed EP-1873) that was used to identify thrombi in a rabbit model of plaque rupture. EP-2104R is a probe similar to EP-1873 but employs the more stable Gd-DOTA chelate. After evaluation in a number of preclinical rabbit [26] and swine models [27–31], EP-2104R advanced to human studies (Fig. 2). EP-2104R binds equally to two sites on human fibrin (K_d = 1.7 μM), with excellent specificity for fibrin over fibrinogen (over 100-fold) and for fibrin over serum albumin (over 1000-fold) [12]. The relaxivity of EP-2104R bound to fibrin at 37° C and 1.4 T was 71.4 mM-1 s-1 per molecule of EP-2104R (17.4 per Gd), about 25 times higher than that of Gd-DOTA measured under the same conditions [12].

In a clinical trial, EP-2104R was evaluated for detection of thrombus in six territories: the deep veins, pulmonary

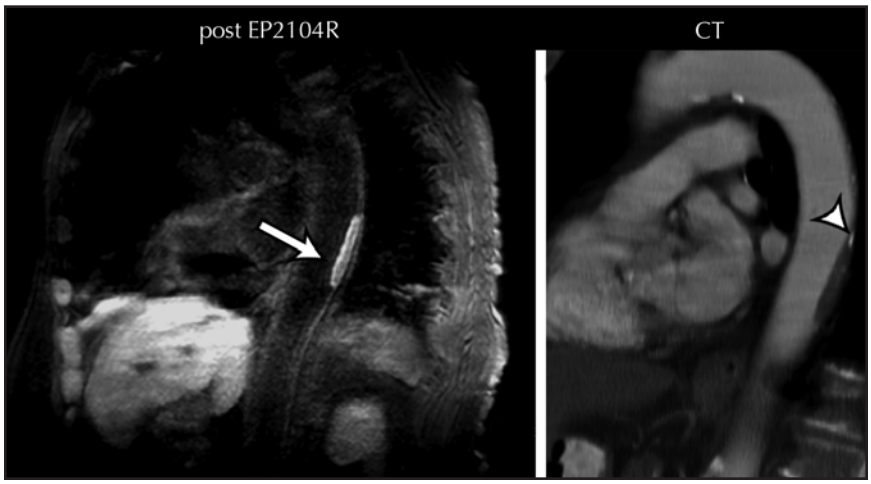

Figure 2. Molecular MRI of thrombus in the descending thoracic aorta in an 82-year-old female injected with EP-2104R. An inversion recovery black-blood gradient-echo sequence was used to generate positive T1 contrast from the gadolinium-containing probe. The high local signal amplification allows for definitive localization of thrombus in the thoracic aorta clot (*arrow*). A corresponding multiplanar reconstruction from a contrast-enhanced multislice CT demonstrates a filling defect consistent with a thrombus in the aortic wall. At the cranial end of the plaque a small calcification is also visible (*arrowhead*). (*From* Spuentrup et al. [10••]; with permission.)

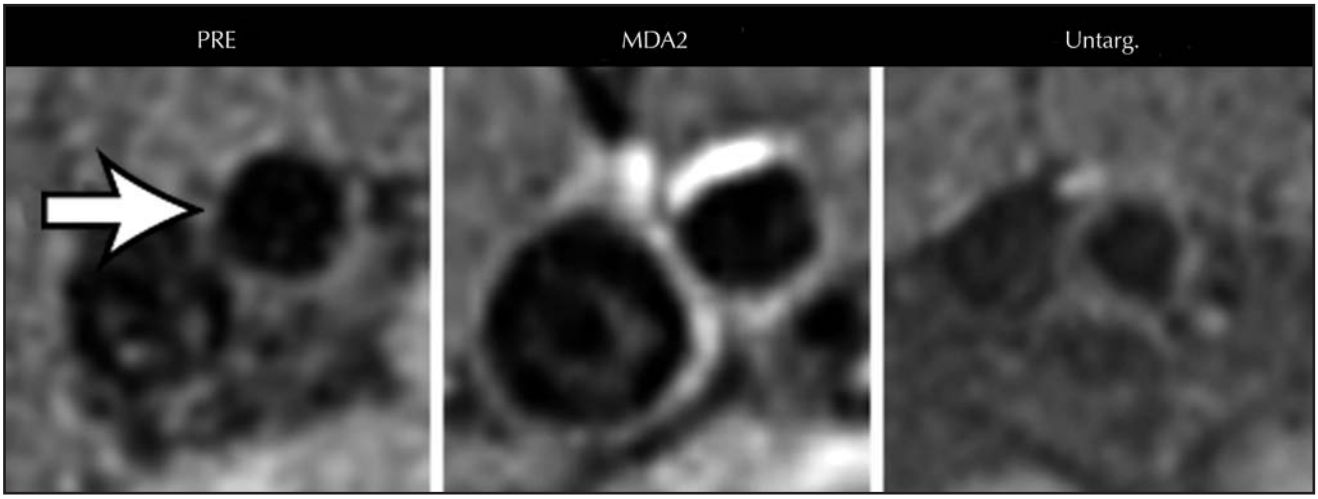

Figure 3. Molecular MRI of oxidized low-density lipoprotein (LDL) with an antibody (MDA2) targeted to oxidized murine LDL. The *left panel* shows the abdominal aorta of an ApoE$^{-/-}$ mouse prior to injection of the contrast agent. In this black-blood sequence the vessel wall of the aorta (*arrow*) is isointense with the surrounding muscle. The *middle panel* shows an image taken 72 hours after injection of the particles labeled with the MDA2 antibody. Note significant enhancement in the wall of the aorta but not in the wall of the vena cava. For comparison, an animal injected with immunoglobulin G–labeled particles is shown at 72 hours postinjection (*right panel*), where vessel wall enhancement is modest. (*From* Briley-Saebo et al. [4••]; with permission.)

arteries, carotid arteries, the aortic arch, the left ventricle, and the atria of the heart [10••]. Figure 2 shows one example from this study, in this case a thrombus in the thoracic aorta. An inversion recovery black-blood gradient-echo sequence was used to maximize the positive T1 contrast from the Gd-containing probe. Contrast-enhanced CT in this subject demonstrates a filling defect consistent with a thrombus in the aortic wall; however, the positive enhancement of this mass following administration of the fibrin-targeted magnetic resonance probe confirms the presence of thrombus.

Nanoparticles for targeting macrophage and oxidized LDL

Fayad and coworkers have used an imaging platform based on mixed micelles (Fig. 3). They initially showed that untargeted micelles could enhance plaques in ApoE knockout mice, whereas no vessel wall enhancement was seen in wild-type mice [15]. By incorporating an antibody that targets the macrophage scavenger receptor, plaque uptake was further increased [16••]. The targeted probe provided a 79% increase in signal intensity of atherosclerotic aortas in ApoE$^{-/-}$ mice, compared with only 34% using untargeted micelles at 24 hours postinjection. Ex vivo fluorescence microscopy revealed that the probe colocalized with macrophage in the plaque [16••]. In a more recent report, this group targeted oxidized LDL using antibodies that bind oxidation-specific epitopes [4••]. They prepared particles containing either antibodies to murine (MDA2, E06) or human (IK7) oxidized LDL, as well as particles with nonspecific immunoglobulin G (IgG) or particles without antibodies. All particles also contained a rhodamine tag for microscopy. In ApoE$^{-/-}$ mice, antibody-targeted probes showed substantial plaque enhancement (125% to 230% signal enhancement in the arterial wall) at 72 and 96 hours postinjection, and this was significantly higher than that achieved with the nonspecific IgG-labeled particles or the antibody-free

particles (15% to 20% enhancement, $P < 0.001$ for difference in targeted vs nontargeted), as shown in Figure 3. Confocal microscopy confirmed that MDA2, IK17, and E06 micelles accumulated within atherosclerotic lesions and specifically within macrophages.

Iron Oxide–Based Imaging

The physical properties of iron-oxide nanoparticles have been extensively described elsewhere [32]. In brief, these agents consist of a 2- to 3-nm core of superparamagnetic iron oxide surrounded by a coating material, usually dextran. The final construct reaches 20 to 30 nm in size, remains inert in the bloodstream, and has long circulatory half-life [32]. The small size and long half-life of these agents allow them to penetrate atherosclerotic plaques, where they can be taken up by macrophages or targeted to a specific component of the plaque [3••,5••,8,9]. Micron-sized nanoparticles have also recently been used to target surface receptors accessible from the blood pool [33,34].

Imaging of plaque macrophage content

High macrophage content is one of the hallmarks of vulnerable atherosclerotic plaque. Several investigators have shown in experimental animal models of atherosclerosis, such as the rabbit aorta [5••], that iron-oxide nanoparticles accumulate in plaque macrophages. Initial studies also suggested that carotid plaque inflammation could be imaged in humans in vivo with this strategy [8,9]. The feasibility of imaging plaque macrophage content on the basis of iron-oxide accumulation has been confirmed in several recent studies [5••,35], which have also advanced our understanding of the mechanisms of nanoparticle uptake and the optimal strategy with which to image them [5••,35].

The uptake of iron-oxide nanoparticles by macrophages was believed to be the obligate response of a phagocytic cell encountering a nanoparticulate foreign body. However, it has been recently shown that minor modifications to the surface of these nanoparticles can significantly alter their uptake [36]. Nanoparticle libraries containing constructs with both high and low affinity for either resting or activated macrophages have been constructed [36]. The uptake of iron-oxide nanoparticles is also not confined to plaque macrophages [35]. Inflamed endothelial and smooth muscle cells in the plaque also take up these agents, but to a significantly lesser degree [35]. The uptake of iron-oxide nanoparticles is thus predominantly a marker of plaque macrophages but, more accurately, a marker of composite plaque inflammation.

MRI of iron-oxide nanoparticles has traditionally been performed with T2*-weighted gradient echo sequences [32]. These sequences generate signal hypointensity (negative contrast) in the vicinity of the iron-oxide nanoparticles, which creates robust contrast in parenchymal organs such as the myocardium, liver, and pancreas [37,38]. Imaging the vessel wall with this approach, however, is significantly more complex. The structures surrounding the vessel wall in both the thorax and the neck frequently contain large amounts of air, which does not provide a suitable background for negative contrast. Cine sequences can be used to create a positive signal in the blood pool of the vessel, but, at the longer echo times needed for T2* weighting, these sequences often suffer from flow-related dephasing and other artifacts. As a result, there are concerns regarding the accuracy of a negative contrast-based strategy for imaging the vessel wall [5••].

Sequences to generate positive contrast from iron-oxide nanoparticles have been developed and used successfully to image labeled stem cells [39], macrophage infiltration in the infarcted myocardium [40], and atherosclerotic plaque. Intraplaque hemorrhage and the generation of ferritin have been detected with these techniques [41]. More recently, these positive contrast techniques have been used to detect plaque macrophage infiltration in hyperlipidemic rabbits [5••]. Tissue not containing iron oxide is nulled with these techniques, while areas of plaque containing iron oxide or ferritin appear bright. Within the first 24 hours of injection, the high concentration of circulating iron-oxide nanoparticles can be used to create robust angiograms [5••]. However, within 72 hours these agents are completely cleared from the blood, and positive contrast is only seen in areas of the vessel wall where macrophages have taken up the nanoparticle [5••]. A positive contrast approach is particularly suited to the imaging of iron oxide–based nanoparticles in the vessel wall and is likely to become the default approach in future clinical studies.

Imaging of specific receptors and targets

The expression of adhesion molecules such as vascular cell adhesion molecule (VCAM)-1 is one of the earliest events in the atherosclerotic cascade. Several generations of VCAM-1–targeted magnetofluorescent contrast agents have been synthesized and used to image VCAM-1 in vivo [1]. The most recent version of this agent is based on a linear peptide, identified using phage display, that has high affinity for VCAM-1 [3••]. This peptide was conjugated to the magnetofluorescent nanoparticle, CLIO-Cy5.5, to provide both an MRI and fluorescent readout of VCAM-1 expression. Fluorescence microscopy of the injected probe showed that the accumulation of the agent within plaque was due to binding to VCAM-1, and not due to macrophage uptake of the CLIO-Cy5.5 nanoparticle [3••]. This underscores the importance of using a ligand with high affinity for the molecular target and tailoring the blood half-life of the agent to the diagnostic question. Targeting an endothelial surface receptor such as VCAM-1 is optimal when the blood half of the agent is 1 to 3 hours, which favors ligand-mediated binding to the target while reducing the exposure of the probe to nonspecific uptake by plaque macrophages.

In vivo imaging of VCAM-1 was performed in $ApoE^{-/-}$ mice fed a high-cholesterol diet [3••]. These mice predictably develop the most robust atheromatous plaques on their aortic roots, which provides an ideal location for molecular MRI. The aortic root is shielded by the left and right atria,

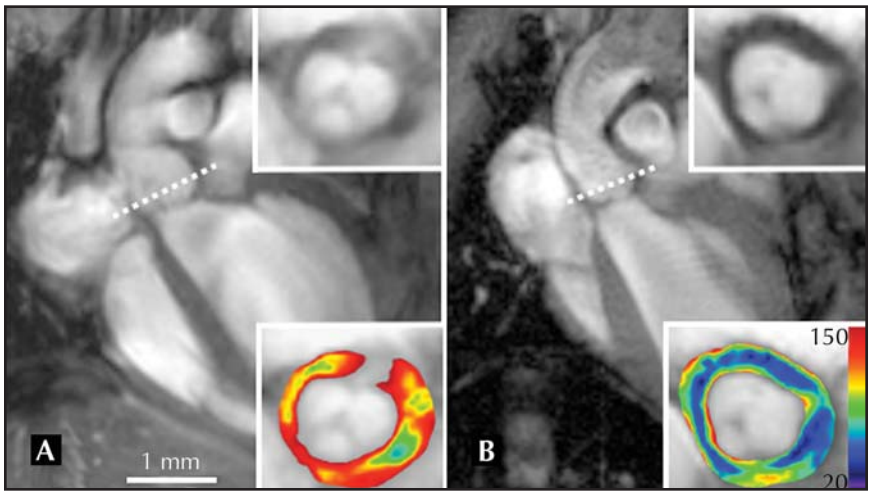

Figure 4. Molecular MRI of vascular cell adhesion molecule (VCAM)-1 expression in ApoE$^{-/-}$ mice [3••]. The MNP CLIO-Cy5.5 has been labeled with a peptide with high affinity and specificity for VCAM-1. **A**, T2*-weighted gradient echo images in the long and short axes of the left ventricle and the aortic root are shown. The short axis plane through the aortic root, an area of high plaque burden, is demarcated by the *dashed line*. **B**, T2*-weighted images acquired at the identical locations after the injection of the VCAM-1–sensing probe. Significant negative contrast, consistent with VCAM-1 expression and accumulation of the probe, is seen. Signal intensity in the aortic root has been mapped to a color scale in the lower pair of color insets. (*From* Nahrendorf et al. [3••]; with permission.)

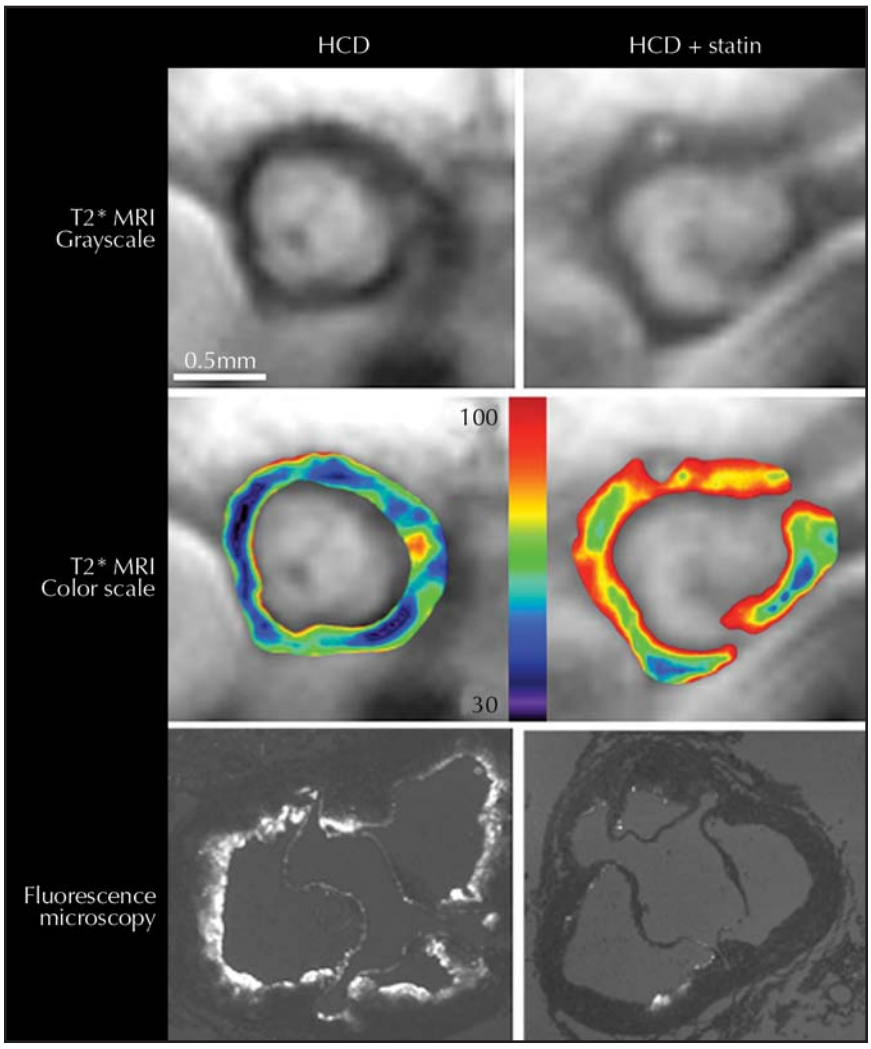

Figure 5. Molecular MRI of vascular cell adhesion molecule (VCAM)-1 expression in vivo in the aortic roots of ApoE$^{-/-}$ mice fed a high-cholesterol diet (HCD), and ApoE$^{-/-}$ mice fed the identical diet but treated with statins [3••]. VCAM-1 expression (signal hypointensity and negative contrast in the aortic root) is shown by in vivo MRI to be significantly higher in the non–statin-treated mice. Ex vivo fluorescence images of the aortic root confirm the in vivo MRI findings, with significantly more probe accumulation seen in the untreated mouse than the statin-treated mouse. (*From* Nahrendorf et al. [3••]; with permission.)

and is thus not affected by susceptibility artifacts from the lungs. In addition, the valve cusps of the aortic root provide an excellent anatomical landmark for serial imaging studies. Significant uptake of the VCAM-1–sensing nanoparticle in the aortic root of the ApoE$^{-/-}$ mice could be detected in vivo with T2*-weighted MRI (Fig. 4). Ex vivo fluorescence imaging and histology confirmed the uptake of the probe in regions of the aortic root with high levels of VCAM-1 [3••]. Moreover, treatment of the mice with statins significantly reduced uptake of the agent, which could be robustly visualized with in vivo MRI (Fig. 5). The VCAM-1–sensing agent thus produced adequate sensitivity and dynamic range to detect the effects of treatment on an important marker of plaque vulnerability [3••].

Several potentially useful targeted nanoparticles have also been developed and tested in conditions other than atherosclerosis. Molecular MRI of apoptosis, which plays a significant role in plaque rupture, has been performed in the myocardium [38]. In addition, the experience with micron-sized iron-oxides is growing, adding further to the potential imaging armamentarium. Micron-sized iron-oxide particles have been targeted to VCAM-1 and P-selectin [33] and co-injected to detect inflammation in the aortic roots of $ApoE^{-/-}$ mice. An antibody to the activated glycoprotein II_b/III_a receptor has also been conjugated to micron-sized iron-oxide and used to image activated platelets in vivo [34]. Micron-sized particles have extremely high relaxivities, which aids in their detection. However, the toxicity and elimination of these agents, and hence their potential for human use, remains unclear.

Conclusions

Iron-oxide nanoparticles have been used extensively to image hepatic and lymph node metastases in humans, and have an extensive safety record [42]. Targeted iron-oxide nanoparticles have yet to be tested in humans, although no preclinical evidence of toxicity has been reported with these agents. Initial human data with a targeted Gd-based probe have now been obtained [10••]. The fibrin-detecting gadolinium chelate, EP-2104R, has to date been given to 52 patients, with excellent short-term safety results [10••]. However, concerns regarding the long-term accumulation of targeted Gd-based probes will need to be addressed, particularly in the case of Gd-based nanoparticles and micelles.

One of the advantages of larger constructs such as liposomes, however, is their potential to carry a therapeutic payload. Winter and colleagues [6] have shown that a Gd-loaded liposome can be targeted to the $\alpha_V\beta_3$ integrin, involved in plaque angiogenesis. Incorporating the anti-angiogenic agent fumagillin into the liposome reduced angiogenesis and subsequent uptake of the probe [43••]. Recent data from Mulder and colleagues (presented, Society for Molecular Imaging 2008) also suggest that liposomes loaded with corticosteroids can significantly reduce plaque inflammation. These constructs thus facilitate a theranostic (therapeutic and diagnostic) approach, in which molecular imaging is performed not only to diagnose disease, but also to follow and monitor therapy.

The breadth and comprehensive nature of MRI are amongst its most appealing attributes. Nevertheless, multimodality MRI strategies are being developed and hold great promise. The Weissleder group [44••] has conjugated ^{64}Cu to a magnetofluorescent nanoparticle to yield a PET-, fluorescence-, and magnetic resonance–detectable probe. Uptake of this multimodal nanoparticle by plaque macrophages in the aortic roots of $ApoE^{-/-}$ mice could be imaged in vivo with both MRI and PET [44••]. Moreover, the PET signal generated by this agent was significantly stronger than that generated by ^{18}FDG. Integrated magnetic resonance–PET systems have been constructed, and it is likely that such systems will play an important role in the clinical translation of molecular imaging.

Targeted molecular MRI of atherosclerosis is already playing an important role in basic science investigation, and the development and testing of novel pharmaceuticals. Although the clinical translation of these techniques holds much promise, safety, regulatory, and economic hurdles will need to be addressed. The recent human experience with EP-2104R is in many ways encouraging, and it is likely that additional selected agents will move into clinical trials. Further advances in the molecular imaging of atherosclerotic plaque will also be significantly facilitated by advances in therapy, specifically additional data showing the long-term safety of drug-eluting stents. The optimal approach to prevent cardiovascular events remains an area of significant debate and activity. It is unlikely, however, that a genomic- and biomarker-based approach alone will suffice, and complementary information provided by imaging techniques such as molecular MRI will thus be crucial.

Acknowledgments

David E. Sosnovik has been funded in part by the following National Institutes of Health grants: R01 HL093038 and K08 HL079984.

Disclosure

Peter Caravan acknowledges support from Siemens Medical Solutions.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Sosnovik DE, Nahrendorf M, Weissleder R: **Molecular magnetic resonance imaging in cardiovascular medicine.** *Circulation* 2007, **115:**2076–2086.
2. Jaffer FA, Libby P, Weissleder R: **Molecular and cellular imaging of atherosclerosis: emerging applications.** *J Am Coll Cardiol* 2006, **47:**1328–1338.
3.•• Nahrendorf M, Jaffer FA, Kelly KA, et al.: **Noninvasive vascular cell adhesion molecule-1 imaging identifies inflammatory activation of cells in atherosclerosis.** *Circulation* 2006, **114:**1504–1511.

An important study showing the feasibility of imaging VCAM-1 expression in vivo with molecular MRI.

4.•• Briley-Saebo KC, Shaw PX, Mulder WJ, et al.: **Targeted molecular probes for imaging atherosclerotic lesions with magnetic resonance using antibodies that recognize oxidation-specific epitopes.** *Circulation* 2008, **117:**3206–3215.

A study describing the use of gadolinium-loaded immunomicelles to image oxidized LDL.

5.•• Korosoglou G, Weiss RG, Kedziorek DA, et al.: **Noninvasive detection of macrophage-rich atherosclerotic plaque in hyperlipidemic rabbits using "positive contrast" magnetic resonance imaging.** *J Am Coll Cardiol* 2008, **52:**483–491.

A new technique to image iron oxide nanoparticles in the vessel wall is described. This technique is likely to facilitate the clinical study and translation of iron oxide nanoparticles.

6. Winter PM, Morawski AM, Caruthers SD, et al.: **Molecular imaging of angiogenesis in early-stage atherosclerosis with alpha(v)beta3-integrin-targeted nanoparticles.** *Circulation* 2003, **108:**2270–2274.

7. Botnar RM, Perez AS, Witte S, et al.: **In vivo molecular imaging of acute and subacute thrombosis using a fibrin-binding magnetic resonance imaging contrast agent.** *Circulation* 2004, **109:**2023–2029.

8. Trivedi RA, U-King-Im JM, Graves MJ, et al.: **In vivo detection of macrophages in human carotid atheroma: temporal dependence of ultrasmall superparamagnetic particles of iron oxide-enhanced MRI.** *Stroke* 2004, **35:**1631–1635.

9. Kooi ME, Cappendijk VC, Cleutjens KB, et al.: **Accumulation of ultrasmall superparamagnetic particles of iron oxide in human atherosclerotic plaques can be detected by in vivo magnetic resonance imaging.** *Circulation* 2003, **107:**2453–2458.

10.•• Spuentrup E, Botnar RM, Wiethoff AJ, et al.: **MR imaging of thrombi using EP-2104R, a fibrin-specific contrast agent: initial results in patients.** *Eur Radiol* 2008, **18:**1995–2005.

This is the first clinical study involving the use of a targeted gadolinium contrast agent. The safety and potential utility of the agent are demonstrated.

11. Flacke S, Fischer S, Scott MJ, et al.: **Novel MRI contrast agent for molecular imaging of fibrin: implications for detecting vulnerable plaques.** *Circulation* 2001, **104:**1280–1285.

12. Overoye-Chan K, Koerner S, Looby RJ, et al.: **EP-2104R: a fibrin-specific gadolinium-based MRI contrast agent for detection of thrombus.** *J Am Chem Soc* 2008, **130:**6025–6039.

13. Chen JW, Pham W, Weissleder R, Bogdanov A Jr: **Human myeloperoxidase: a potential target for molecular MR imaging in atherosclerosis.** *Magn Reson Med* 2004, **52:**1021–1028.

14. Lancelot E, Amirbekian V, Brigger I, et al.: **Evaluation of matrix metalloproteinases in atherosclerosis using a novel noninvasive imaging approach.** *Arterioscler Thromb Vasc Biol* 2008, **28:**425–432.

15. Lipinski MJ, Amirbekian V, Frias JC, et al.: **MRI to detect atherosclerosis with gadolinium-containing immunomicelles targeting the macrophage scavenger receptor.** *Magn Reson Med* 2006, **56:**601–610.

16.•• Amirbekian V, Lipinski MJ, Briley-Saebo KC, et al.: **Detecting and assessing macrophages in vivo to evaluate atherosclerosis noninvasively using molecular MRI.** *Proc Natl Acad Sci U S A* 2007, **104:**961–966.

Plaque macrophage content is imaged in this study using an immunomicelle targeted to the macrophage scavenger receptor.

17. Mulder WJ, Strijkers GJ, Briley-Saboe KC, et al.: **Molecular imaging of macrophages in atherosclerotic plaques using bimodal PEG-micelles.** *Magn Reson Med* 2007, **58:**1164–1170.

18. Li H, Gray BD, Corbin I, et al.: **MR and fluorescent imaging of low-density lipoprotein receptors.** *Acad Radiol* 2004, **11:**1251–1259.

19. Frias JC, Williams KJ, Fisher EA, Fayad ZA: **Recombinant HDL-like nanoparticles: a specific contrast agent for MRI of atherosclerotic plaques.** *J Am Chem Soc* 2004, **126:**16316–16317.

20. Meding J, Urich M, Licha K, et al.: **Magnetic resonance imaging of atherosclerosis by targeting extracellular matrix deposition with Gadofluorine M.** *Contrast Media Mol Imaging* 2007, **2:**120–129.

21. Barkhausen J, Ebert W, Heyer C, et al.: **Detection of atherosclerotic plaque with Gadofluorine-enhanced magnetic resonance imaging.** *Circulation* 2003, **108:**605–609.

22. Yeon SB, Sabir A, Clouse M, et al.: **Delayed-enhancement cardiovascular magnetic resonance coronary artery wall imaging: comparison with multislice computed tomography and quantitative coronary angiography.** *J Am Coll Cardiol* 2007, **50:**441–447.

23. Fuster V, Moreno PR, Fayad ZA, et al.: **Atherothrombosis and high-risk plaque: part I: evolving concepts.** *J Am Coll Cardiol* 2005, **46:**937–954.

24. Shah PK: **Pathophysiology of coronary thrombosis: role of plaque rupture and plaque erosion.** *Prog Cardiovasc Dis* 2002, **44:**357–368.

25. Virmani R, Burke AP, Kolodgie FD, Farb A: **Vulnerable plaque: the pathology of unstable coronary lesions.** *J Interv Cardiol* 2002, **15:**439–446.

26. Sirol M, Aguinaldo JG, Graham PB, et al.: **Fibrin-targeted contrast agent for improvement of in vivo acute thrombus detection with magnetic resonance imaging.** *Atherosclerosis* 2005, **182:**79–85.

27. Botnar RM, Buecker A, Wiethoff AJ, et al.: **In vivo magnetic resonance imaging of coronary thrombosis using a fibrin-binding molecular magnetic resonance contrast agent.** *Circulation* 2004, **110:**1463–1466.

28. Spuentrup E, Buecker A, Katoh M, et al.: **Molecular magnetic resonance imaging of coronary thrombosis and pulmonary emboli with a novel fibrin-targeted contrast agent.** *Circulation* 2005, **111:**1377–1382.

29. Spuentrup E, Fausten B, Kinzel S, et al.: **Molecular magnetic resonance imaging of atrial clots in a swine model.** *Circulation* 2005, **112:**396–399.

30. Spuentrup E, Katoh M, Wiethoff AJ, et al.: **Molecular magnetic resonance imaging of pulmonary emboli with a fibrin-specific contrast agent.** *Am J Respir Crit Care Med* 2005, **172:**494–500.

31. Spuentrup E, Katoh M, Buecker A, et al.: **Molecular MR imaging of human thrombi in a swine model of pulmonary embolism using a fibrin-specific contrast agent.** *Invest Radiol* 2007, **42:**586–595.

32. Sosnovik DE, Nahrendorf M, Weissleder R: **Magnetic nanoparticles for MR imaging: agents, techniques and cardiovascular applications.** *Basic Res Cardiol* 2008, **103:**122–130.

33. McAteer MA, Schneider JE, Ali ZA, et al.: **Magnetic resonance imaging of endothelial adhesion molecules in mouse atherosclerosis using dual-targeted microparticles of iron oxide.** *Arterioscler Thromb Vasc Biol* 2008, **28:**77–83.

34. von zur Muhlen C, von Elverfeldt D, Moeller JA, et al.: **Magnetic resonance imaging contrast agent targeted toward activated platelets allows in vivo detection of thrombosis and monitoring of thrombolysis.** *Circulation* 2008, **118:**258–267.

35. Jaffer FA, Nahrendorf M, Sosnovik D, et al.: **Cellular imaging of inflammation in atherosclerosis using magneto-fluorescent nanomaterials.** *Mol Imaging* 2006, **5:**85–92.

36. Weissleder R, Kelly K, Sun EY, et al.: **Cell-specific targeting of nanoparticles by multivalent attachment of small molecules.** *Nat Biotechnol* 2005, **23:**1418–1423.

37. Sosnovik DE, Nahrendorf M, Deliolanis N, et al.: **Fluorescence tomography and magnetic resonance imaging of myocardial macrophage infiltration in infarcted myocardium in vivo.** *Circulation* 2007, **115:**1384–1391.

38. Sosnovik DE, Schellenberger EA, Nahrendorf M, et al.: **Magnetic resonance imaging of cardiomyocyte apoptosis with a novel magneto-optical nanoparticle.** *Magn Reson Med* 2005, **54:**718–724.

39. Stuber M, Gilson WD, Schar M, et al.: **Positive contrast visualization of iron oxide-labeled stem cells using inversion-recovery with ON-resonant water suppression (IRON).** *Magn Reson Med* 2007, **58:**1072–1077.

40. Farrar CT, Dai G, Novikov M, et al.: **Impact of field strength and iron oxide nanoparticle concentration on the linearity and diagnostic accuracy of off-resonance imaging.** *NMR Biomed* 2008, **21:**453–463.

41. Mani V, Briley-Saebo KC, Hyafil F, Fayad ZA: **Feasibility of in vivo identification of endogenous ferritin with positive contrast MRI in rabbit carotid crush injury using GRASP.** *Magn Reson Med* 2006, **56:**1096–1106.

42. Harisinghani MG, Barentsz J, Hahn PF, et al.: **Noninvasive detection of clinically occult lymph-node metastases in prostate cancer.** *N Engl J Med* 2003, **348:**2491–2499.

43.•• Winter PM, Neubauer AM, Caruthers SD, et al.: **Endothelial alpha(v)beta3 integrin-targeted fumagillin nanoparticles inhibit angiogenesis in atherosclerosis.** *Arterioscler Thromb Vasc Biol* 2006, **26:**2103–2109.
In this review, a theranostic (therapeutic and diagnostic) nanoparticle is described to both detect and treat angiogenesis.

44.•• Nahrendorf M, Zhang H, Hembrador S, et al.: **Nanoparticle PET-CT imaging of macrophages in inflammatory atherosclerosis.** *Circulation* 2008, **117:**379–387.
Strong demonstration of the power of multimodal imaging platforms. An MRI, PET, and fluorescence detectable agent is used to image plaque macrophage content.