

The Promise of Molecular MRI in Cardiovascular Imaging

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The term “Molecular Imaging” refers to the noninvasive imaging of certain cellular and subcellular events in vivo. The advantages of an MRI-based approach to cardiovascular molecular imaging include its high spatial resolution, excellent soft tissue contrast and ability to simultaneously image cardiovascular anatomy and physiology [1]. The role of molecular MRI in cardiovascular medicine has been extensively described in several recent reviews, and the interested reader is referred to these articles for a more comprehensive discussion of the field [1, 2]. In this article, the reader is provided with a brief introduction to cardiovascular molecular imaging. The opportunities and challenges pertaining to the use of gadolinium-based probes, magnetic (iron-oxide) nanoparticles, positive and negative contrast imaging, and the application of these contrast agents and techniques to cardiovascular disease are discussed.

Contrast agents and imaging techniques

The principal challenge of molecular MRI lies in its lower sensitivity, compared, for example, to nuclear imaging techniques. Conventional gadolinium chelates have a sensitivity in the micromolar range, which is usually inadequate for molecular MRI. Conventional extracellular gadolinium chelates also have extremely short intravascular half-lives and rapid renal excretion, which further limits their utility for most molecular imaging applications. Several novel gadolinium constructs have been developed to address these limitations including gadolinium-loaded liposomes, micelles and lipoproteins. These constructs are heavily loaded with paramagnetic gadolinium chelates and in general have longitudinal relaxivity (R1) values ranging from 10-20 s⁻¹ mM⁻¹ [3,4]. The relaxivity of

an MR contrast agent reflects its ability to interact with adjacent protons and strongly influences its detectability. The higher the longitudinal relaxivity (R1) of an agent, the brighter tissue in its vicinity becomes, while the higher the transverse relaxivity (R2), the darker the tissue becomes.

Gadolinium based probes are generally imaged with T1-weighted sequences at standard clinical field strengths, such as 1.5 and 3 Tesla. However, the R1 of these agents decreases rapidly at high field strengths, while the R2 of paramagnetic gadolinium-based probes increases with field strength. Increasing the dose of a gadolinium-based probe at higher field strengths may thus increase the dominance of the T2 effects and makes the generation of positive contrast even more difficult.

Although no acute toxic effects have been reported with most of the novel gadolinium constructs, the long-term effect of these agents remains less well understood. Prolonged tissue retention of low amounts of a targeted gadolinium-based probe may be safe, particularly in the absence of dechelation, but will require significant further study and testing. The recent description of a systemic fibrosis syndrome in those with renal dysfunction, and thus prolonged tissue retention of conventional gadolinium chelates, will likely require any new gadolinium construct to be extensively tested for similar types of chronic toxicity.

Magnetic iron oxide nanoparticles

Magnetic iron oxide nanoparticles (MNP) are typically superparamagnetic and can be imaged with T1, T2, T2* and steady state free precession techniques [5-7]. MNP agents typically have a central core of iron-oxide, measuring 3-5 nm in diameter, surrounded by a carbohydrate (for macrophage targeting) or polymer coat (often for targeting to

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other cells) [8, 9]. A citrate coated iron-oxide nanoparticle has also recently been developed and used as a blood pool agent [10]. Selected MNP have been used extensively in the clinical arena to image the liver and lymphatic system [11], and their established safety record thus makes them a highly appealing platform for molecular MRI. MNP do not contain any inert or non-degradable moieties, and it has been shown that the iron-oxide core of these agents is metabolized within two weeks, after which the released iron is used in the synthesis of new red blood cells. However, the ability of the body to excrete excess iron is limited, which places a limit on the frequency with which these contrast agents can be given. It is thus likely that limitations will be imposed on the frequency of repeat dosing for most MNP.

The R2 values of the superparamagnetic MNP range from 50 to > 600 s⁻¹ mM⁻¹ [9,12], and remain constant over all field strengths > 0.5 Tesla. The R1 values of these agents, however, decrease with field strength much like paramagnetic gadolinium constructs. First generation MNP, such as Feridex™ (Advanced Magnetics, Cambridge, MA, USA), contain relatively thin dextran coats and have the propensity to form polycrystalline clusters, which are rapidly cleared from the blood stream by the reticulo-endothelial system. This agent can thus be used to detect the replacement of normal liver tissue by neoplasm and after intravenous injection, and has been FDA-approved for this since 1993. The metabolism, pharmacokinetics and toxicity of MNP taken up by cells in the reticulo-endothelial system have been well studied [13]. Histologic and serologic studies have not revealed any toxic effects related to MNP administration. Iron radiotracer (59Fe) and MR relaxivity studies have also shown that the iron-oxide core of the MNP is broken down into other forms of iron and then incorporated normally into hemoglobin in newly formed erythrocytes [13]. More recently, several groups have used Feridex™ and other iron oxide nanoparticles to label exogenous stem cells prior to their in vivo administration [14, 15]. The pharmacokinetics, metabolism and safety profile of Feridex™ when used for cell labeling, however, requires a separate and detailed evaluation.

MION

Second and third generation MNP have been synthesized with more extensive polymer coatings

and remain monodisperse in solution [8]. The term monocrystalline iron oxide or MION is thus often applied to these agents. Unlike Feridex™, these agents were designed to have a much longer blood half life (24 hours in humans, 11 hours in mice) and typically have a homogenous uniform size distribution in the 30 - 50 nm range [8, 9,16]. The small size, long blood half lives and high relaxivities of these MNP constitute a powerful combination that allows them to penetrate deep tissue spaces, such as the interior of atherosclerotic plaque and the myocardium [17, 18], and detect sparsely expressed targets in the low nanomolar range. The MNP Ferumoxtran (Combidex™ or Sinerem™), a preparation similar to the experimental MION-47, has been used to image lymph node micrometastases in several phase 3 clinical trials [11]. This agent has also been used to image inflamed vulnerable plaque in humans [19,20], although the experience in this regard is very preliminary.

CLIO

A highly stabilized and cross-linked derivative of MION-47, known as CLIO-47, has also recently been developed for targeted molecular imaging applications [9, 21]. CLIO contains amine groups on cross-linked dextran chains, allowing a large variety of ligands to be conjugated to the nanoparticle with a high degree of flexibility, stability and ease. Near infrared fluorochromes, for instance, can be attached to the amine groups on the probe to form a dual modality magnetofluorescent nanoparticle [22, 23]. In addition, many copies of the targeting ligand can be attached to the CLIO-fluorochrome conjugate to form a multivalent (> 1 targeting ligand) magnetofluorescent nanoparticle. Examples of two recently used such ligands include annexin for apoptosis imaging [6, 23], and a peptide specific for the adhesion molecule VCAM-1 [24, 25]. More recently an experimental MNP with even higher relaxivity (MION-48, CLIO-48, R2 > 180 s⁻¹mM⁻¹) has been synthesized, and has the potential to enhance the sensitivity of these and other targeted probes even further.

Specificity of agents

The specificity of both gadolinium and iron oxide based probes is influenced by their size, potential for non-specific binding to extracellular proteins and other substances, and their degree of uptake

MNP do not contain any inert or nondegradable moieties – the iron-oxide core of these agents is metabolized within two weeks, after which the released iron is used in the synthesis of new red blood cells.

Multivalent magnetofluorescent nanoparticles can be used for molecular imaging of apoptosis or adhesion molecules like VCAM-1.

Table 1: Recognition of artifacts in cardiovascular molecular MRI. Potential solutions to these artifacts are shown in parenthesis.

Gadolinium Constructs	Comment
Incomplete fat suppression	Short T1 of fat may mimic probe uptake in the vascular wall. (Improve shim or use inversion recovery techniques.)
Incomplete suppression of signal from blood pool due to slow flow	Residual positive signal from blood may compromise evaluation of probe uptake by atherosclerotic plaque. (Use of diffusion encoded stimulated echo techniques to improve signal loss in blood pool.)
B ₁ inhomogeneity	Variable flip angle can produce inconsistent contrast in image. Problem in humans and larger animals at higher field strengths, such as 3 Tesla and higher. (Adiabatic excitation pulses or RF shimming with multiple transmit coils.)
Ghosting from chest wall fat into region of interest	(Parallel acquisition to reduce breathhold duration and/or use of fat suppression.)
Iron Oxide Nanoparticles	
Calcification	Low signal may mimic MNP uptake. (MNP produce bright signal with T1 or positive contrast techniques. Ultrashort echo or UTE techniques may also play a role.)
Air	Low signal may mimic MNP uptake. (MNP produce bright signal with T1 or positive contrast techniques. Ultrashort echo or UTE techniques may also play a role.)
Susceptibility Artifacts	
Hemorrhage	Endogenous iron products may mimic exogenous MNP accumulation. Exceedingly rare in mouse heart and atheromatous plaque. Incidence in humans will require study. (Dual modality imaging combining MRI with Fluorescence or PET will resolve issue.)
Motion	Can produce spin dephasing and MR signal loss, particularly with the long echo times needed for T2*-weighting. (Reduce echo time, use gradient moment nulling, change to off-resonance technique with short echo time.)

by immune cells such as macrophages. Larger gadolinium-containing liposomes for instance, may become retained non-specifically in the interstitial space of the myocardium, and gadolinium-containing micelles may bind non-specifically to lipophilic or hydrophilic components of the extracellular matrix. MNP may be taken up non-specifically by macrophages in inflamed tissues, but high throughput chemical screens have recently revealed that minor surface modifications of the MNP may reduce this [26].

When T2, T2* or SSFP sequences are used the MNP is imaged through the generation of negative contrast or relative signal hypointensity. Concerns have been raised that this negative contrast could be non-specific and difficult to differentiate from signal hypointensity due to calcification, susceptibility artifacts, flow related signal loss or air (Table 1). Several off-resonance or positive contrast techniques have thus recently been developed by Cunningham, Mani, Stuber and others to potentially address these issues. The sensitivity of the positive contrast sequences for MNP may approach that of conventional T2* based techniques, (nanomolar) [27, 28], if performed under optimal conditions and with parameters producing low specificity. However, under most circumstances the sensitivity of the off-resonance techniques is lower than that of conventional gradient echo imaging. In addition, these techniques work less efficiently at higher field strengths and are fairly nonlinear, particularly if the echo time is not kept extremely short [28].

Artifacts in cardiovascular molecular MRI

Several important artifacts, likewise, need to be considered when using gadolinium based probes (Table 1). Fat has a high R1 value and appears bright on T1-weighted sequences. Incomplete suppression of the perivascular fat, for instance, can thus mimic uptake of the probe in the vessel wall. Slow flow within the vessel lumen can also result in incomplete suppression of the blood signal and thus mimic uptake of the probe within the endothelium [29]. Finally B1 inhomogeneity becomes a significant problem at higher field strengths and can produce significant variation in signal intensity due to inconsistent flip angles. This B1 inhomogeneity will need to be addressed with techniques such as adiabatic pulses or B1 shimming if T1-weighted sequences are to be used optimally in humans and large animals at higher field strengths [30].

A strong awareness of the sensitivity, specificity and artifacts produced by each imaging technique (T1, T2*, off-resonance) is thus needed (Table 1). Methods to recognize and potentially eliminate some of these possible artifacts are provided in the parentheses within Table 1. In extreme cases multi-modality imaging may be needed. However, one of the strengths of MRI lies in its ability to generate multiple forms of contrast, which can usually be used to differentiate any potential artifacts from a true molecular signal.

Cardiovascular applications

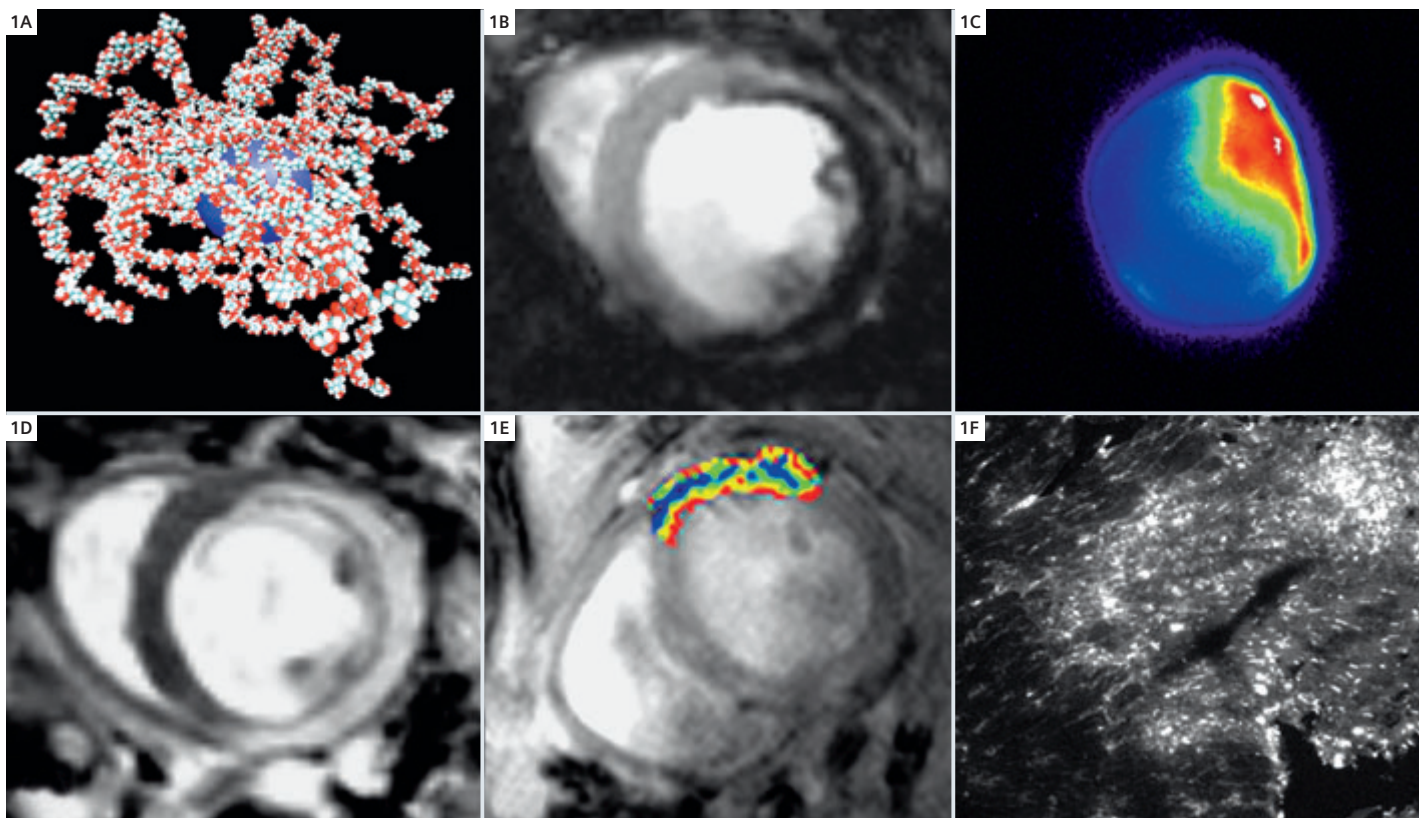
MNP have been used to image molecular targets in atherosclerosis [17, 25], myocardial injury [6, 18, 31], and stem cell therapy [14, 15]. The properties of MNP make them ideal agents with which to image myocardial macrophage infiltration in healing infarcts, transplant rejection and myocarditis [18, 31, 32]. This was recently demonstrated in a mouse model of post-infarction macrophage infiltration. The mice were injected intravenously with 3–20 mg Fe/kg of the MNP, CLIO-Cy5.5, 48 hours after the infarct and then imaged with conventional T2*-weighted MRI 48 hours later. Negative contrast, consistent with the uptake of the probe by infiltrating macrophages, was seen in the infarcted anterolateral myocardium of all mice and at all doses [18], as shown in Figure 1.

Targeted imaging of cardiomyocyte injury has been performed using apoptosis and necrosis detecting MNP probes. Cardiomyocyte apoptosis has been imaged in vivo by MRI in a mouse model of transient coronary ligation [6]. No significant changes were seen in myocardial signal intensity when mice were injected with an unlabeled control probe. However, injection of the annexin-labeled probe (AnxCLIO-Cy5.5) produced significant negative contrast enhancement [6]. As shown in Figure 1, T2* could be measured in the injured myocardium and was significantly lower in those mice injected with the annexin labeled probe [6]. Cardiomyocyte necrosis has been imaged by MRI in the rat heart ex-vivo with an antimyosin antibody conjugated to MION [33]. The use of this probe in conjunction with apoptosis detecting probes could thus provide powerful insights into the pathogenesis of cell death during acute myocardial injury.

The myocardium is highly suited to multimodal molecular imaging approaches. Fluorescence tomography of the myocardium, for instance, has recently

MNP have been used to image molecular targets in atherosclerosis, myocardial injury, and stem cell therapy.

The myocardium is highly suited to new multimodal imaging approaches such as fluorescence tomography or MR-PET.



1 Molecular MRI of myocardial injury in mouse models [1, 18].

(A) Schematic of a second-generation iron oxide magnetic nanoparticle (MNP), showing the iron oxide core and surrounding dextran coat. The composite size of the nanoparticle reaches 30-50 nm and remains monodisperse in solution.

(B) Accumulation of the MNP CLIO-Cy5.5 in macrophages infiltrating a healing myocardial infarct [18]. Negative contrast due to CLIO-Cy5.5 accumulation can be seen in injured anterolateral wall of the myocardium.

(C) Conjugation of a fluorochrome, such as Cy5.5, to the MNP allows fluorescence imaging to be performed to confirm the MRI findings [18]. The distribution of the MNP in panel (B), signifying macrophage infiltration, corresponds well to the area of delayed enhancement after gadolinium injection (D).

(E) T2* map of AnxCLIO-Cy5.5 accumulation in apoptotic myocardium following transient coronary artery ligation in a mouse [6]. The T2* values in the hypokinetic myocardium are significantly lower than in the remote myocardium, indicating the presence of cardiomyocyte apoptosis and uptake of the probe. The construction of T2* maps allows serial noninvasive quantification of the molecular process to be performed in-vivo [6].

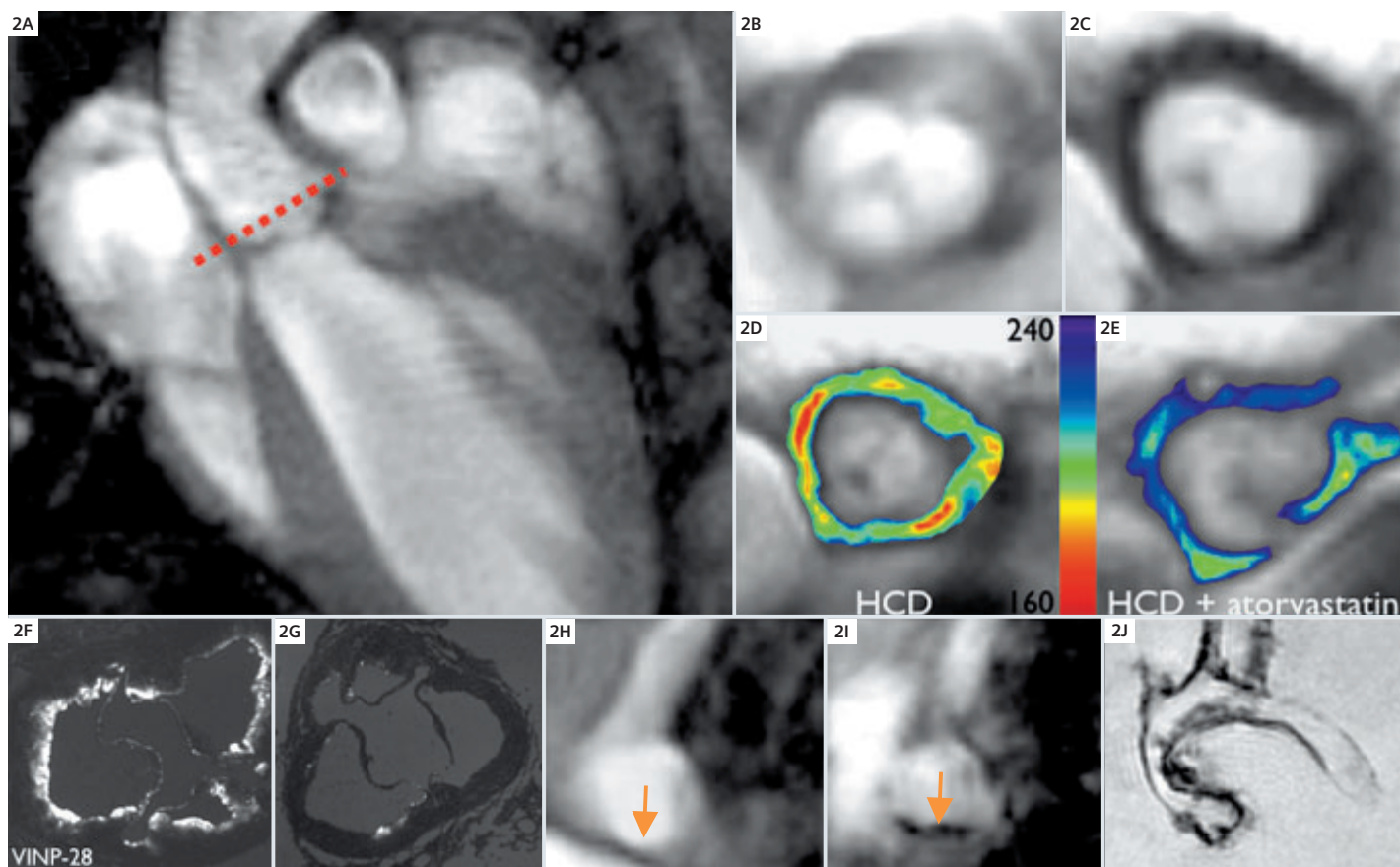
(F) Since the attachment of a fluorochrome to a MNP does not affect its pharmacokinetics, this can be used to evaluate the cellular localization of the MNP by fluorescence microscopy [1, 18]. This is a distinct advantage of molecular MRI with MNPs over radioisotope techniques, where cellular and microscopic localization of the probe is not possible.

Studies demonstrate the potential of targeted magnetic nanoparticles for diagnostic and therapeutic use.

been performed in the mouse heart in vivo [18, 31]. This is a completely non-invasive technique that is easily performed in conjunction with the MRI of magnetofluorescent MNP in small animals. In large animals and humans MRI can be combined with PET imaging either sequentially or simultaneously, using novel MR-PET systems.

Molecular MRI approaches to the imaging of atherosclerosis have focused on the detection of plaque lipid content, plaque inflammation, plaque thrombosis and plaque angiogenesis [2]. Gadofluorine is a novel chelate of gadolinium, which is more lipophilic and has a longer circulation half-life than con-

ventional chelates, and has been used to detect lipid rich plaques in rabbits [29, 34]. Labeled gadolinium-containing liposomes have been used in rabbits to target the v3 integrin and also used as a platform for the anti-angiogenic agent fumagillin [35]. This construct demonstrates the potential of using a targeted magnetic nanoparticle in both a diagnostic and therapeutic capacity. Gadolinium containing HDL-like nanoparticles have also been shown to accumulate in atherosclerotic plaques [4, 36]. Plaques rich in macrophages tend to take up these lipoprotein nanoparticles quicker than those with a low macrophage content [4]. Plaque macrophage



2 (A–G) Molecular MRI of VCAM-1 expression in apoE-/- mice imaged with a VCAM-1 sensing probe [25].

(A) T2*-weighted gradient echo image of the long axis of the left ventricle and thoracic aorta. The short axis plane through the aortic root is shown by the dashed red line. The images were acquired on a 9.4T horizontal bore MR scanner (Biospec, Bruker, Billerica, MA, USA).

(B, C) Short axis images of the aortic root before (B) and after (C) the injection of the probe are shown. Significant accumulation of the probe, consistent with VCAM-1 expression, is seen in the aortic root, which is an area rich in plaque.

(D, E) VCAM-1 expression in the aortic root of a cholesterol fed apoE-/- mouse (D) is shown by in vivo MRI to be significantly higher than in a statin treated apoE-/- mouse (E). Ex-vivo fluorescence images of the aortic root confirm higher probe accumulation in the untreated mouse (F) than the statin treated mouse (G).

(H–J) MRI of macrophage infiltration in atherosclerotic plaque in vivo [17, 44]. A cross section of the aortic arch of an apoE-/- mouse is imaged in (H, I) showing a smooth vessel outline when the mouse is injected with saline (H), but evidence of MNP accumulation in plaque when the mouse is injected with CLIO-Cy5.5 (I). The in vivo distribution of MNP in plaque can be confirmed by ex-vivo MRI (J).

content has also been imaged with a gadolinium containing micelle decorated with an antibody to the macrophage scavenger receptor [37].

A fibrin specific peptide has been conjugated to a conventional gadolinium-containing chelate to yield a small molecular weight probe. This probe has been used to image both acute and subacute thrombi in a variety of large animal models [38–41]. The low background uptake of this peptide and the very high levels of fibrin expression within thrombi allowed this agent to be conjugated to a low relaxivity conventional gadolinium chelate. This served both to make the agent potentially

safe in humans (initial results in 4 patients have been reported) but also required large amounts of the targeting peptide to be synthesized per dose, raising logistical concerns and barriers. At the present time further development of this agent has thus been stopped. Conjugation of a lower dose of the peptide ligand to a higher relaxivity contrast agent could potentially solve this issue.

The largest and perhaps most clinically applicable experience in the imaging of atherosclerotic plaque inflammation has been with MNPs [2]. Long circulating MNPs are able to penetrate an atherosclerotic plaque and are then taken up by its cellular compo-

Long circulating MNPs are able to penetrate an atherosclerotic plaque.

nents (Figure 2). Plaque inflammation has been imaged with MNPs in mice [17], large animal models [42], and in human carotid arteries in-vivo [19, 20]. Several generations of VCAM-1 targeted MNPs have also been developed. With the latest generation (linear peptide) of this probe, VCAM-1 expression could be successfully imaged in the aortic roots of apoE^{-/-} mice in-vivo (Figure 2) [25]. In addition, the effect of statin therapy on VCAM-1 expression could be imaged in-vivo with this probe (Figure 2) [25]. Molecular MRI of VCAM-1 expression was thus able to detect a sparsely expressed molecular marker early in a disease process and also demonstrate adequate dynamic range to detect a treatment effect. The use of stem cells for cardiac regeneration is highly promising and molecular MRI may play an important role in the development of this therapy. The MNP, Feridex™, has been used to label and image stem cells injected directly into the myocardium [14, 15]. Recently stem cells have been labeled with gadolinium and fluorine containing constructs too, allowing both proton and fluorine MRI to be performed. While MRI offers superior spatial resolution, its sensitivity for stem cells remains lower than SPECT [43]. MRI of labeled cells is also not able to determine cell viability or metabolic state. The advantage of MRI of labeled stem cells, therefore, lies in its ability to precisely delineate the infarct, guide the intramyocardial injection of the cells and track their movement over time [14, 15].

Conclusion

Molecular MRI is currently playing an increasing role in basic science research and pharmaceutical development. Examples showing the utility of molecular MRI in the imaging of biological processes in the myocardium and atherosclerotic plaque have been provided in this article. First generation polymer-coated MNP are already FDA approved and subsequent generations of MNP (Combidex™) have completed phase-3 clinical trials [11]. Molecular MRI in the cardiovascular system thus has the potential to become a powerful tool in both the basic science as well as the clinical settings.

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