The treatment of myocardial infarction (MI) has focused heavily on early reperfusion of the infarct-related artery. However, the inflammatory processes in the infarct that occur shortly after MI and govern wound healing in the heart have not been targeted therapeutically. Perhaps this has resulted from our inability to visualize these processes. In contrast to coronary angiography, which has clearly depicted stenotic coronary arteries for decades and initiated the era of percutaneous coronary interventions, imaging inflammation in the heart of patients with MI has been difficult. In this issue of Circulation: Cardiovascular Imaging, Alam et al describe important progress to overcome this hurdle. Their article reports the first human study in which an iron oxide nanoparticle (ferumoxytol, AMAG Pharmaceuticals, Lexington, MA) was used to image leukocytes in the infarcted tissue by T2* weighted magnetic resonance imaging (MRI). Building on previous preclinical data in mice with MI,2 and the use of ferumoxtran in patients with inflammatory disease,3–5 the authors imaged 16 patients within the first week after MI, 10 of whom received intravenous ferumoxytol. R2* values tripled in the infarct and doubled in the remote zone 24 to 48 hours after ferumoxytol administration. Because all patients in this small pilot study survived the subacute infarct period, corroborating histologic data on the tissue uptake of ferumoxytol were not available.

The uptake of ferumoxtran and ferumoxytol by leukocytes in vivo occurs without active targeting and is caused by the size, blood half-life, and surface properties of these agents. In a recent issue of the journal, Richards et al6 report another clinical first with the agent ferumoxides, an iron oxide nanoparticle designed and used clinically for liver imaging. Here, they use ferumoxides to label human monocytes ex vivo and then reinject the labeled cells to image their homing to sites of injury. The publication of these 2 clinical articles is an extremely welcome development and nicely demonstrates the safety, feasibility, and value of molecular MRI.

**Imaging of Myocardial Inflammation Post ST-Segment Elevation Myocardial Infarction**

Ischemic myocardial injury triggers a robust inflammatory response.7 After a rapid and short-lived spike of neutrophil recruitment, macrophages and monocytes, their immediate circulating progenitors, dominate the inflammatory cell population during the first 2 weeks after the injury.8 Preclinical work has shown that the monocyte/macrophage response can be subdivided into an early phase dominated by inflammatory monocytes and a later phase, starting around day 4 in the mouse, which is dominated by the noninflammatory subset.9 Clinical data on blood monocyte levels suggest similar kinetics in patients.10 These monocyte subsets are recruited by different chemokines and, importantly, pursue different functions during wound healing. In mice, many of these cells derive from a splenic monocyte reservoir,11,12 which may relate to the intriguing signal changes in the spleen observed by Alam et al.1 Given the central role of macrophages in wound healing, the activity of monocyte/macrophages shortly after MI has been found to be closely related to left ventricular remodeling and the evolution of heart failure in preclinical studies. Insufficient macrophage numbers, but perhaps more importantly, also a prolonged and increased presence of these cells in the infarct, both resulted in exaggerated left ventricular remodeling.11,13 Interestingly, these cells are centrally involved in chronic atherosclerosis because they promote plaque growth and vulnerability,14 providing a worthwhile therapeutic target. Targeting CC-chemokine receptor 2, the chemokine receptor that monocytes rely on for migration, modulates inflammation.15,16 Although the role of infarct monocyte/macrophages is fairly well understood, the presence of these cells in the remote myocardium has been reported only recently.17

Alam et al1 report significant changes in R2* values in the remote myocardium, but not in skeletal muscle. This would seem to corroborate the fact that inflammatory cells do invade the nonischemic, remote myocardium. The current study is also a very faithful translation of previous murine studies, which confirmed that the T2* changes seen in infarcted myocardium were indeed caused by macrophage infiltration.2,18 By using a fluorescently labeled analogue of ferumoxtran, it could be shown that the majority of the nanoparticles in the infarct were indeed taken up by monocytes and macrophages. More recently, serial R1 mapping after the intravenous injection of gadolinium-labeled...
liposomes has been used to characterize myocardial inflammation in a mouse model of MI. An increase in R1 in the remote zone, consistent with monocyte infiltration, was seen in these mice as well. Taken together, these findings strongly suggest that the increase in R2* in the remote zone seen by Alam et al reflects the infiltration of immune cells. However, before this can be definitively stated 2 important caveats must be raised.

An extensive body of evidence has shown that ferumoxtran is avidly taken up by immune cells. In addition to the preclinical studies discussed above, several clinical studies in which imaging was followed by histology have shown that the majority of this agent accumulated within macrophages. In contrast, the ability of ferumoxylot to image tissue macrophage infiltration, particularly in the heart, has not been extensively studied. Although ferumoxtran and ferumoxylot share many key properties, important differences also exist between the 2 agents (dextran versus carboxymethyl-dextran surfaces). A recent preclinical study suggests that the ability of ferumoxylot to track tissue macrophage infiltration is identical to that of ferumoxtran. However, the histological demonstration of this in the heart would be highly valuable.

The blood half-life of an imaging agent is extremely relevant in the myocardium because its intravascular volume fraction (≈10%) is far higher than in other tissues. Imaging should thus ideally be performed 5 half-lives after injection to ensure that the agent is completely cleared from the blood. In this study a dose of 4 mg Fe/kg of ferumoxylot was injected and imaging was performed 24 and 48 hours later. The blood half-life of ferumoxylot in humans, however, is ≈15 hours. A significant amount of residual ferumoxylot was thus present in the intravascular space at the time of imaging, particularly the 24-hour time point. The changes in R2* seen in the remote zone after the injection of the agent thus likely reflect some contribution from residual ferumoxylot in the very high intravascular space of the myocardium. We suspect that this contribution was not dominant, but the definitive determination of this would require the inclusion of an additional control arm in which normal volunteers were injected with ferumoxylot and imaged at the identical time points.

Notwithstanding the minor concerns above, the study by Alam et al is an excellent one that will hopefully lead to additional and larger studies. In particular, it would be interesting to investigate the correlation between the degree and time course of myocardial inflammation post-MI and the evolution of infarct size. Likewise, the relationship between myocardial inflammation post-MI, particularly in the remote zone, and the subsequent development of heart failure would be intriguing to assess. The ability of MRI to characterize the myocardium in humans is unmatched. Myocardial energetics, mass, function, strain, edema, iron/fat infiltration, perfusion, and viability are all routinely assessed in volunteers and patients. The study by Alam et al adds another tool to this impressive armamentarium by moving the imaging of inflammation in the heart with iron oxide nanoparticles from the preclinical into the clinical realm.

**MRI of Labeled Cells**

The use of iron oxide nanoparticles to label a variety of cells has been extensively reported in the preclinical literature. A large number of these studies have used ferumoxides to label the cells. Ferumoxides is rapidly removed from the blood stream after intravenous injection and has been Food and Drug Administration-approved for liver imaging for well over a decade. The article by Richards et al, however, is the first to investigate the safety of cell labeling with ferumoxides in humans.

Richards et al isolated peripheral monocytes from human volunteers, labeled these cells ex vivo with ferumoxides, and then injected the labeled cells intravenously. They were able to show in vitro that the viability and migratory function of the cells was not affected by labeling. In addition, they were able to show that the injected cells were well tolerated, migrated to sites of inflammation (produced by tuberculin skin testing) and could be robustly detected with T2-weighted MRI and R2* maps.

The study by Richards et al stresses the important point that the safety of a clinically approved imaging agent cannot be assumed when its route of administration is changed. This is particularly relevant when cells without the degradative machinery of monocytes are labeled. Having proven the safety of this approach, what are the next steps? Ferumoxides is no longer being manufactured and alternative agents such as ferumoxylot will need to be tested. The role of cell labeling will also need to be carefully studied. It is extremely likely that image-guided delivery of labeled cells will offer significant benefit over their blind injection, but this will need to be proven on a case-by-case basis.

Richards et al also convincingly show that the homing of labeled cells to the site of injury after intravenous injection in humans can be detected by MRI. It should be noted, however, that preclinical work with dual labeled cells in infarcted dogs showed that the sensitivity of this approach compared with radiolabeling was limited. The role of cell labeling in following the survival of injected cells is more controversial. Concerns have been raised that the presence of the label in a given tissue does not necessarily reflect cell survival. Ultimately, we believe, it is most likely that reporter imaging approaches will be needed to address cell survival.

**Clinical Translation at the Tipping Point**

After years of chemical development and methodical preclinical investigation, the study by Alam et al clearly brings us to a translational tipping point. Ferumoxylot is Food and Drug Administration-approved and now widely available to the clinical imaging community. How then should the clinical imager with little or no experience with these agents interpret and build on the preclinical literature? First, it is imperative to realize that a large number of iron oxide particles have been studied preclinically and that significant differences exist among them. Iron oxide microparticles, for instance, have very different properties from iron oxide nanoparticles and significant caution must thus be used when basing...
clinical studies with nanoparticles on preclinical study with microparticles.

Likewise, it is vital to be familiar with the different properties of the available nanoparticles. Ferumoxides, for instance, forms large aggregates after intravenous injection and has a blood half-life of only a few minutes. Ferumoxtran has more extensive dextran coating, remains monodisperse, and has a blood half-life of ≈15 hours in humans. Importantly, the blood half-lives of these agents in animals are frequently significantly shorter than those seen in humans, and this can be quite variable from agent to agent. An appreciation of these pharmacokinetic differences is thus vital for the rational design of clinical studies based on prior preclinical experience.

Many of the preclinical studies with iron oxide nanoparticles have been performed at high field strengths and the clinical investigator may wonder how these agents will perform at clinical field strengths. The answer to this is that they are equally effective at clinical field strengths. The transverse relaxation rate (r2) of superparamagnetic agents plateaus at 0.5 Tesla and their effect on R2* (transverse relaxation rate) is thus identical at 1.5 Tesla and 9.4 Tesla. High field strengths and gradients have been used in mice to achieve adequate signal to noise ratio (SNR) and deal with rapid motion, but are not needed to detect iron oxide nanoparticles.

The studies by both Alam et al and Richards et al used a T2* based approach to produce signal hypointensity in the region of the nanoparticles. The clinical researcher accustomed to the signal hypointensity produced by T1 weighted imaging of gadolinium chelates may wonder whether a similar approach can be employed with ferumoxytol. The answer is yes, and in fact the longitudinal relaxation (r1) of these nanoparticles is 4 times higher than that of the clinically used gadolinium chelates. The r2/r1 ratio of ferumoxytol at 1.5 to 3 Tesla, however, remains higher than that of gadolinium, and care must thus be taken to minimize the echo time to minimize R2 effects.

The large r1 of the iron oxide nanoparticles allows these agents to be robustly imaged with T1 weighted sequences but also complicates the accurate interpretation of late gadolinium enhancement in someone already injected with ferumoxytol. We recommend that the scheme used by Alam et al, who performed late gadolinium enhancement before the injection of ferumoxytol, thus be used. This restriction does not apply at very high field strengths because the r1 of iron oxide nanoparticles decreases significantly with field and essentially becomes negligible at 9.4 Tesla. Off-resonance sequences have also been used to image iron oxide nanoparticles in the myocardium, but we would recommend this approach only for the expert user.

In conclusion, we would like to congratulate Alam et al, Richards et al for 2 excellent and very valuable contributions to the literature. Molecular MRI of myocardial inflammation with ferumoxytol has reached a vital milestone and its translational tipping point. Clinical studies of cell labeling with this agent are sure to follow shortly. Although some have recently questioned the commercial feasibility of molecular imaging, Alam et al, Richards et al have clearly shown that molecular MRI of the cardiovascular system is on the move.

Disclosures

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References


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