Iron Oxide Particles for Atheroma Imaging

Tjun Y. Tang, Karin H. Muller, Martin J. Graves, Zhi Y. Li, Stewart R. Walsh, Victoria Young, Umar Sadat, Simon P.S. Howarth, J.H. Gillard

Abstract—The selection of patients for vascular interventions has been solely based on luminal stenosis and symptomatology. However, histological data from both the coronary and carotid vasculature suggest that other plaque features such as inflammation may be more important in predicting future thromboembolic events. Ultrasmall superparamagnetic iron oxide (USPIO) contrast agents have been used for noninvasive MRI assessment of atherosclerotic plaque inflammation in humans. It has reached the stage of development to have been recently used in an interventional drug study to not only assess inflammatory progression but also select patients at high risk. This article reviews the basic science behind the use of USPIO contrast agents in atheroma MR imaging, experimental work in animals, and how this has led to the emergence of this promising targeted imaging platform for assessment of high risk carotid atherosclerosis in humans. (Arterioscler Thromb Vasc Biol. 2009;29:1001-1008.)

Key Words: carotid atheroma • atherosclerosis • USPIO • MRI • inflammation • plaque vulnerability

Atherothrombosis is a major cause of morbidity and mortality in the western world and is now widely accepted as a chronic systemic vascular inflammatory disorder. The concept of the vulnerable atheromatous plaque, initially derived from the coronary circulation, is increasingly accepted in the carotid territory. Inflammation is a recognized risk factor and is fundamental to lesion progression and destabilization. The macrophage is one of the key cellular mediators of this process, and histological studies have shown that the degree of macrophage infiltration, typically either at the shoulders of the plaque or in the necrotic lipid core, increases the risk for plaque rupture and subsequent thromboembolism. Detection of macrophage activity and hence inflammation within atheroma could potentially distinguish between vulnerable and more stable states and remains one of the key goals of atheroma imaging in translational cardiovascular research.

Although evaluation of the coronary circulation is the ultimate goal, the required technology for assessing coronary plaque itself is still some years off. The carotid vessels are superficial, larger, and more amenable to noninvasive assessment, and they not only provide a window to detect early systemic atherosclerosis but also the impact on the downstream cerebral circulation. Hence this review will concentrate on imaging data pertaining to carotid atheromatous plaque.

It has recently been possible to identify carotid plaque inflammation noninvasively with Ultra small Superparamagnetic Iron Oxide (USPIO)-enhanced magnetic resonance (MR) imaging in both animal and in vivo human studies. The use of a USPIO agent, Sinerem (Guerbet; Ferumoxtran-10), has allowed the direct visualization of macrophage infiltration of carotid atheroma in vivo.

The hypothesis that Sinerem may be useful in the assessment of inflammatory activity in atherosclerotic plaque is strongly supported by the endothelial dysfunction theory. Dysfunctional endothelium initiates and sustains an inflammatory reaction within the arterial wall and allows for the accumulation of plasma components, such as low-density lipoproteins (LDL), in the subendothelial space. Oxidized LDL particles are then phagocytosed by macrophages ultimately forming foam cells. USPIO particles of a diameter comparable to that of LDL (15 to 25 nm) enter atherosclerotic plaques with an increased endothelial permeability and accumulate in atheroma with high macrophage content, visualized as a signal dropout or voids on MRI (Figure 1).

The main aim of this review is to describe how USPIO contrast agents such as Sinerem have been used for noninvasive MRI assessment of atherosclerotic plaque inflammation. We describe the basic science behind their use, experimental work in animals, and how this has led to the emergence of this promising targeted imaging strategy for carotid atherosclerosis in humans.

SPIO & USPIO Characteristics

Three classes of iron oxide particles are currently used for atheroma imaging. They can be classified as:

1. Superparamagnetic Iron Oxide (SPIO) particles
2. Ultrasmall Superparamagnetic Iron Oxide (USPIO) particles
3. Ferumoxides (Ferumoxtran-10)

SPIO & USPIO particles are water-soluble, non-immunogenic, and have a relatively short T1 relaxation time. They are typically administered intravenously and accumulate in the macrophages of the atheroma.

In the carotid territory, SPIO particles have been shown to accumulate in the macrophages of vulnerable plaques, which are associated with an increased risk of plaque rupture and subsequent thromboembolism. This has led to the development of targeted imaging strategies for identifying high-risk plaques in vivo.

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It has recently been possible to identify carotid plaque inflammation noninvasively with Ultra small Superparamagnetic Iron Oxide (USPIO)-enhanced magnetic resonance (MR) imaging in both animal and in vivo human studies. The use of a USPIO agent, Sinerem (Guerbet; Ferumoxtran-10), has allowed the direct visualization of macrophage infiltration of carotid atheroma in vivo.

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In the carotid territory, SPIO particles have been shown to accumulate in the macrophages of vulnerable plaques, which are associated with an increased risk of plaque rupture and subsequent thromboembolism. This has led to the development of targeted imaging strategies for identifying high-risk plaques in vivo.
a. Micron-sized iron oxide particles (these have been used for targeted imaging of selectins and activated platelets).
b. Short circulating iron oxide nanoparticles such as Ferumoxides. These are superparamagnetic iron oxide (SPIO) particles and have a thin and incomplete dextran coat. This causes them to aggregate in solution into large clusters that are rapidly cleared from the circulation by the reticulo-endothelial system (RES). The blood half-life of these agents in humans is thus about 5 minutes (generally not suited to atheroma imaging).
c. Long circulating iron oxide nanoparticles such as Ferumoxtran. “USPIO” such as ferumoxtran have a thick and complete dextran coat. They thus remain monodisperse in solution and are cleared from the blood very slowly by the RES. Ferumoxtran in humans has a blood half-life of around 24 hours. It is this long blood half-life that makes it suited to macrophage and lymph node imaging (highly suited to atheroma imaging).

SPIO and USPIO agents consist of microcrystalline magnetic cores coated with dextran or siloxanes. After injection they accumulate in the RES of the liver and the spleen. Iron oxide particles function as contrast-enhancing agents by creating a large dipolar magnetic field gradient that acts on the water molecules that diffuse close to the particles.14 SPIO and USPIO particles have a very large r2/r1 ratio, where r1 and r2 refer to the longitudinal and transverse relaxivities (lmmol⁻¹s⁻¹) respectively, so that although they increase both T1 and T2 relaxation, in general, the T2 effects predominate in the atherosclerotic plaque to the extent that negative contrast is observed in both T1-weighted and T2-weighted imaging. The predominant T2 effects create an area of hypointensity on conventional spin-echo MR sequences that is enhanced further by compaction, for example within cells. The intracellular compaction also appears to reduce the T1 effect considerably.14,15 as discussed further below. Gradient echo MR sequences appear to be more sensitive than spin-echo sequences for detecting the contrast agent, by virtue of the inherent T2* sensitivity of these sequences.14–16 The degree of magnetic susceptibility effect (blooming) can be controlled by specifying data acquisition parameters that have more or less T2 and T2* dependence. It should therefore be noted that the extent of signal drop is not directly proportional to SPIO concentration, because the blooming effect may extend some distance depending on the imaging parameters.

Recently a group from Japan investigated the potential of MR carotid plaque imaging using the SPIO Ferucarbotran (Resovist).17 They performed MR plaque imaging on 10 patients scheduled for carotid endarterectomy before and 24 to 43 hours after administration of SPIO. Three-dimensional gradient recalled acquisition in the steady state (3D-GRASS) was used for detecting macrophages within plaques. Signal loss on postcontrast GRASS image was found in 50% of the subjects, and the accumulation of SPIO particles in the vessel wall was confirmed in 80% subjects. Intracytoplasmic localization of SPIO particles within recruited macrophages was verified by double stain analysis. This was the first study to use a SPIO to look at inflammatory carotid plaques and may help elucidate the differences in the internalization of iron particles within plaques between SPIO and USPIO. USPIO agents, typically with a mean diameter less than 50 nm, have a different biodistribution compared to SPIO. They do not accumulate in the RES so rapidly, which results in a longer plasma half-life. This feature together with the fact that the r2/r1 ratio decreases with decreasing particle size, has led to some USPIO agents being proposed as potential blood pool agents for magnetic resonance angiography,18 where the vascular blood pool appeared bright on short TE/TR gradient echo imaging sequences. The majority of studies that have used USPIO particles have used long TE (10 to 20 ms) gradient echo sequences in which the regions containing USPIO particles typically demonstrate signal hypointensity. However if the local concentration of the agent is lower, ie, it is relatively disperse and short TE and repetition times (TR) are used then there is the possibility of obtaining signal hyperintensity. This is due to the fact that at short TE, and with disperse nanoparticles, relaxation is dominated by T1 effects. Conversely, when the nanoparticles cluster together then the r2 increases, whereas the effect on r1 is minimal,19 hence the r2/r1 ratio increases and T2/T2* relaxation effects predominate. Therefore depending on the local concentration of the agent and the pulse sequence parameters the T1 signal enhancing effect may confound with the signal decay attributable to T2/T2* resulting in the presence of the agent being obscured.20 Therefore the optimal visualization of the agent with negative contrast, ie, signal hypointensity, should involve the use of a T2*-weighted gradient echo sequence where the effects of T1 can be minimized by, for example, the use of a long TR and low flip angle. The optimal choice of echo time is also difficult to determine a priori because too short a TE may not allow sufficient susceptibility-induced phase spread and hence signal loss, whereas too long a TE may result in unnecessary sacrificing of image signal to-noise ratio. Multi-echo gradient echo sequences are very useful in these applications because they allow multiple images acquisitions at different echo times.

**USPIO Uptake Into Macrophages**

Whereas the accumulation of USPIO in macrophages is well established,21 their route of transport is not yet well defined.
Both the degree and mode of uptake are dependent on particle size, nature and charge of the particle coating, as well as the cell type. In phagocytic cells, larger particles are often internalized more efficiently than smaller ones, whereas in nonphagocytic T-cells intermediate sized particles were internalized more efficiently than any other size. As to the mode of uptake, smaller particles may be internalized by pinocytosis, whereas with increasing size, receptor-mediated endocytosis and phagocytosis can become more prominent. Specific interaction with cell surface receptors, such as the scavenger receptor A, and the integrin Mac-1 (CD11b/CD18), have also been involved in iron oxide particle uptake. Our results indicate that Sinerem particles are internalized by human monocytic-macrophages in a nonsaturable manner, suggesting uptake by fluid-phase transport. After internalization, most iron oxide nanoparticle species are found in the endosomal compartment, and Ferumoxtran-10 was found to persist for several days within lysosomes.

Several mechanisms have been suggested: (1) USPIOs are endocytosed by activated blood monocytes, which migrate into the pathological tissues; for example, the long blood half-life of ferumoxtran-10 allows sufficient time for the blood monocytes to endocytose the nanoparticles and for the progressive migration of these cells; (2) transcytosis of USPIOs through the endothelium and migration of the USPIOs into the tissue, followed by progressive endocytosis of these USPIOs by in situ macrophages; and (3) transport of USPIOs into the pathological tissue, in some cases via the inflammatory neovascularure (vasa vasorum) irrigating the media and adventitia in atherosclerotic lesions.

USPIOs have a good biocompatibility profile, their uptake by macrophages is not associated with cell activation. Furthermore, USPIOs, which are biodegradable, have no long-term toxicity.

The blood half-life of USPIO differs in human and animal species. At 30 or 45 μmol Fe/kg, the blood half-life of ferumoxtran-10 is 2 hours in rats, whereas in humans it varies from 24 to 36 hours. In rats, increasing the dose caused a longer blood half-life because of the progressive saturation of uptake by the liver and spleen. Because the access of USPIO to deep compartments is favored by prolonged blood residence time, animal imaging experiments are generally performed using high doses of USPIO (200 to 1000 μmol Fe/kg) compared with the human clinical dose of 45 μmol Fe/kg.

Toxicity
Release of free iron from the core of iron oxide nanoparticles could potentially be cytotoxic because of its catalytic function in the production of reactive oxygen species (ROS) in the Fenton reaction, which in turn can cause lipid peroxidation, protein oxidation, and DNA damage. However, most in vitro studies have shown iron oxide nanoparticles to be innocuous to cells in culture. In our study, Ferumoxtran-10 was not toxic to human monocyte-macrophages at extremely high concentrations and key activities, such as cytokine production, Fc-receptor-mediated phagocytosis, and chemotaxis, remained unaltered in the presence and after uptake of Ferumoxtran-10.

Statins
Ferumoxtran-10 was used in a randomized controlled study to monitor the effect of atorvastatin therapy on macrophage activity in carotid plaques as an indicator of high risk. However, a literature report stated that statins may inhibit macrophage uptake of iron oxide nanoparticles, an effect which would jeopardize the suitability of Ferumoxtran-10 to monitor macrophage burden in patients receiving statin treatment. In an in vitro study, we showed that Ferumoxtran-10 uptake by human monocyte-macrophages is not affected by nontoxic concentrations of atorvastatin. Furthermore, the intracellular distribution of Ferumoxtran-10 as judged by transmission electron microscopy and T1 and T2* relaxation times remained unchanged by atorvastatin treatment.

Animal Studies
Animal studies using atheromatous rabbits or ApoE knockout mice have revealed that USPIO particles are taken up by macrophage-laden aortic plaques as intracellular inclusions. This causes areas of focal signal loss on T2*-weighted MRI within the vessel wall when compared with precontrast images. In experimental models of double-balloon injury of the infrarenal aorta in hypercholesterolemic rabbits, ferumoxtran-10 was found to allow MRI quantitative assessment of macrophage neointimal infiltration. Interestingly, the exposure period of atherosclerotic plaque to circulating USPIO seems to be critical for the uptake of nanoparticles. In a comparative study in hyperlipidaemic rabbits, the in vivo MRI signal intensity was significantly higher with the long half-life agent ferumoxtran-10 than with another USPIO of similar size, ferumoxytol (T1/2 ≈ 6 hours in rabbits), whereas in vitro macrophage phagocytosis was greater with ferumoxytol.

Clinical Studies
Two preliminary clinical studies using ferumoxtran-10 have also shown USPIO-induced signal loss in aorta and pelvic arteries or in carotid atheromatous plaques. Another clinical study confirmed the targeting of ferumoxtran-10 by macrophages in stenotic carotid plaques. In this study, histological analysis showed USPIO in 27/36 (75%) of the ruptured or rupture-prone plaques and 1/14 (7%) of the stable atheromatous lesions.

Areas of focal signal loss on in vivo MR images have been shown to correspond to accumulation of iron particles in ex vivo specimens. USPIOs are thought to accumulate predominantly in macrophages in ruptured and rupture-prone human atherosclerotic lesions. Kooi initially showed that USPIO uptake induces significant signal decreases in the in vivo T2*w MR images obtained using an ECG-triggered gradient echo sequence with a TE of 20 ms, acquired 24 hours after intravenous administration of USPIO. Moreover clustering of iron oxide particles in tissue may lead to additional T2* shortening and the “blooming effect.” This signal decrease was found to be attenuated in images acquired after 72 hours. This suggested that there is an active process of accumulation and excretion of USPIO particles. Trivedi et al went on to investigate in more detail the in vivo temporal relationship of signal intensity reduction on MRI after USPIO administration in symptomatic patients scheduled for carotid endarterecto-
An ECG-triggered quadruple inversion recovery (QIR) prepared spiral sequence was used. The QIR preparation suppresses the high luminal signal both before and after Sinerem administration, whereas the spiral acquisition provides a highly time-efficient method for data collection. Two acquisitions with effective echo times of 5.6 ms and 15.0 ms were acquired. Eight consecutive patients with severe internal carotid artery stenosis underwent multi-sequence MR imaging of the carotid bifurcation before and 24, 36, 48, and 72 hours after USPIO administration. There was a distinct temporal variation in the size of the area showing signal intensity loss between images from any one patient. The earliest discernible signal loss was evident by 24 hours becoming more visually obvious at 36 hours after infusion and remaining so at 48 hours after infusion (Figure 2). The area of signal loss began to decrease after 48 hours but was still visible on images taken 96 hours after infusion. The area of signal intensity loss localized to the fibrous cap region on histological coregistration and localization suggesting that it is the macrophages that are taking up the USPIO. This was confirmed with double staining techniques using Perls (an iron stain) and anti-CD68 (for macrophages). This temporal change in the resultant signal intensity reduction on MRI suggests an optimal time window for the detection of macrophages on postinfusion imaging.

This study was later validated with a larger cohort of 30 patients whereby more than 90% of symptomatic patients who were also scheduled for carotid endarterectomy showed USPIO enhancement.

Howarth et al also used USPIO to compare patients with symptomatic and asymptomatic carotid stenosis. They reported that USPIO appeared to show a dual contrast effect with signal enhancement being seen in plaques with little inflammation and large fibrous caps. Although seen best on $T_1$-weighted imaging, the effect was also seen on $T_2^*$-weighted imaging. They concluded that this phenomenon may be attributable to the signal enhancement seen on gradient echo imaging at low concentrations of USPIO. Symptomatic patients had more focal areas of signal drop than asymptomatics, thus suggesting that their plaques had large inflammatory infiltrates. Asymptomatic plaques showed significantly more enhancement in both $T_1$-weighted and $T_2^*$-weighted images than symptomatic plaque suggesting more stability as a result of thicker fibrous caps. However some asymptomatic plaques also showed focal areas of signal drop, suggesting an occult macrophage burden. It is perhaps this group that would benefit most from this technique, allowing identification of inflammation within otherwise morphologically “stable” plaques. The conclusion was that, if validated by larger studies, USPIO particles may prove a useful dual contrast medium able to enhance the risk stratification of patients with carotid stenosis thus improving patient selection for intervention.

The contralateral sides of symptomatic patients given USPIO were also analyzed. It was found that 95% patients showed bilateral USPIO uptake suggesting an inflammatory burden within their carotid atheroma bilaterally. Only one patient with USPIO signal decrease on the symptomatic side showed no signal decrease on the asymptomatic side. This finding highlights the truly systemic nature of vulnerable atheroma and that patients showing inflammatory activity on one side may be more likely to have it contralaterally than truly asymptomatic patients. However, there is currently no literature to support the notion that the risk of stroke from asymptomatic disease contralateral to a symptomatic stenosis is any higher than 1% to 2%, which remains the predicted risk in the medically treated group from the Asymptomatic Carotid Atherosclerosis Study. Numbers are however small, and no proper natural history study has been published with regard to contralateral carotid disease.

Further USPIO studies have been performed subsequently in asymptomatic patients with carotid stenosis. Tang et al explored whether there was a difference in the degree of MR-defined inflammation using USPIO particles, between truly asymptomatic carotid atheromatous plaque and asymptomatic...
The hypothesis was that inflammatory atheroma is a systemic disease and that one inflamed symptomatic vascular bed is likely to increase the risk of other arterial vessels becoming inflamed. Although both groups of patients would be classified as asymptomatic from a clinical viewpoint, USPIO enhanced MRI may allow improved risk stratification of the subgroup of asymptomatic patients who harbor vulnerable plaque and who are at likely increased risk of developing stroke.

The primary findings were: (1) Patients with contralateral asymptomatic disease showed more inflammatory activity than did the completely asymptomatic cohort, as demonstrated by normalized signal loss in significantly more quadrants and a significant decrease in mean signal intensity after USPIO infusion, despite a mean lower grade of luminal stenosis (46% versus 63%); (2) Completely asymptomatic individuals had significantly more quadrants with signal enhancement (possibly implying a larger fibrous cap component and hence possibly greater plaque stability) but there was also a subset of quadrants showing signal loss. The issue regarding plaque enhancement arose again in this publication. The completely asymptomatic plaques showed an overall normalized mean signal enhancement. Enhancement may be related to low concentrations of USPIO within the plaque (as $T_1$ shortens at low concentrations before a $T_2^*$ susceptibility effect produces an exponential drop in signal at higher concentrations). As there is a lack of phagocytic activity in these plaques, it is likely that USPIO concentrations locally will be reduced and little clumping of particles will occur in phagolysosomes and hence reduced number of signal voids will be seen on $T_2^*$-weighted MR imaging. Asymptomatic enhancement may therefore be related to plaque stability (Figure 3). It is possible that this may go some way to understanding the relatively low event rate from the asymptomatic contralateral side as well as from a truly asymptomatic carotid stenosis. They concluded that truly asymptomatic plaques seem to demonstrate inflammation but not to the extent of the contralateral asymptomatic stenosis to the symptomatic side and that inflammatory activity may be a significant risk factor in asymptomatic disease.

Another USPIO-based study performed by the same group went on to explore whether or not there was a difference in the degree of MR-defined inflammation between asymptomatic plaques in patients awaiting coronary artery bypass grafting (CABG) and those in individuals with a carotid stenosis who were completely asymptomatic in all vascular territories. Patients awaiting CABG had more inflammatory activity within their carotid atheroma than did the completely asymptomatic cohort despite a mean lower degree of luminal stenosis (59% versus 65%). Completely asymptomatic individuals had significantly more quadrants with signal enhancement, possibly implying a larger fibrous cap component and hence possibly greater plaque stability. However, there was also a subset of quadrants showing signal loss, too. The authors concluded that inflammatory activity may be a significant risk factor in asymptomatic disease and may help risk stratify this patient cohort in the future.

The problems with these types of USPIO based studies are that they are cross-sectional in nature and only accounts for the inflammatory status at one moment in time. The hypothesis that inflammatory activity as defined by USPIO-enhanced MRI may be a risk factor in these patients can only be validated after large scale prospective natural history studies. Unfortunately at the time of writing no study has been performed, and therefore, although these studies represent interesting preliminary work, they require validation not only in terms of a larger number of patients but also corroborative natural history data. Were such validation to be possible, there might be a strong argument in the future, for example, for following a group of CABG patients prospectively in a future USPIO study to determine the number of cerebrovascular events that occur perioperatively. An interesting question is whether or not there is a correlation between the degree of USPIO uptake preoperatively and the development of stroke subsequently. This is highly clinically relevant as patients awaiting coronary revascularization who are found to have a degree of carotid artery stenosis may still be offered CEA before CABG. It remains unclear whether this is clinically appropriate or whether a synchronous procedure is indicated.

The only longitudinal USPIO based study in humans assessed the feasibility and repeatability of performing sequential USPIO studies over a period of 1 year at intervals of 0, 6, and 12 months with no drug intervention. This is currently being submitted for peer-review. Ten patients with a moderate asymptomatic carotid stenosis underwent multi-
sequence MR imaging before and 36 hours after USPIO infusion at 0, 6, and 12 months. All patients were able to undergo multiple MR imaging sessions at multiple time-points and tolerated multiple USPIO infusions with no adverse events indicating that this technique may feasibly be incorporated into larger-scale prospective sequential studies. Furthermore, on analysis of the MR imaging datasets, no significant difficulty was found in coregistration of the images either between longitudinal time-points or between pre- and post-USPIO imaging (Figure 4).

The results revealed no statistical difference in USPIO uptake between the 3 time points. There was a good agreement of quadrant signal pre-USPIO infusion between 0 and 6 months (0.71) and 0 and 12 months (0.70). Good agreement of quadrant signal after USPIO infusion was shown between 0 and 6 months (0.68) and moderate agreement between 0 and 12 months (0.40). The relatively good agreement in signal change before and after USPIO over 0 and 6 months and moderate agreement between 0 and 12 months suggests that the process of inflammation within carotid atheroma is a dynamic and cyclic one (Figure 4). The findings of this study suggested that not only is this technique quantitatively reproducible but also that USPIO particles have been cycled out of the plaque within this 6-month timeframe, having important implications for future longitudinal studies involving pharmacological intervention.

ATHEROMA was a study undertaken to investigate the effects of low-dose (10 mg) and high-dose (80 mg) atorvastatin on macrophage activity in carotid atherosclerotic plaques using serial USPIO-enhanced MRI. Forty-seven patients with carotid stenosis ≥40% on duplex ultrasonography and who demonstrated intraplaque accumulation of USPIO on MRI at baseline were randomized in a balanced interventional drug trial. Simultaneously it facilitated the enrichment of a trial population so that only individuals with plaque inflammation were selected for enrollment into the study. As validation continues, USPIO-enhanced MRI methodology may be a useful imaging biomarker for the screening and assessment of therapeutic response to “anti-inflammatory” interventions in patients with carotid atherosclerotic lesions.

Methods of Analysis

T$_2$*-Weighted Imaging: The Region of Interest (ROI) Approach

Although T$_2$*-weighted imaging does allow the visualization of the effect of USPIO particles, there has been much debate in the literature as how best to quantify this and whether the degree of signal loss is in any way proportional to the inflammatory load within the plaque. Trivedi et al$^{12}$ used manually delineated regions of interest and calculated the normalized signal change between pre- and post-USPIO images. The signal was normalized to the signal in the adjacent sternocleidomastoid muscle, and any ROIs drawn that showed an actual normalized signal drop were taken to indicate USPIO uptake. This showed only a moderate correlation with macrophages staining positively for USPIO on Perls staining (Pearson product moment 0.6). Although useful, this technique has an inherent problem of bias and observer error leading to some question as to its usefulness in quantification of inflammatory burden.

The Quadrant Approach

A more recent approach has been to arbitrarily divide the vessel wall in each slice into quadrants by constructing perpendiculars to the horizontal axis across the image. This technique has the advantage that data points come from the whole vessel and every section that has plaque rather than the rather biased population of ROIs. Thus, a quadrant showing signal loss, once normalized to adjacent muscle, is taken to mean USPIO uptake in that quadrant.

Although eliminating operator bias, this technique has a number of problems associated with it, including that small focal areas of signal loss may be lost in a quadrant when regions of signal enhancement are found around it, limiting the spatial resolution of the analysis.

T$_2$* Quantification

Although T$_2$*-weighted imaging allows visualization of USPIO uptake, the need to normalize the signal in quadrants to the
adjacent muscle is problematic. It makes assumptions that the signal in the muscle should not change after USPIO infusion and that any change in muscle signal will be related to varying sensitivity of the surface coil across the field of view between the 2 imaging sessions, 36 hours apart. This is clearly an inaccurate assumption, and the need to normalize the signal to the adjacent muscle is a potential source of significant error and variability. Further, the signal response at the muscle ROI may very well be different to the signal response at the carotid wall quadrant, and the two may not be directly comparable.

An accurate and reliable quantitative assessment of in vivo inflammatory atheroma burden is crucial for studies designed to look at the efficacies of new pharmacological interventions currently aimed at the stabilization of vulnerable plaque and target inflammatory activity. One potential solution is to measure the carotid wall $T_2^*$ before and after administration of USPIO particles as an index of macrophage quantification. It should be noted that $T_2^*$ relaxometry for quantitative imaging is strongly hampered by large-scale field inhomogeneities, which lead to signal losses and an underestimation of the $T_2^*$.

**Limitations of USPIO**

Although the clinical studies performed by our institution and others have demonstrated the technical feasibility of USPIO-enhanced MRI, the diagnostic accuracy and potential effect on clinical management are yet to be defined in larger scale studies. Moreover, the disadvantages with USPIO-MRI include the fact that at least 2 MRI studies are required before and after infusion of contrast medium. The relatively modest mean percentage reduction in signal loss can sometimes only be appreciated with the use of dedicated quantitative algorithms and can be masked, for instance, in plaques with heavy calcification and blood degradation products, which can both have strong susceptibility effects on gradient echo sequences. New image-acquisition methods, eg, GRASP, that allow USPIO to be detected as a strong positive contrast enhancement have been developed in vitro with promising results but are yet to be implemented in human carotid atheroma imaging.

With regards to quantification of signal reduction, there is currently no consensus to which methodology to use. Relative signal intensity where the signal change is referenced to the adjacent muscle is reliant on the assumption that the muscle does not uptake USPIO. Furthermore, the signal reduction is dependent on reproducibility of coil positioning and on image coregistration before and after USPIO infusion. From our experience it can be sometimes difficult to null the blood before and after contrast even with quadruple inversion recovery, especially in patients who do not have good ECG gating and whose necks are rather large and short.

Reservations remain of the specificity of Sinerem. Internalization of iron-oxide nanoparticles into cells increases their signal but is not required for their visualization. There are reports suggesting entrapment within a densely packed environment such as thrombus or engorged vessels may be enough for the characteristic enhancement to be detected.

**The Future for USPIO**

Although showing great promise for the noninvasive assessment of carotid atheroma, it is likely that the future for USPIO compounds will lie with more targeted agents. Work on agents of this sort thus far in humans has been limited to fibrin-targeted gadolinium-based imaging. However, molecular targeting of contrast agents to either membrane bound receptors or free proteins will eventually allow “in vivo microscopy” at an exquisite resolution with the use of high-field clinical systems. Macrophage markers, well known in histological circles such as MAC387 and CD68, could well be used as targeted moieties for specific USPIOs to further aid localization of USPIOs to these cells, and lastly USPIO-tagged HDL may well be a possibility in the future. As novel sequences are developed and more clinical work is done, a consensus may finally be reached in the literature as to how to best quantify USPIO uptake in inflammatory atheroma and how this may change over time and after pharmaceutical intervention. It is quite clear that USPIO-enhanced MR imaging will have a major role to play in the development of atheroma stabilizing drugs of the future and may have a place in the risk stratification of asymptomatic atheroma and moderate stenotic atheroma and selection for subsequent intervention.

Many site-specific MR contrast agents are being developed to target the biological processes of atherosclerosis such as angiogenesis and biomarkers of plaque instability, eg, fibrin binding gadolinium-labeled peptide, but these remain very much experimental in animals.

**Disclosures**

J.H.G. is a consultant to GSK.

**References**


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