Nanoparticles as Contrast Agents for MRI of Atherosclerotic Lesions

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Abstract: Nanoparticle contrast agents for MRI may aid in identifying atherosclerotic lesions that give rise to ischemic events by means of penetration and retention in the plaque. These imaging agents may provide valuable information regarding plaque characteristics which can help determine the risk of plaque rupture. By increasing molecular flexibility or adding a means of specifically targeting ligands via antibody or peptide, nanoparticles can enhance certain regions of the atherosclerotic plaque. The development of single contrast agents detectable with multiple imaging modalities may further improve our ability to detect and characterize atherosclerosis in clinical and preclinical applications. These exciting developments may help in the realization of MRI as a powerful tool in the prevention of cardiovascular morbidity and mortality.

Keywords: MRI, contrast agents, atherosclerosis

Atherothrombotic events, such as myocardial infarction, stroke and ischemic peripheral vascular disease, represent the single greatest cause of morbidity and mortality in Western society. However, identification of atherosclerotic lesions in clinical practice that will progress to plaque rupture remains challenging and is frequently based on the degree of luminal stenosis (Topol et al. 1995). Imaging technologies reliant on determining lesion severity by the degree of luminal stenosis can fail to appreciate the presence of atherosclerotic plaque due to compensatory arterial enlargement (Glagov et al. 1987). The compensatory enlargement in regions of atherosclerotic plaque is believed to occur in order to maintain adequate blood flow distal to the lesion. Another clinical challenge is that the majority of acute coronary syndromes result from lesions with mild to moderate stenosis (Little et al. 1988; Ambrose et al. 1988), which may help explain the epidemiological finding that the first manifestation of coronary disease in half of individuals is unheralded sudden death or myocardial infarction (Kannel, 1976). Thus, the challenge is to identifying lesions prone to rupture in asymptomatic individuals since lesions that produce angina due to myocardial ischemia tend to be greater than 70% stenosed (Gould et al. 1974).

Experimental, pathological and clinical studies have clearly demonstrated the heterogeneity of atherosclerotic lesions (Fuster et al. 1992; Naghavi et al. 2003). Three major aspects of plaque morphology are considered to be important: 1) plaque size, thickness, eccentricity and distribution along the vascular bed; 2) plaque tissue composition, including the lipid/necrotic core, dense and loose fibrous matrix, hemorrhage and calcifications; and 3) plaque inflammation (Stara, 2000; Yuan et al. 2006). Early diagnosis and risk stratification of atherosclerotic lesions can help to identify individuals at elevated risk and direct therapies that may help stabilize the plaque and prevent atherothrombotic events (Choudhury et al. 2004).

Magnetic resonance imaging (MRI) is a noninvasive technique which has been shown to be capable of detecting lesions (Fayad and Fuster, 2000). This powerful technique is capable to provide at real time soft-tissue and functional information by exploiting proton density, perfusion, diffusion, and biochemical contrast. It also offers a superb resolution (<1 mm) and offers a good depth penetration (>10 cm) (Rink, 2003; Merbach and Coth, 2001). The quality of the images can be further increased by the employ of contrast agents.
These chemicals mainly based on gadolinium complexes cause a large increase in the water proton relaxation rate which enhances the differences between healthy and diseased tissues. The main drawback of this technique is its inherent low sensitivity that can be overcome by signal amplification strategies that generate a high concentration of contrast agent at the region of interest. Attachment of the contrast agents into linear polymers (Aime et al. 1999), dendrimers (Kobayashi and Brechbiel, 2004), micellar structures (Lipinski et al. 2006), lipoproteins (Frias et al. 2006) and protein bound chelates (Aime et al. 2001) have been employed to amplify the signal and deliver enough quantity of contrast agent to image (Caravan, 2006) in vivo the presence and biologic activity of atherosclerosis. Additionally, these contrast agents can be modified by the attachment of antibodies or peptides that specifically target components of atherosclerotic plaque to improve plaque characterization (Lipinski et al. 2004).

Micelles and Liposomes

Micelles and liposomes are supramolecular adducts formed generally with phospholipids and a surfactant. In general, micelles have a small diameter (<25 nm) and are formed by a monolayer of phospholipids. In contrast liposomes have a larger size (>50 nm) and are comprised of a phospholipid bilayer. While the liposome core usually contains water, other substances such as contrast agents, perfluorochemicals, drugs, quantum dots, or other agents may be included. In order to create a micelle or liposome-based contrast agent, the platform should incorporate a phospholipid containing a moiety capable of chelating gadolinium or a lipophilic gadolinium contrast agent. These intravascular contrast agents have long circulating time that allows enables adequate exposure to atherosclerosis and may also serve to determine luminal characteristics (Torchilin, 1997; Anelli et al. 2001).

Several studies describe the detection and characterization of atherosclerotic lesions using mixed micelles or liposomes in genetically modified mice. Briley-Saebo et al. 2006 and Mulder et al. 2006 have reported the employment of micelles and liposomes in an apolipoprotein E knockout (Apo E−/−) murine model of atherosclerosis to image the vessel wall. These data showed that the type of nanoparticles employed did not affect the in vivo MR efficacy with respect to uptake in the vessel wall of the mice and provided a significant enhancement of the vessel wall. Gadofluorine M, a contrast agent developed by Schering AG, has a tendency to form micelles in water and has been evaluated as an imaging agent to detect atherosclerotic plaques (Fig. 1). MRI on Watanabe heritable hyperlipidemic (WHHL) rabbits revealed that Gadofluorine M enhanced the imaging of atherosclerotic plaques (Sirol et al. 2004) and even enabled improved plaque detection of nonstenotic lesions that are not visible on unenhanced MRI (Barkhausen et al. 2003). A recent paper by Meding and colleagues elegantly demonstrated that following intravenous injection, Gadofluorine M micelles breakdown and bind to albumin. The Gadofluorine M is then carried into atherosclerotic plaque where it accumulates within the extracellular, fibrous parts of the plaque by binding to collagens, proteoglycans and tenascin but had little interaction with LDL and the lipid-rich plaque (Meding et al. 2007).

Although mixed micelles or liposomes demonstrated the capability to enhance the vessel wall, a strategy to target specific components present in the atherosclerotic lesion can be achieved by incorporating into the contrast agent platform antibodies or peptides that target ligands present in the plaque. This will increase the amount of contrast agent retained in the tissue and will further enhance the resolution of the images. Due to the flexibility of micelles and liposomes it is possible to incorporate modified phospholipids that possess moiety that will react via a covalent bond (amide bond, disulfide bond, or thioether bond), by a noncovalent linkage (avidin-biotin linkage) and by nonspecific surface adsorption with antibodies or peptides.

Selection of a target molecule that has much higher expression in atherosclerosis than in surrounding tissues remains a challenge. Since macrophages play an integral role and have elevated levels in plaque, targeted imaging of macrophages may enable improved imaging of atherosclerosis (Lipinski et al. 2006b). An example of targeted imaging of the macrophage was achieved by using micelles linked to antibodies against CD-204 (Lipinski et al. 2006a; Amirbekian et al. 2007), the macrophage scavenger receptor A (MSR-A). MSR-A is important in the progression of atherosclerosis (Suzuki et al. 1997) and is expressed at elevated levels in lesions (Takahashi et al. 2002). These data demonstrated that immunomicelles targeting CD-204 improved the detection and characterization...
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of atherosclerosis and the degree of signal enhancement correlated with macrophage density (Lipinski et al. 2006a; Amirbekian et al. 2007). Another example of targeted imaging was the use of nanoparticles linked to antibodies targeting \(\alpha_v\beta_3\)-integrin (Winter et al. 2003), which is associated with angiogenesis. As the vasa vasorum attempts to supply the growing atherosclerotic plaque, angiogenesis is increased compared with normal vessels (Moreno et al. 2006). Nanoparticles targeting \(\alpha_v\beta_3\)-integrin provided good quality images of the vessel wall in a rabbit model of atherosclerosis (Winter et al. 2003). In order to avoid the use of antibodies but keeping the same selectivity towards the targets, some peptides have been used to image atherosclerotic lesions. These molecules, with a length of less than 50 amino acids, mimic the function of the antibodies that target the selected tissue and can be easily synthesized. For example, 37pA is an amphiphatic peptide that mimics apolipoprotein A-I (ApoA-I), which plays a key role in the removal of excess cholesterol from peripheral tissues. It was recently demonstrated that immunonanoparticles containing a mimic peptide 37pA (Cormode et al. 2007) can also serve as a potential imaging agent.

Lipoproteins

Lipoproteins are endogenous macromolecular aggregates of lipids and proteins that are responsible for the transport of water insoluble nutrients through the vascular and extravascular spaces. Lipoproteins comprise a heterogeneous population of nanoparticles traditionally classified according to their density: chylomicrons, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL), and high density lipoproteins (HDL).

Although there are a large number of references of lipoproteins as contrast agents for imaging atherosclerotic lesions, there are only a few that employ MR imaging while the majority focus on nuclear imaging (Frias et al. 2007). Modified HDL has been used to image atherosclerotic mice (Frias et al. 2004; Frias et al. 2006). The results showed that the nanoparticles enhanced the vessel wall with a maximum of enhancement at 24 h (Fig. 2). Until the date and despite the potential of modified LDL as MRI agent for detection and characterization of atherosclerosis, no experiments in animals have been published (Mitsumori et al. 2004; Corbin et al. 2006).
Proteins and Polymers
Macromolecular gadolinium-based contrast agents have proved reliable as imaging agents at magnetic field strengths employed in clinical practice. Researchers have exploited this property by attaching covalently-linked contrast agents to proteins and polymers to amplify the signal intensity. This concept of using a strand of contrast agents is a commonly employed means of loading the carrier molecule with paramagnetic ions. Gustafsson et al. 2006 made use of the broad ligand specificity of the scavenger receptor class A (SR-A), a receptor highly expressed on macrophages, for a diverse array of polyanionic macromolecules by preparing a contrast agent based on maleylated bovine serum albumin (mal-BSA). Although they fully characterized the contrast agent and demonstrated in vivo macrophage accumulation, no studies were performed on animal models of atherosclerosis. Chaabane et al. 2004 used P717, a slow-clearance blood-pool agent (carboxymethyl-dextran derivative CMD-A2-Gd-DOTA, from Guerbet) in apolipoprotein E knockout mice. They demonstrated that the degree of signal enhancement and time course of P717 uptake varies with the staging of the atherosclerotic lesion. Therefore, modification of peptides or proteins through covalent attachment of Gd-DTPA or other paramagnetic ions may serve as another strategy to characterize atherosclerosis with either targeted or non-targeted molecular MRI.

Iron Oxide Particles
Iron oxide nanoparticles, which contain thousands of iron atoms surrounded by a dextran, starch or polymer coat, are clinically classified according to their size into superparamagnetic iron oxide (SPIO) particles (diameter >50 nm) and ultrasmall superparamagnetic iron oxide (USPIO) particles with a smaller hydrodynamic diameter (Corot et al. 2006). They result in signal loss on T2* imaging sequences and can therefore identify regions of uptake. SPIOs aggregate in vivo and are rapidly cleared from the bloodstream. Subsequent iron oxide nanoparticles have been synthesized with more extensive polymer coatings and remain monodisperse. The term monodisperse iron oxide (MION) is thus often applied to these agents. A highly stabilized and cross-linked derivative of MION, known as CLIO, has recently been developed for targeted molecular imaging applications. These nanoparticles are commonly taken up by mononuclear cells via the MAC-1 receptor (von zur Mulhen et al. 2006), enabling the nanoparticles to serve as an MR contrast agent that detects atherosclerosis through uptake by resident macrophages. Additionally, SPIOs and USPIOs enable imaging of other inflammatory disorders in which mononuclear cells play a major role. Ruehm et al. 2001 reported that after MR angiography of the thoracic aorta with conventional paramagnetic contrast agents that failed to reveal any abnormalities, administration of USPIO contrast agent revealed increased signal in the aortic lumen. A marked susceptibility effect became evident on day 4 within the aortic wall of the hyperlipidemic rabbits. Ex vivo imaging of aortic specimens confirmed the in vivo results. Multiple studies have demonstrated a role for USPIO imaging for predicting the degree of inflammation in carotid atherosclerosis (Trivedi et al. 2006, 2004a, 2004b, 2003; Tang et al. 2006, 2008; Kooi et al. 2003). These studies have shown correlation of signal alteration on T2*-weighted imaging signal and high-risk atherosclerotic plaques with regions of signal alterations closely correlating with macrophage density.

Targeted imaging with a USPIO-based contrast agent was accomplished by Kelly and colleagues by creating iron oxide nanoparticles coupled with a phage-display derived peptide (the VHSPNKK motif), a specific ligand of VCAM-1 and injected the contrast agent into cholesterol fed apoE−/− mice (Kelly et al. 2005). The contrast agent was found to have very high affinity for endothelial cells expressing VCAM-1 and enabled detection of extensive areas of neovascularization throughout the lesion. Impressive decreases in signal intensity associated with iron oxide nanoparticles accumulation were observed in atherosclerotic lesions, particularly in the aortic arch and bifurcation regions.

Figure 2. In vivo MRI of the abdominal aorta at 9.4 T in apo E−/− mouse after administration of gadolinium loaded HDL. With permission from Frias JC, et al. JACS 2004; 124: 16316–7.
of large vessels (Kelly et al. 2005). Recently, microparticles of iron oxide have also been linked to antibodies targeting VCAM-1 for detection of acute brain inflammation (McAteer et al. 2007) and atherosclerosis (McAteer et al. 2008).

Multimodal Contrast Agents

As previously mentioned, the main drawback of MRI contrast agents is the low inherent sensitivity. Therefore, several strategies have been devised in order to increase the signal intensity. Recently, a great deal of interest has been shown in the development of multimodal probes that combine several imaging modalities (MRI, optical, PET, ultrasound, CT, SPECT) (Frullano and Meade, 2007; Jaffer et al. 2007). The imaging group of Weissleder has made enormous progress developing this class of multimodal contrast agents which combine MRI with optical imaging. Based on a superparamagnetic iron oxide core coated with dextran, these nanoparticles are modified by adding a far-red fluorochrome for fluorescence detection. These magnetofluorescent nanoparticles are targeted with a linear peptide homologous to the integrin very late antigen-4 to detect VCAM-1 expression in vivo. Molecular imaging experiments detected proteolytic and osteogenic activity in early aortic valve disease (Aikawa et al. 2007).

Quantum dots (QDs) are nanocrystals of inorganic semiconductors that typically have a diameter of 2–10 nm and contain 200–10,000 atoms. These tiny light-emitting particles are emerging as a new class of fluorescent probe for in vivo biomolecular and cellular imaging due to the extreme brightness and resistance to photobleaching. They are robust fluorescence emitters with size-dependent emission wavelengths (Alivisatos et al. 2005; Gao et al. 2005). QDs have been coated with phospholipids that incorporate a paramagnetic complex for MRI of plaques and modified phospholipids for later attachment to antibodies or peptides. Mulder et al. 2007 used this approach in apo E−/− mice. The MR images demonstrated significant enhancement of the atherosclerotic plaques. The excised aortas were illuminated with UV-light in order to identify regions with a high contrast uptake. These regions were clearly identified by a green fluorescence originating from the QDs.
from the QDs and correlated with regions of enhancement on MRI.

**Cellular Trafficking**

Interest has arisen in loading of stem cells with MR contrast agents as this may serve as a realistic means of tracking the migration of these cells. Recently, SPIO-labeled mesenchymal stem cells were injected into a rat model of myocardial infarction and demonstrated localization of SPIO-labeled cells within the myocardial scar (Amsalem et al. 2007). This may also be applicable to atherosclerosis by tracking the migration of inflammatory cells into atherosclerotic plaque. SPIO-labeled stem cell trafficking with migration to atherosclerosis has been observed with Inversion-recovery with ON-resonant water suppression (IRON) sequences which generate positive contrast with iron oxide nanoparticles (Stuber et al. 2007). This enables detection of the contrast agent without the signal loss seen with T2* that may also arise from other sources, such as motion, tissue absence, or calcification. Additionally, in vivo trafficking of SPIO-loaded monocytes with migration into atherosclerotic plaques has been characterized with MRI (Litovsky et al. 2003).

**Conclusion**

The numerous means of modifying nanoparticles to improve penetration and retention enables the customization of contrast agents to improve MR imaging of atherosclerotic plaque. Techniques such as the addition of antibodies or peptides that provide specific targeting of plaque components may also serve as a tool in studying the complex cascade of events involved in atherosclerosis formation and aid the rapidly evolving field of vascular biology. Finally, multi-modality imaging contrast agents hold great promise in furthering plaque characterization but also risk assessment in patients with subclinical atherosclerosis.

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