Targeted Contrast Techniques

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Current imaging methods for atherosclerosis often focus on detecting luminal stenosis, but most clinical events occur in vessels that do not show any appreciable stenosis. Instead, plaques consisting of large lipid cores and thin fibrous caps, known as vulnerable plaques, are prone to rupture, forming thrombi that impede blood flow or embolize to downstream vessels. The vast majority of targeted contrast agents designed for cardiovascular applications are designed to bind to plaque features indicative of vulnerability, including lipid components, inflammatory cells, vascular markers and thrombi. Specific targeting of MRI contrast agents to biomarkers of disease, such as atherosclerosis, is often called molecular imaging.

Introduction

Targeted contrast agents are generally divided into two groups, superparamagnetic (such as iron oxide particles) and paramagnetic (such as gadolinium chelates). Superparamagnetic agents shorten the T2 relaxation time of water by inducing local magnetic field inhomogeneities. MRI is particularly sensitive to magnetic field disturbances, making these superparamagnetic particles an ideal choice for molecular imaging applications. Iron oxide nanoparticles can be coated with dextran, phospholipids, or other compounds to inhibit aggregation and enhance in vivo stability. The particles are typically 15-60 nm in diameter and exhibit a very long blood half-life (>24 hours). Iron oxides can be targeted to cellular receptors by conjugating targeting ligands to the surface or they can be entrapped in specific cell populations, such as macrophages or stem cells. As a T2 agent, iron oxide particles are typically detected as a dark spot by MRI (negative contrast), however a number of bright spot imaging techniques have recently been developed.[1-3]

Paramagnetic contrast agents, on the other hand, shorten the T1 relaxation time of water. The sensitivity of MRI techniques to conventional gadolinium chelates, however, is only on the order of micromolar concentrations, making them unsuitable for most molecular imaging applications. In order to overcome this limitation, contrast agents that carry numerous gadolinium ions have been developed, including liposomes, micelles, polymer particles, dendrimers and viral particles. Liposomes are uni- or multi-lamellar vesicles that consist of lipid bilayer membranes surrounding an aqueous interior and are typically 50-700 nm in diameter. Emulsions are similar to, but chemically distinct from liposomes. They consist of oil-in-water mixtures forming particles of 200-400 nm in diameter that are stabilized with surfactants. Micelles, such as high-density lipoprotein (HDL) or low-density lipoprotein (LDL) particles, are comprised of phospholipids with the hydrophobic groups sequestered in the particle core. Polymers can be formulated from one or multiple molecular components in linear, branched, or globular conformations ranging in size from 40-200 nm. Their size and shape can be tightly controlled, and the surface can be functionalized to bind a variety of targeting agents. Dendrimers are highly branched globular polymers that are sequentially assembled in covalent interactions. The branches provide a number of reactive sites for conjugating imaging agents or targeting ligands. Synthetic viral particles can be designed to self-assemble into a protein cage structure. These nanoparticles can be manipulated under certain chemical conditions to create pores that permit encapsulation of imaging agents. As a T1 agent, gadolinium chelates are typically detected as increased signal intensity by MRI (positive contrast).

While small molecule Gd chelates have relaxivity values of 4-5 $(ms*mM)^{-1}$, particulate carriers have relaxivities in the 15-40 $(ms*mM)^{-1}$ range, due to the slow tumbling of the particle. However, the relaxivity per particle is more indicative of the signal enhancement achieved per binding site and the large payload of Gd on the particle surface allows particulate relaxivities to reach more than 1,000,000 $(ms*mM)^{-1}$, allowing detection of picomolar concentrations of

targeted particles per imaging voxel. This feature has allowed sensitive detection epitopes as diverse as integrins in plaque angiogenesis, fibrin in disrupted lesions, macrophage scavenger receptors, interstitial collagen exposed by angioplasty in the media of disrupted vessels, and other targets.[4,5]

Applications

The macrophage is the most prominent inflammatory cell type in atherosclerotic lesions. Targeted agents have been designed to label macrophages directly, or by binding to surface receptors, such as VCAM-1, E-selectin, scavenger receptors and others. Uptake of iron oxide particles has been shown to be more prevalent in vulnerable plaques (75%) compared with stable lesions (7%).[6] MRI has shown a very high sensitivity (92.5%) and a moderate specificity (64%) for detecting uptake of iron oxide, using histology as a gold standard.[7] Typically, the clinical dose of iron particles is 2.6 mg Fe/kg which leads to the maximal signal change at 24-36 hours post infusion and loss of all detectable signal by 72 hours.[6,8] In animal studies, a much higher dose of iron oxide is typically used (~20 times higher), which leads to identification of particle uptake only after the blood pool signal has decreased, ~4 days post infusion.[9]

Several iron oxide particles have been targeted, through the attachment of an affinity ligand, to important processes in atherosclerotic plaques. The expression of VCAM-1 on the endothelium is an early event in the atherosclerotic process and has been successfully imaged in the ApoE-/- mouse in vivo with a targeted iron particle. The therapeutic effect of statin therapy on VCAM-1 expression in vivo could also be imaged with this probe. Iron particles have also been conjugated to annexin and used to image myocardial apoptosis in vivo. An iron oxide particle conjugated to the RGD peptide has also been demonstrated for imaging plaque angiogenesis through detection of integrin expression.[10]

The earliest hallmark of plaque rupture is fibrin deposition. The diagnosis of disrupted plaque by detection of small deposits of fibrin in erosions or microfractures could allow characterization of a potential "culprit" lesion before a high-grade stenosis has been formed that is detectable by traditional imaging methods, such as cardiac catheterization. Specific imaging of fibrin has been demonstrated with paramagnetic MR contrast agents. Perfluorocarbon nanoparticles bearing tens of thousands of Gd chelates have been targeted to thrombi utilizing antibodies that are highly specific for cross-linked fibrin peptide domains. This agent provides ample signal enhancement to detect vascular clots in animal models using clinical imaging equipment.[11] In a similar fashion, Epix Pharmaceuticals has developed a fibrin-specific contrast agent named EP-2104R. Clinical trials are currently assessing this agent for molecular MRI of thrombosis in patients at 3.0 T. Early results of these trials indicates that the agent provides selective and high-contrast visualization of thrombi inside the chambers of the heart and attached to the aortic or carotid wall.[12] The detection of thrombi may be of high clinical impact, especially in patients with embolic stroke and unknown origin of embolism.

Expression of integrins on vascular endothelial cells is a general marker of angiogenesis, which plays an important role in a wide variety of disease states, including atherosclerosis. Integrins play a critical part in smooth muscle cell migration and cellular adhesion, both of which are required for the formation of new blood vessels. Angiogenesis plays a critical role in plaque growth and rupture. In regions of atherosclerotic lesions, angiogenic vessels proliferate from the vasa vasorum to meet the high metabolic demands of plaque growth. Integrin–targeted perfluorocarbon nanoparticles have been demonstrated for the detection and characterization of angiogenesis associated with atherosclerosis.[11] Integrin targeted paramagnetic nanoparticles produced significant enhancement of the atherosclerotic lesions in cholesterol-fed rabbits. Animals on a control diet exhibited no increased signal and background was minimal. Integrin expression in the adventitia was confirmed by colocalized histological staining of integrin and platelet endothelial cell adhesion molecule, which is a general endothelial marker.

Recombinant paramagnetic HDL-like particles have also been shown to enhance atherosclerotic plaques in apolipoprotein E-deficient mice.[13] These particles are formed through the delipidation of isolated human HDL particles, followed by reconstitution with phospholipids

including a phospholipid conjugated to Gd-DTPA for signal enhancement. Typically, 15 to 20 gadolinium chelates can be incorporated onto each 9 nm particle. These particles are preferentially taken up by the atherosclerotic plaques and produce signal enhancement in animal models of atherosclerosis.

In some cases, these same particles designed for molecular imaging applications have been used to deliver therapeutic agents.[14,15] The combination of biomarker imaging and drug delivery allows monitoring of drug efficacy for inhibition of angiogenesis, post-angioplasty restenosis, fibrinolysis, etc.[16]

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