MR Molecular Imaging of Atherosclerosis

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Introduction

Magnetic resonance (MR) molecular imaging aims to identify and map the expression of important biomarkers on a cellular scale. Typical MR techniques lack sufficient resolution and sensitivity to directly detect these molecular signatures due to their very low concentrations in vivo. Important metabolites and biomolecules, such as glucose, adenosine triphosphate (ATP), lactate and others, can be detected through MR spectroscopy, but in vivo mapping of these compounds is not widely available for routine biomedical applications due to the need for specialized instrumentation or very high concentrations. Instead, MR molecular imaging relies on the development of a new class of contrast agents, which are specifically targeted to the biomarker of interest and are capable of amplifying the signal enhancement to generate sufficient image contrast. These contrast agents often take the form of nanoparticle constructs, which offer a large surface area for the incorporation of multiple binding ligands (to improve targeting efficacy) and multiple paramagnetic chelates (to amplify the signal enhancement). In some cases, the nanoparticle may also serve as a drug delivery agent, providing diagnostic and therapeutic information via noninvasive MR imaging.

A number of other medical imaging modalities have been employed for molecular imaging, including PET, SPECT and optical imaging [1-3]. Like MR imaging, these modalities typically rely on specifically designed contrast agents that bind to the molecular biomarker of interest. For PET and SPECT imaging, the contrast agents consist of a radioactive element for detection. In optical imaging, the contrast agent will utilize fluorescence or bioluminescence for mapping expression of cellular receptors. MR imaging, however, has certain important advantages over these other modalities for molecular imaging applications. MR imaging offers higher spatial and temporal resolution than PET or SPECT and provides greater tissue penetration than optical imaging. This allows MR imaging to combine anatomical and/or functional information with molecular imaging, much like the multimodality benefits of PET/CT. In addition, MR imaging may be better suited for serial tracking of disease progression or therapeutic response than the nuclear imaging methods due to concerns of cumulative radiation dose.

Paramagnetic and Superparamagnetic Contrast Agents

Typically, molecular imaging contrast agents are based on either paramagnetic gadolium chelates or superparamagnetic iron oxide particles [4]. Gadolinium agents have been grafted onto targeting molecules, such as antibodies or peptides, to directly bind to important biomarkers of disease, such as fibrin. One of the hallmarks of a ruptured atherosclerotic plaque is the accumulation of fibrin-rich thrombi in the vessel lumen. Identifying and localizing ruptured plaques would have an enormous benefit in the clinical diagnosis and treatment of myocardial infarction and stroke. Phage display has been used to develop a novel peptide that specifically binds to fibrin. This ligand was modified with four Gd-DTPA chelates per peptide to generate signal enhancement on MR images. The abundance of fibrin in clots allows imaging thrombi in the left atrium, pulmonary arteries, and coronary arteries in animal models [5-11] and in clinical patients [12].

Another biomarker of clinical importance that has been pursued with paramagnetic gadolinium contrast agents is the uptake of lipoproteins within atherosclerotic plaques. Recombinant paramagnetic HDL particles were used to image atherosclerotic regions in apoE-deficient mice. The HDL particles are formulated with 15-20 Gd-DTPA chelates per particle to provide ample MR signal enhancement [13]. This agent has also been coupled to a macrophage scavenger receptor antibody for specific imaging of macrophages infiltrating the plaques [14]. Another contrast agent that selectively accumulates in fatty deposits of atherosclerotic plaques is gadofluorine. This gadolinum chelate contains fluorinated side chains that are hydrophobic. The side chains cause gadofluorine to form nanometer sized micelles when dispersed in water. These small lipophilic particles have been shown to preferentially accumulate in the lipid-rich areas of vascular plaques in cholesterol-fed rabbits [15].

The therapeutic uses of stem cells have become a rapidly growing field of research. A number of new MR contrast agents and methods have been developed with the aim of tracking the delivery, migration, viability and fate of labeled stem cells. Iron oxide contrast agents have dominated cell tracking studies [16-19]. In most ceases, cultured stem cells are labeled in vitro with iron oxide particles. Cell

labeling is often accomplished with exogenous transfection agents that induce uptake of the particles by endocytosis. The labeled cells are subsequently injected into the tissue of interest and detected in the MR image based on the loss of signal due to enhancement of T2* relaxation. The use of iron oxide particles provides abundant image contrast, which allows detection of even a single labeled cell [20].

In addition to in vitro labeling of stem cells, iron oxide particles have been utilized for in vivo imaging of macrophages associated with atherosclerotic plaques. Activated macrophages spontaneously uptake particulate contrast agents by phagocytosis in animal models [21-24] and clinical atherosclerosis [25-26]. One limitation of MR imaging after systemic injection of iron oxide contrast agents is that they stay in the blood pool for a long time, requiring a delay between injection and imaging on the order of hours to days. Clinical imaging of carotid plaques required 24 hours between injection and imaging, which resulted in a 24% decrease in the image intensity of the plaques [25]. The negative image contrast associated with iron oxide particles has been considered a drawback for these methods. As an alternative approach, new pulse sequences and image processing routines have been developed to generate positive contrast in the MR images [27-34]. For example, a method called "Inversion-recovery with ON-resonant water suppression", abbreviated as IRON, has been demonstrated to generate bright image contrast that was correlated with the number of labeled cells [33].

Another novel application of iron oxide particles is the construction of magnetic relaxation switches that change their MR relaxation properties based on interactions with biomolecules associated with various disease states [35]. For example, particles have been formulated with numerous copies of high-affinity ligands that bind myeloperoxidase, an enzyme produced by inflammatory cells that is associated with plaque vulnerability [36]. In another cardiovascular application, iron oxide agents conjugated to a2AP peptides have been used to detect activated Factor 13, an enzyme that crosslinks fibrin and stabilizes clots [37].

Molecular Imaging and Targeted Drug Delivery

Paramagnetic nanoparticles that bind to the $\alpha_v\beta_3$-integrin, a biomarker of angiogenesis, have been produced by incorporating a highly specific targeting ligand onto the surface of perfluorocarbon (PFC) nanoparticles. This agent carries 94,000 gadolinium chelates per particle, generating longitudinal relaxivities (relative to the concentration of nanoparticles) of 1,690,000 $(s*mM)^{-1}$ at 1.5T and 910,000 $(s*mM)^{-1}$ at 4.7T [38]. This extremely high relaxivity results in a minimum detection limit, defined as the concentration of particles required to generate a contrast to noise ratio equal to 5, of 113 pM, which is within the range of the biological abundance of many important biomarkers. The development of atherosclerotic plaques requires active angiogenesis to meet the high metabolic demands of the abnormal tissue. In atherosclerosis, angiogenesis occurs in the vasa vasorum of large caliber arteries, such as the aorta, carotids and coronaries. Atherosclerotic rabbits were injected with either $\alpha_v\beta_3$-targeted or nontargeted paramagnetic nanoparticles at a dose of 1 mL/kg (0.0046 mmol Gd^{3+}/kg) [39]. As a point of reference, the standard dose of conventional gadolinium agents for clinical MR scans is 0.1 mmol Gd^{3+}/kg, which is 20-fold higher than the dose delivered with paramagnetic nanoparticles. MR imaging of the abdominal aorta showed higher signal enhancement following injection of the targeted nanoparticles compared to animals receiving the nontargeted nanoparticles. MR signal enhancement generally was highest in the proximal aorta segments, which typically displayed an increased burden of atherosclerotic plaques by histopathology.

Combining a molecularly targeted imaging agent with a therapeutic drug could provide a range of benefits in the clinical management of patients with cardiovascular disease. The imaging agent would allow confirmation and quantification of local drug delivery, and enable personalization of treatment protocols based on the pharmacokinetics in the target tissue. In addition, specific targeting of the agent could improve uptake and retention of the drug at the sites of disease while lowering the exposure to other susceptible organs and reducing the occurrence of side effects. The acute therapeutic response to an anti-angiogenic drug was studied in atherosclerotic rabbits treated with $\alpha_v\beta_3$-targeted paramagnetic nanoparticles containing the drug fumagillin [40]. MR imaging was performed on a 1.5 T clinical scanner to estimate drug deposition in the aortic wall. One week later, the level of neovascular $\alpha_v\beta_3$-integrin expression was assessed using $\alpha_v\beta_3$-targeted paramagnetic nanoparticles without fumagillin. Aortic areas displaying high MR enhancement at the time of treatment had the largest subsequent reduction in $\alpha_v\beta_3$-targeted MR signal 1 week later, suggesting that combining imaging with therapy may not only confirm

and quantify the local delivery of chemotherapeutics but may also provide early predictions of the subsequent treatment effects.

In addition to the acute response to therapy, MR molecular imaging could provide serial monitoring of the end organ effects with the ultimate goal of optimizing drug treatment regiments and customizing patient protocols. The anti-angiogenic effect of atorvastatin with and without targeted delivery of fumagillin was serially monitored for eight weeks by MR imaging in atherosclerotic rabbits [41]. Atorvastatin was dosed continuously via the animal's feed, while fumagillin treatment was provided once every four weeks. Rabbits were imaged on a clinical 1.5T scanner before and three hours post injection of $\alpha_v\beta_3$-targeted nanoparticles. Histology revealed that the image enhancement was strongly correlated to the microvascular density in the aortic wall in a logarithmic fashion. During eight weeks of study, atorvastatin treatment did not reduce $\alpha_v\beta_3$-integrin expression in the aortic wall. Fumagillin treatment, on the other hand, resulted in a transient (2-3 week) reduction in image enhancement, indicating successful anti-angiogenic treatment. Combining the fumagillin and atorvastatin treatments yielded a persistent decrease in the MR signal, suggesting that chronic statin treatment could be used to prolong the effects of discrete doses of an anti-angiogenic agent. An effective and sustained anti-angiogenic treatment may lead to stabilization of atherosclerotic plaques due to decreased intramural hemorrhage.

Direct Detection of MR Contrast Agents

While changes in the proton signal intensity can be used to detect contrast agent uptake, this is an indirect measure of the contrast agent. An alternative method is direct detection of a signal originating from the agent itself, much as PET, SPECT and optical imaging directly map a nuclear or fluorescent tag incorporated onto the agent. There are a number of important nuclei that are visible by MR techniques, such as ^{19}F, [23]Na, ^{31}P, and ^{13}C, however, the sensitivity of these elements tend to be quite low. Typical clinical MR imaging utilizes the proton (^{1}H) signal, which represents a concentration of 110M and a relative MR sensitivity of 1.0. In comparison, the relative sensitivities of ^{19}F, [23]Na, ^{31}P, and ^{13}C are 0.83, 0.093, 0.066 and 0.016, respectively. Furthermore, the concentrations of these elements tend to be very low in biological samples, typically less than 10mM, lowering their sensitivity by orders of magnitude compared to proton.

[19]F MR of Perfluorocarbon Nanoparticles

Direct detection of ^{19}F has been explored in a number of research studies because it has a relatively high sensitivity, 83% compared to ^{1}H, and there is virtually no native background signal. Thus, fluorinated contrast agents can provide a definitive and quantitative MR signature. For example, ^{19}F MR imaging of fibrin-targeted PFC nanoparticles could be used to map the formation of thrombi on ruptured atherosclerotic plaques and quantify the extent of ruptures in the fibrous cap. Human carotid endarterectomy samples were treated with fibrin-targeted paramagnetic nanoparticles and imaged at 4.7 T [42]. [1]H MR imaging showed high levels of signal enhancement along the luminal surface due to nanoparticle binding to fibrin deposits. A ^{19}F projection image of the artery, acquired in less than five minutes, displayed an asymmetric distribution of nanoparticles around the vessel wall corroborating the ^{1}H signal enhancement. Spectroscopic quantification of nanoparticle binding allowed calibration of the ^{19}F MR signal intensity. Co-registration of the quantitative nanoparticle map with the ^{1}H image permitted visualization of both anatomical and pathological information in a single image. Combining information from ^{1}H and ^{19}F MR imaging could allow prediction of subsequent occlusion or distal embolization from unstable or disrupted plaques, and aid clinical decision-making for acute invasive intervention vs. pharmaceutical therapies.

[19]F MR imaging is able to independently detect two different populations of PFC nanoparticles formulated with perfluorooctylbromide (PFOB) or perfluoro-15-crown-5-ether (CE) based on their unique spectral signatures [43]. Both imaging and spectroscopy could distinguish nanoparticles containing either PFOB or CE as the core material. The signal to noise for PFOB was lower than CE (10 vs. 25, respectively), presumably due to the single CE peak (20 equivalent fluorine atoms) compared to the multiple PFOB peaks (17 fluorine atoms distributed over 5 peaks). A clear linear relationship between the ^{19}F signal intensity and PFC concentration was found for both PFOB and CE using both imaging and spectroscopy. From this demonstration on fibrin clots, it follows that multiple PFC nanoparticle agents could be used to target different epitopes and achieve a noninvasive analogy to immunohistochemistry. For example, simultaneous quantification of angiogenesis in the vessel wall and fibrin deposition on the plaque cap could be used to evaluate the pathophysiological stage of an obstructive lesion.

Angiogenesis is also a prominent feature in the progression of aortic valve stenosis. Cholesterol-fed rabbits develop aortic valve sclerosis, characterized by gross thickening, macrophage infiltration, calcification, and eventual bone formation that mimics the clinical presentation of the disease [44-47]. Cholesterol-fed rabbits underwent ^{19}F MR imaging after injection of $\alpha_v\beta_3$-targeted nanoparticles to quantify angiogenesis in the aortic valve leaflets [48]. The cholesterol feeding caused gross thickening of the aortic valves accompanied by extensive foam cell infiltration, non-calcified bone deposition, activation of myofibroblasts, abnormal microvascular proliferation and upregulation of $\alpha_v\beta_3$-integrin expression. None of these abnormalities were observed in the normal valve tissue from control animals. Rabbits received IV injections of $\alpha_v\beta_3$-targeted nanoparticles, nontargeted nanoparticles or in vivo competitive inhibition of $\alpha_v\beta_3$-integrin binding via pretreatment with $\alpha_v\beta_3$-targeted safflower oil nanoparticles. Two hours after nanoparticle injection, the aortic valve leaflets were excised for ^{19}F MR spectroscopy at 11.7T.

The CE signal arising from the nanoparticle contrast agent was readily detected and distinguished from a PFOB quantification reference based on the chemical shifts of these PFC species, allowing quantification of the total volume of bound nanoparticles. The volume of targeted nanoparticles bound to the valves was 19.5 nL, which was more than three times higher than the amount of nontargeted nanoparticles (5.6 nL). Competitive inhibition of $\alpha_v\beta_3$-integrin binding reduced the amount of nanoparticles in the valves by about half (10.3 nL). Valves from healthy rabbits treated with targeted nanoparticles contained almost nine times fewer nanoparticles (2.3 nL) than the valves from cholesterol-fed rabbits. These techniques may be useful for assessing atherosclerotic components of preclinical aortic valve disease in patients and could assist in defining efficacy of medical therapies. The sensitivity of this approach for molecular detection of sparse quantities of inflammatory epitopes in very thin structures at high field strengths establishes a basis for future efforts to develop localized spectroscopic methods at clinical field strengths that could be useful for detecting disease and monitoring therapies.

Quantitation of Metabolic Flux

Another important physiological marker of disease is metabolic flux rates. For example, the creatine kinase (CK) reaction is responsible for replenishing adenosine triphosphate (ATP) by using phosphocreatine (PCr). Quantitative ^{31}P spectroscopy studies have shown significant reductions in cardiac PCr and ATP concentrations in MI patients compared to healthy controls [49]. However, these measurements do not distinguish between cell death, reduced substrate availability or impaired enzyme activity. MR spectroscopy can be used to measure the rate of metabolic reactions by tracing the exchange of saturated spins from one molecule to another. The pseudo-first-order CK rate constant, k, reflects the intracellular CK reaction kinetics and is independent of myocyte number, while CK flux is defined as the product of [PCr] and k. The value of k can be interpreted as the fraction of the PCr pool used to create ATP via the CK reaction each second, which is a measure of intracellular metabolic function. Therefore, k depends only on the surviving cells that contribute to the ^{31}P MRS signal and is not confounded by myocyte loss. On the other hand, reduced myocardial CK flux can be due to a loss of total enzyme activity, altered intracellular substrate levels, or allosteric modifications of the enzyme.

In a clinical study of myocardial infarction patients, the CK kinetics were measured noninvasively using ^{31}P spectroscopy. The tissue concentrations of ATP and PCR were measured and CK kinetics (k and CK flux) were measured by magnetization transfer on a 1.5T clinical scanner [50]. Myocardial [ATP] and [PCr] were 39% to 44% lower in MI patients compared to healthy controls, however the myocardial CK rate constant, k, was normal in these patients. As a result of the lower tissue PCr levels, the CK flux was reduced by 50% in the MI patient population. These results demonstrate that ATP loss following MI is a direct result of PCr depletion, most likely due to myocyte loss. The maintenance of normal k values indicates that intracellular CK metabolism is maintained in the surviving myocytes. These results reinforce the use of therapies for MI patients that combat substrate loss or reduce energy demand, rather than those that increase workload in the surviving tissue. For example, beta blockers are routinely prescribed for MI patients because they reduce the heart rate and myocardial oxygen consumption. Using similar ^{31}P spectroscopy techniques, a 50% reduction in CK flux has been measured in patients with non-ischemic dilated cardiomyopathy and mild-to-moderate chronic heart failure (CHF) [51] and a 65% decrease in CK flux has been reported in patients with pressure-overload left ventricular hypertrophy and CHF [52].

Contrast-Enhanced MR Imaging of Inflammation

Serial contrast-enhanced cardiac magnetic resonance (CE-CMR) was used to characterize inflammation in the coronary vessel wall of patients after acute myocardial infarction (AMI) [53].

Inflammation plays a key role in the development of atherosclerosis [54] and is closely linked to plaque rupture, the underlying cause of myocardial infarctions and strokes [55]. Plaque vulnerability is determined more by the plaque composition and amount of inflammation rather than the degree of luminal narrowing [56]. Clinical MR contrast agents nonspecifically distribute throughout the extracellular space, but the amount of leakage out of the capillary bed is dependent upon the permeability and the surface area of the vasculature. Inflammation causes increased vascular permeability, resulting in increased extravasation of the contrast agent. CE-CMR has been used to characterize acute inflammation within the vessel wall in giant cell arteritis and Takayasu's arteritis [57-58]. CE-CMR of the coronary artery wall was performed in 10 patients with AMI 6 days and 3 months after coronary intervention and in 9 volunteers without coronary artery disease [53]. All subjects were imaged on a 1.5T scanner 30-40 minutes after an IV bolus of 0.2 mmol/kg of Gd-DTPA. Contrast enhancement within the coronary wall was calculated based on the contrast-to-noise ratio (CNR) between the image intensity of the coronary wall and the blood signal in the aorta. Noise was measured in a region of interest placed outside the chest wall.

Six days after AMI, the image enhancement in the coronary wall averaged 7.8, which was significantly higher than the enhancement observed in normal subjects, 5.3. Coronary vessel enhancement was highly correlated with the angiographic severity of lumen narrowing. Signal enhancement in the stenotic coronary artery segments, defined as greater than 25% luminal narrowing via x-ray angiography, was significantly higher (CNR = 10.9) than the enhancement measured in the nonstenotic segments (CNR = 6.4). Three months after AMI, the average CNR in the coronary artery wall was 6.5, a significant decrease compared to the value 6 days after AMI. This reduction in average CNR was caused by a decrease in the CNR from the stenotic vessels, CNR = 6.8, while the CNR in the angiographically normal segments did not change between the acute and chronic phase of infarction. The spatial extent of hyperenhanced segments, vessels displaying CNR values above 9.7, decreased from 70% at six days to 25% at three months post AMI. Mirroring the results from CE-CMR, the levels of a general inflammatory marker, C-reactive protein, were significantly higher at six days post AMI compared to the three month timepoint, 2.6 vs. 0.8 mg/dl, respectively. The observed image enhancement pattern during the post-infarction period may be associated with transient inflammation or edema in the pathologically altered coronary vessel wall. Serial CE-CMR could be used to quantify the spatial extent and intensity of coronary inflammation in patients after AMI. Further studies will be required to determine the utility of this approach to predict clinical events or monitor the response to therapeutic interventions. Some therapies designed to increase angiogenesis in the ischemic regions may also significantly increase vascular permeability and dramatically increase CE-CMR image enhancement. Under these conditions, CE-CMR may not accurately reflect local inflammation, but rather the combined physiological effects of both the disease as well as the therapy.

Conclusions

MR molecular imaging encompasses a wide range of developing technologies, such as site-targeted contrast agents, drug delivery vehicles, activatable MR probes and direct mapping of tissue metabolites. As demonstrated by the research studies cited in this review, the clinical application of these techniques could offer far reaching benefits to patient populations, including early detection of therapeutic response, localizing ruptured atherosclerotic plaques, stratifying patients based on biochemical disease markers, tissue-specific drug delivery, confirmation and quantification of end-organ drug uptake, and noninvasive monitoring of disease recurrence. In particular, molecular imaging with PFC nanoparticle agents has demonstrated a number of applications in animal models of cardiovascular disease. The ability to combine ^{1}H and ^{19}F MR imaging offers anatomical localization as well as definitive and quantitative mapping of nanoparticle uptake. Utilizing the nanoparticle platform has proven to be highly flexible, enabling selection of various contrast mechanisms, targeting ligands or therapeutic drugs based on the requirements of the specific application. Eventually, such agents may play a leading role in reducing the human burden of cardiovascular disease, by providing early diagnosis, noninvasive monitoring and effective therapy with reduced side effects.

Further Reading

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