Molecular Targeted Functional, Cellular and Molecular Imaging of Atherosclerosis With Antibody-Conjugated Superparamagnetic Particles Using Magnetic Resonance

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ABSTRACT

Background: Magnetic Resonance Imaging (MRI) is a powerful non-invasive technique that provides high-resolution anatomical images of biological tissue based on their inherent physio-chemical properties. The signal from MRI can be modulated by contrast agents, which can be specifically directed to cells and molecules of interest, thereby providing an opportunity for targeted imaging.
Aims: We propose to extend previous work that has used very high-resolution MRI to characterize atherosclerotic plaque in mice. Through targeted imaging of cells and molecules involved in mouse atherosclerosis, pathological processes and their response to interventions can be imaged in vivo. Aim 1 was to develop and refine gadolinium-apoferritin nano-particles that have shown great potential as relaxivity agents for MR contrast studies. Aim 2 was image antibody-conjugated iron-oxide micro-beads (0.9 μm)). Aim 3 was applied using these imaging strategies using high field strength MRI (11.7 T/21 T) to interrogate processes of inflammation and thrombosis in vascular models. Targeting can be accomplished with established and novel antibodies developed by phage display (against active forms of fibrin and glycoprotein IIbIIIa).
Methods: Molecular imaging probes were synthesized and characterized in vitro. Arterial injury was associated with a sequence of molecular and cellular events that result in intimal hyperplasia and, in the apolipoprotein E-deficient mouse, results in accelerated atherosclerosis.
Results: This model exhibited molecular imaging targets whose spatial and temporal expression was established. These features, in conjunction with straight, superficial course of the femoral artery makes it an ideal imaging target to examine the pathology of atherothrombosis non-invasively in vivo and to measure the effects of interventions.
Conclusion: The approach of molecular imaging by using antibody-conjugated superparamagnetic contrast agents may provide unique opportunity of microimaging in clinical set up.

Keywords: MRI, molecular imaging, contrast agents, atherosclerosis, thrombosis, mice

1 INTRODUCTION

Magnetic resonance imaging is well placed to address these deficiencies since it can provide direct (non-angiographic), non-invasive images of arteries in multiple vascular territories. The objectives of this approach are to use MRI at high field strength (11.7 T – 21 T) ultrawide bore magnet with biologically targeted contrast probes that will allow cells and molecules of interest to be imaged. By combining molecular imaging with novel activation-specific epitopes of fibrin provides an approach of developing functional imaging technique. Magnetic resonance Imaging (MRI) is non-invasive and free from ionizing radiation. It can provide high-resolution images of multiple vascular territories. Using a combination of T1 weighted (T1 w), T2-weighted (T2 w) and proton density weighted images multiple MRI contrast determines both plaque anatomy and composition in experimental animals. In recent years the use of genetically-modified mice has brought a dramatic expansion in the appreciation of the molecular and cellular events of atherogenesis. It requires their determination in vivo through sophisticated imaging techniques, including the targeted imaging of cells and molecules. Targeted imaging techniques proposed here may be useful in the diagnosis and provide much more precise disease characterization that will guide therapeutic efficacy and prognosis. Targeted imaging of molecules and cells requires signal enhancement through the use of superparamagnetic contrast agent. It identifies the target with the high specificity and provides sufficiently intense signal enhancement within the imaged volume to distinguish it from unenhanced tissue. For example, specificity for the target can be achieved through binding of monoclonal antibodies or their immunospecific fragments F(ab)' peptides or, small molecule peptidomimetics. For optimal contrast delivery it is necessary to synthesize nonoparticles that combine target specificity with the ability to carry a substantial payload of a superparamagnetic agent.

2 THEORY OF CONTRAST

In present report, we describe the principle of gadolinium-ferritin proteins as source of contrast agents with preliminary data and highlight the ultrahigh resolution achieved using MRI microimaging technique.

2.1 Apoferritins as contrast agents-

The chelates increase the relaxivity of water protons on proteins in their vicinity. The effects of contrast agents on relaxivity accrue from water protons on the surfaces of protein in proximity to the GdIII chelate. Importantly for MR contrast, small (0.3 nm) pores in the apoferritin shell allow free diffusion of water. The relaxivity property of the apoferritin-GdHPDO3A is 20 times greater than the

corresponding free GdHPDO3A in water, representing the strongest relaxivity agent.

The ferritins are large multi-subunit proteins that are widely distributed across species. These play a role in the transportation and regulation iron which is carried within the hollow interior core of the ferritin molecule [1]. When the ambient pH is lowered to 2, multimeric apoferritin dissociates. Increasing the pH to 7 causes re-association of the subunits through which it is possible to trap small molecules that are in the free solution. Using this property of apoferritin, Aime and colleagues have recently demonstrated that the neutral gadolinium (GdIII) chelate Gadoteridol (GdHPDO3A), Prohance, Bracko, Milan) can be incorporated in to the core of apoferritin with formation of a stable molecule [2]. Based on the concentration of GdHPDO3A in solution and the volume of the apoferritin cavity, we calculate that each molecule will encapsulate ~ 10 GdHPDO3A. Winter et al. have demonstrated contrast enhancement using per fluorocarbon nanoparticles of 200 nm diameter, which carry a payload of ~ 90000 gadolinium ions, equivalent to 1 Gd per 40 nm [3]. Although individual particles are smaller, apoferritin is predicted to convey a comparable quantity of gadolinium (1 Gd per 100 nm3) when bound. Using these small particles with very high relaxivity effects we hope to overcome some of the steric limitations imposed by 200 nm particles. We anticipate that the much smaller apoferritin particles will deliver a greater effective payload to sites of interest.

2.2 Resolution and contrast enhancement with example of coronary artery imaging-

2.2.1 Selection of TE and TR for optimizing contrast between specific components of coronary artery- The atheromatous core and the fibrous cap of injured femoral artery will be discriminated by varying TE(echo time) or TR(repetition time) scan parameters at a time and keeping constant TE or TR value. Using optimized TE and TR parameters, T2-w imaging can generate good contrast between lipid rich core (dark) and fibrocellular tissue (bright) to distinguish atheromatous core and fibrous tissue better than T1-w sequences.

T1-weighted techniques provide a much higher MRI signal than T2-weighted sequences and define atheroma better than T2-weighted method. Hemorrhage is hyperintense to fibrous tissue on T1-weighted sequences but it is hypointense on T2-weighted sequences. We anticipate that different MRI signal intensities may visualize old vs new thrombus tissues.

3 MATERIALS AND METHODS-

3.1 Magnetic Resonance microimaging (MRIm) technique- Fresh excised artery tissues with resealed slits and calibration markers were placed in a 1.5 ml plastic culture tubes containing PBS buffer. The tube cap will be tightened down to exactly 1 mm so as to fit snugly in the imaging probe head of a 11.7 T and 21 T superconducting magnet (89 mm bore). Transverse slices of 0.5 mm thickness will be obtained using a select gradient of 4.65 G/cm and readout gradient of 2.40 G/cm. Image contrast will be typically optimized at TE=30 ms; TR=1500 ms; a 512 x 256 matrix is zero-filled to 2048 x 2048; Number of averages=128. Images will be rendered using VNMR software and an Epson printer, and compared to histologic images by using ImagePro software.

3.2 Resolution enhancement- Pixel resolution was achieved 256 x 256 matrix size with slice thickness 0.4 mm to get 20 microns ultrahigh spatial resolution and 0.5 mm^3 volume of interest.

3.3 Preparation of Gadolinium-apoferritin complexes- Gadolinium loaded equine apoferirtin (Sigma-Aldrich, Gillingham, UK) were prepared by lowering the pH of a 1 x 10-5 M protein solution in the presence of 0.1 M GdHPDO3A (Prohance, Bracco, Milan) and the fluorophore, neutral red (540/640 nm) as part of experiment at animal Columbia University imaging facility by Dr Ed X Wu . Free Gadoteridol and fluorophore was removed by exhaustive dialysis and the apoferritin centrifuged to remove any aggregates and quantified by protein assay.

4 RESULTS

In preliminary experiments, pH drop and monomerization was associated with marked loss of turbidity of apoferritin solution. After pH elevation the solution again became turbid. Following dialysis to remove GdHPDO3A that was free in solution, the apoferritin was introduced to an MR phantom. Figure 1 shows the T1 maps of apoferritin (alone) (1030 $\pm$ 20 ms),

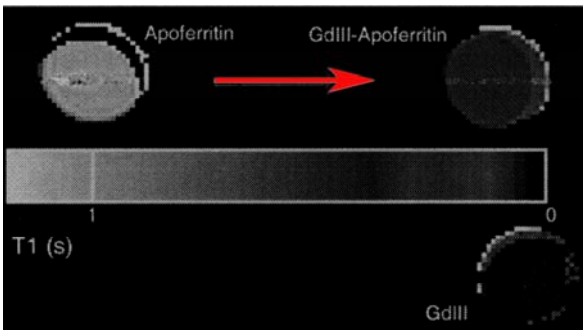

Figure 1: Quantitative T1 maps with pseudocolor obtained from solutions imaged in glass MR tubes. The tubes demonstrate a marked T1 shortening effect by loading GdHPDO3A (Prohance) into apoferritin. The samples on lower right contains 100 mM Prohanceonly. After loading, the apoferritin will be extensively dialysed to remove free Prohance from solution, ensuring that the observed increase in relaxivity will be due to GDIII-apoferritin complexes.

GdII-apoferritin (75.5 ± 1.3 ms) and 100 mM GdHPDO3A (17.5 ± 2.1 ms).

The apoferritin proves to be useful contrast vehicle, it can be synthesized it through recombinant techniques and furthermore to incorporate a fluorescent tag to aid histological analysis. Ultrahigh resolution MRI imager at 21 Tesla magnetic field strength provides microimaging details better than other imager at 12 Tesla strength. The pixel resolution can be achieved up to 15 micron including vascular tissues. At center of Nanomagnetics and Biotechnology, paramagnetic particles using antibody-conjugated contrast agents are prepared. The following image was acquired using myoglobin antibody MION particles to highlight the ultrahigh resolution as shown in Figure 2.

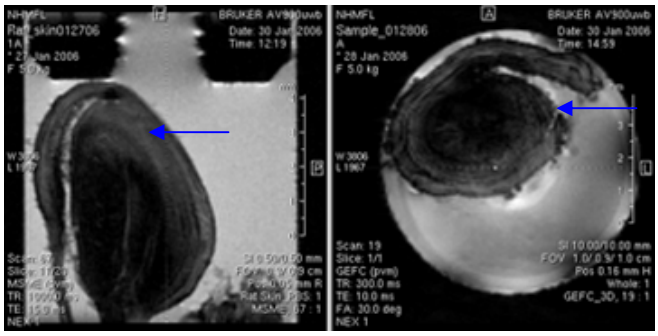

Figure 2: Ultrahigh resolution MRI at 21 T is shown in axial plane and sagittal plane with detail of tissue in an artery placed in 1.5 mm capillary. At the site of injury(arrow), wall layers can be distinguished made of media, intima and adventitia. The resolution will be 23 µm x 23 µm x 30 µm. The approach of Multislice-multiecho PARAVISION imaging technique provided contiguous images in 3 planes.

5. FUTURE OUTCOME

The method here may provide an important non-invasive means of mapping molecular and cellular events in mice. Genetically-modified mice have become the pre-eminent animal model for the study of atherosclerosis and this powerful technique will allow effects of interventions to be followed serially in vivo. Application of these techniques to other mouse models of pathology (such as tumor angiogenesis, inflammatory diseases etc.) is feasible.

Significantly, all of the imaging agents proposed here are actually, or potentially compatible with translation into use in humans with the following opportunities.

i) In keeping with the desire for translation into clinical application, the targeted imaging strategies are biologically compatible with the use in humans. Specifically apoferritin occurs naturally while gadolinium chelates and iron oxide have been used safely in humans imaging previously.

ii) The principles for molecular imaging could easily be applied to cellular imaging, for instance in stem cell therapies.

iii) Finally, the ability to deliver particles with molecular post codes clearly introduces exciting prospects for precise, quantifiable drug treatment.

6 REFERENCES

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