High Resolution in vivo and ex vivo MR Imaging of Experimental Atherosclerosis using Monocrystalline Iron Oxide Nanoparticles (MION)

J. A. Ronald[1], C. Heyn[1], A. Alejski[1], R. Weissleder[2], R. A. Hegele[3], K. A. Rogers[4], B. K. Rutt[1]

[1]Robarts Research Institute, Imaging Research, London, Ontario, Canada, [2]Massachusetts General Hospital, Center for Molecular Imaging Research, Boston, Massachusetts, United States, [3]Robarts Research Institute, Vascular Biology Group, London, Ontario, Canada, [4]University of Western Ontario, London, Ontario, Canada

Introduction

Advanced atherosclerotic lesions are prone to rupture, exposing pro-thrombotic contents such as tissue factor to the bloodstream. A lesion's vulnerability to rupture is partially due to the macrophage content of the lesion, since macrophages are known to degrade the fibrous material that encapsulates the thrombogenic gruel. To be able to image macrophages in lesions using MR, several studies have used superparamagnetic iron oxide (SPIO) nanoparticles that are able to infiltrate into lesions and are phagocytosed by resident macrophages, creating signal voids in vessel wall images where macrophages are present. The use of SPIO in imaging macrophages in atherosclerotic lesions has been accomplished in both patients and balloon-catheterized rabbit models of atherosclerosis; however, its use in an animal model which develops human-type atherosclerotic lesions and has an intact endothelium (i.e. not a balloon-catheterized model) has not been shown and was the focus of the present study.

Methods

New Zealand White rabbits (n=3) were fed a 0.25% cholesterol (CH) diet for 22 months to promote the formation of human-type atherosclerotic lesions in the thoracic aorta. One rabbit was fed a normal chow diet without cholesterol. All rabbits were imaged on a 1.5T CV/i GE MR scanner using a two-channel phased array RF coil prior to, and 60 hours following, the intravenous administration of monocrystalline iron oxide nanoparticles (MION) (200 µmol/kg). During both pre-MION and post-MION imaging sessions a set of T2-weighted "black-blood" fast-spin-echo (FSE) images and "bright-blood" spoiled gradient echo (SPGR) images were collected at the same locations. Immediately following *in vivo* scanning, rabbits were sacrificed and the aortae were dissected fresh and filled with gelatin. High resolution (150 x 150 x 300 μm^3) *ex vivo* imaging using a custom-built gradient coil insert (600 mT/m, peak 2000T/m/s) and solenoid radio-frequency coil was performed. 3DSPGR, 3DFIESTA, 3DFSE (T1W, T2W, PDW) images of the thoracic portion of the aortae were acquired.

Results

In cholesterol-fed animals pre-MION FSE images showed a distinct thickening of the vessel wall that was not apparent in control animals. Post-MION FSE images of the aortae of cholesterol fed animals at the same locations as pre-MION images showed a distinct thinning of the vessel wall, presumably due to the accumulation of MION in lesion and consequent signal reduction on the luminal side of the wall (Figure 1). Similarly, post-MION SPGR images of the vessel wall showed areas of distinct signal loss next to the lumen that were not apparent in pre-MION images (Figure 2). These effects of MION administration were not seen in the control rabbit (Figures 1 and 2). Furthermore, *ex vivo* FIESTA images of aortae from CH-fed rabbits revealed distinct areas of signal loss adjacent to the lumen, which were not seen in the control rabbits. In addition, we believe this signal loss is attributable to MION loading as lengthening the echo time (3 ms to 11ms) in SPGR images increased the amount of signal loss (Figure 3). While T1w, T2w and PDw 3DFSE images of aortae in CH-fed animals were performed to provide compositional data of the thickened vessel wall, signal voids in these images corresponded well with signal voids in both FIESTA and SPGR images (data not shown).

Discussion

Large macrophage content in atherosclerotic lesions is one of the hallmarks of plaque instability. Injected SPIO particles are taken up by macrophages in lesions and can be imaged using MRI; therefore, this technique has the potential to dynamically image macrophage content of lesions over time. Previous studies showing SPIO accumulation in vessel wall have used rabbit models that involve balloon-catheterization, which denudes the endothelial barrier of the vessel and promotes medial hyperplasia; hence, these animal studies are not ideal since these processes are atypical of human atherosclerosis. Here we use high resolution *in vivo* and *ex vivo* imaging to image MION accumulation in aortic atherosclerotic lesions of a non-balloon injured cholesterol fed rabbit model. This work lays the foundation for future studies looking at the effects of interventions upon the macrophage content of atherosclerotic lesions, potentially highlighting important factors that may affect plaque stability.

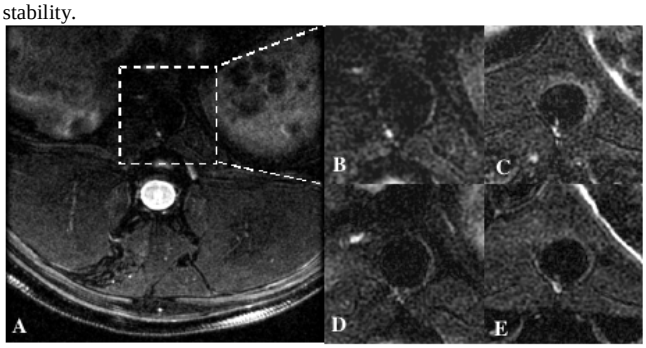

Figure 1: A) Axial "black blood" FSE image of the aorta in a control rabbit at 22 months. B/C) Magnified view of the aorta in a control (B) and CH-fed (C) rabbit prior to MION injection. Note the thicker vessel wall in the CH-fed animal. D/E) Vessel wall appearance at same locations as B/C 60 hours post-MION injection. Note the apparent thinning of the vessel wall in the CH-fed animal following MION administration.

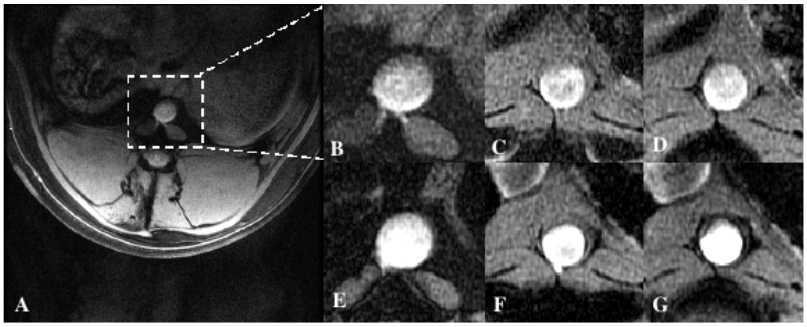

Figure 2: A) Axial "bright blood" SPGR image of the aorta in a control rabbit at 22 months. B/C/D) Magnified view of the aortae in a control (B) and CH-fed (C/D) rabbit prior to MION injection. E/F/G) Images at the same locations as pre-MION images 60 hours following the injection of MION. Note the areas of distinct signal void next to the lumen in aortae of CH-fed animals (F and G) that are not present in control images (E) or in the corresponding pre-MION images (C and D).

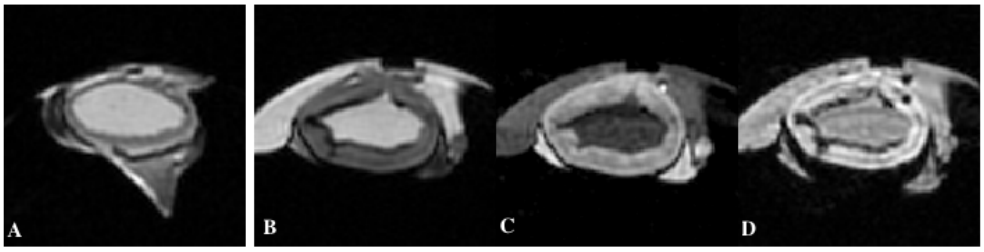

Figure 3: A) *Ex vivo* FIESTA image of aorta filled with gelatin from control rabbit. B) *Ex vivo* FIESTA image of aorta filled with gelatin from CH-fed rabbit. Note the distinct areas of signal void adjacent to the lumen. C/D) Short TE (C) and long TE (D) SPGR images showing blooming artifact indicative of the presence of SPIO.