Evaluation of an iron oxide contrast agent in the visualization of aortic valve sclerosis - a preliminary study

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Purpose: Aortic valve sclerosis (AVS) is a prevalent vascular disease and despite significant clinical consequences, no effective preventative treatment currently exists.[1] Clinical trials of potential pharmaceutical therapies have exemplified the need for early diagnosis in the development of useful AV disease therapies.[2] Macrophage invasion has been identified as a critical pathological factor and early marker of both atherosclerosis and AVS.[3] MRI enhanced with iron oxide has previously been used to evaluate atherosclerotic plaques in hyperlipidemic rabbits.[4] We have shown this animal model also develops typical AVS with macrophage infiltrate, lipid deposition and calcification. The aim of this study was to test the efficacy of identifying early stage AVS in low-level cholesterol fed rabbits using iron oxide-enhanced MRI.

Methods: Ten male New Zealand White rabbits (1.6 – 1.8 kg) were fed cholesterol-supplemented (0.125 – 0.25 %) chow for 30 months to promote aortic valve sclerosis development. Five additional rabbits served as controls and were fed normal chow. Monocrystalline iron-oxide nanoparticle (MION-47) was administered intravenously to five cholesterol-fed and two control-fed anaesthetized rabbits at a concentration of 0.06 mmol/kg (11.2 mg Fe/kg). These rabbits were imaged before and 48 h post-MION-47 administration. Imaging was performed using a 1.5 Tesla MR clinical scanner (CV/i cardiac MRI scanner, GE Healthcare) interfaced with a customized two-channel phased array radiofrequency surface coil. All *in vivo* imaging was executed using CINE fast spoiled gradient echo (fSPGR; FOV = 8 cm, matrix 0.3125 x 0.625 mm, slice thickness = 2 mm, TR = 15.5+/-1.4 ms, TE = 9.0+/-0.6 ms, FA = 20°, BW = +/-31.25 kHz, NEX = 6, 3 slices) sequences gated retrospectively to the cardiac cycle (peripheral trigger, arrhythmia rejection window = 30, minimum trigger delay, 30 cardiac phases, 2 segments per view). The rabbit aortic valve was examined using three oblique sagittal slices each transecting one of the three aortic valve cusps. Seventy-two hours following MION-47 administration, 15 rabbits (with or without contrast) were sacrificed with an intravenous ketamine injection (50 mg). Aortic valve cusps were excised, fixed and embedded in 1% agarose. Ex vivo MR imaging was conducted on a 3.0 Tesla GE EXCITE whole-body MR system with a custom-built gradient insert coil using a customized solenoid radiofrequency coil. Specimens were visualized in cross-section using a 3D FIESTA pulse sequence (TR = 9.4 ms, TE = 4.6 ms, FA = 20°, BW = +/-16 kHz, 4 phase cycles, NEX = 2) at 100x100x200 μm^3 resolution. After ex vivo MR imaging, valve cusps were embedded in OCT by freezing in liquid-nitrogen cooled isopentane. Tissue blocks were serially sectioned and stained with each of the following: anti-rabbit activated macrophage RAM11 for macrophage invasion, anti-mouse CD31 for endothelial cells and, anti-mouse smooth muscle alpha actin (SMA) for smooth muscle and myofibroblasts. All stained sections were counterstained with Perl's Prussian Blue in order to determine which cell type had taken up the contrast agent. In vivo MR image analysis was performed off-line using the Ontario Consortium of Cardiac Imaging (OCCI) Viewer. Images were reviewed by three independent blinded observers and valve thickness was measured in the middle third of the cusp using digital calipers. Measurements from all three cusps were averaged to generate final thickness measurements for control and cholesterol-fed rabbit cusps pre- and post- MION-47. To analyze the percent area of voids seen in ex vivo MR images, five random slices of each cusp in cross-section were analyzed using Adobe Photoshop CS2. Valves were manually traced to determine total pixel area and the void area was determined by thresholding. The number of positive pixels was reported as a percentage of valve pixel area. All data was analyzed with one-way ANOVA followed by Tukey's posthoc test. Values represent mean +/- standard error. Significance where indicated was $P<0.05$.

Results: The administration of MION-47 improved the visibility of both control and cholesterol-fed rabbit aortic valve cusps during in vivo imaging (Figure 1), thereby suggesting that the contrast agent was being taken up in both tissue states. Ex vivo imaging confirmed the existence of significant signal voids in MION-47 administered control and cholesterol-fed rabbit valves (Figure 2). No signal voids were observed in control or cholesterol-fed aortic valves without iron oxide administration. Histopathological analysis of MION-47 administered rabbits revealed iron staining in smooth muscle cells and macrophages on the fibrosal side of cholesterol-fed aortic valves (Figure 3). Iron staining was also found in MION-47 positive control valve cusps and was localized to smooth muscle cells on the fibrosal side of the cusp. Contrast agent-free rabbits did not exhibit any Perl's Prussian blue positive staining.

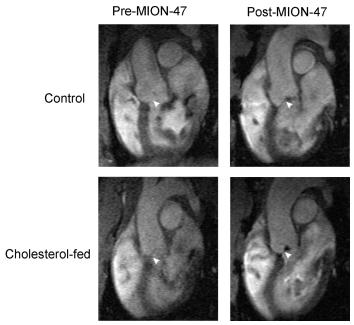

Figure 1: In vivo oblique sagittal images of aortic valve cusps pre- and post-MION-47.

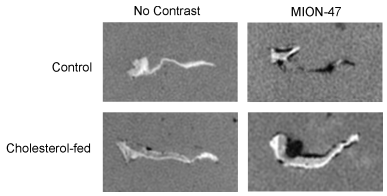

Figure 2: Ex vivo images of control and cholesterol -fed cusps with and without MION-47.

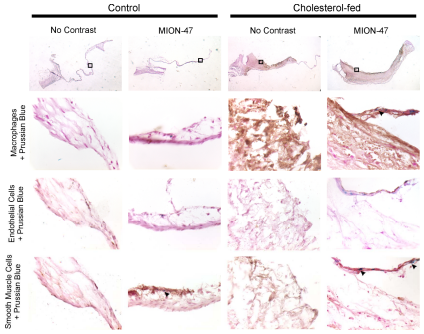

Figure 3: Immunohistochemical analysis of aortic valve cusps with and without MION-47.

Discussion: In atherosclerosis MR imaging, active intraplaque macrophages are known to readily phagocytose iron oxide particles that diffuse or migrate into the plaque.[4] Much like atherosclerosis, macrophage invasion begins early in aortic valve disease progression and macrophages can accumulate prior to substantial valve thickening.[3] It stands to reason that early imaging of macrophage invasion would be a particularly useful tool in the investigation of early valve disease for the development of preventative therapy. Our investigation of iron oxide-enhanced MR imaging yielded some unexpected results. Control rabbit aortic valve cusps are devoid of macrophages[5] and thus, we anticipated that iron oxide particles would not be taken up by these control valves. Surprisingly, iron staining smooth muscle cells were found in both control and cholesterol-fed rabbits. Cholesterol-fed rabbit aortic valves also contained the expected iron staining macrophages. All MION-47 uptake was observed on the fibrosal side of the valve which is the side of the valve in which disease progression predominantly occurs.[3,5] In 2003 Rong *et al* showed that smooth muscle cells can take on a macrophage-like phagocytotic phenotype when stressed with high cholesterol levels.[6] Our findings here suggest that smooth muscle cells on the fibrosal surface may also express this phagocytotic phenotype naturally due to the stressful environment on the aortic (fibrosal) side of the valve. Our findings also suggest that passive targeting of macrophages in the valve is insufficient and more specific macrophage-targeted contrast agents should be investigated in order to image early macrophage invasion in the aortic valve.

References: 1. Otto CM *et al.* (1997) Circulation. 95:2262-70. 2. Cowell ST *et al.* (2005) N Engl J Med. 352:2389-97. 3. Otto CM *et al.* (1994) Circulation. 90(2):844-53. 4. Briley-Saebo KC *et al.* (2007) J Magn Reson Imaging. 26:460-79. 5. Cimini M *et al.* (2005) J Heart Valve Dis. 14(3) :365-75. 6. Rong JX *et al.* (2003) Proc Natl Acad Sci. 100(23):13531-6.