In Vivo & Ex Vivo Micro-MRI in Detection of Mouse Atherosclerotic Plaques: An MRI-Histopathology Correlation Study

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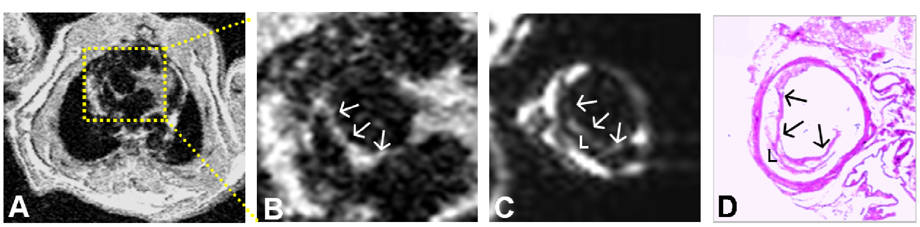
PURPOSE: Atherosclerotic cardiovascular disease remains the leading cause of death in the developed countries. Continuous efforts are warranted to understand more about the pathogenesis and efficient management of atherosclerotic cardiovascular disease. To this end, it is essential to establish animal models with atherosclerosis. $ApoE^{-/-}$ mice have been used as excellent models with atherosclerotic plaques. To date, several imaging techniques, such as digital subtraction aniography (DSA), ultrasound, CT, and magnetic resonance imaging (MRI), are available to evaluate atherosclerotic vessels. MRI has several advantages superior to other imaging modalities, including (i) generating high-resolution imaging of atherosclerotic vessel walls; (ii) providing both morphological and functional assessments of early ischemic organs; and (iii) lack of radiation (1-3). Recent development of molecular and cellular MR techniques offers both high spatial resolution and whole-body coverage, which should provide the basic science a great tool for non-invasive investigation on atherosclerotic cardiovascular disease (4). The aim of this study was to evaluate the capability of using in vivo and ex vivo micro-MRI to detect atherosclerotic plaques in animal models, which was confirmed by histopathological correlation.

METHODS: Twenty-five $ApoE^{-/-}$ mice (8-10 weeks old, Jackson Laboratories) were fed an atherogenic diet for approximately 9-11 weeks to create atherosclerosis. The atherosclerotic lesions of mouse ascending aortas were first detected, in vivo, using a 4.7T MR imager (Bruker Biospin) with a 35-mm birdcage coil (Bruker Biospin). The mice were positioned in the supine position and a pressure-sensitive pad for respiratory gating was placed on the abdomen. Electrocardiograms (ECG) were observed by insertion of two ECG needles (SA Instruments, Inc) to the right-upper and left-lower extremities of the animals. The ECG and respiratory signals were used to synchronize image acquisition to reduce imaging artifacts due to cardiac and respiratory motions. The in vivo imaging parameters included a fast spin-echo (FSE) sequence, repetition time (TR) = 2 seconds, echo time (TE) = 13.4 msec, echo train length (ETL) = 4, four signal averages, slice thickness = 1 mm, 12 slices with no gap, field of view (FOV) = 22 mm ×22 mm, matrix = 256×256, and an in-plane resolution = 0.08 ×0.08 mm^2. The total imaging time was approximately 15 minutes depending on the respiratory and heart rates of each animal.

To further confirm the accuracy of MRI in detection of atherosclerotic plaques, we performed ex vivo 3-dimentional (3D), high-spatial-resolution MRI using an 11.7 T scanner (Bruker Biospin). After in vivo 4.7T MRI, the animals were anesthetized by intraperitoneal injection of 100-150 μL pentobarbital (0.25-0.38 mg/g) and then euthanized by perfusing the animal with 4% paraformaldehyde via an open-chest, left-cardiac-ventricle puncture approach. This perfusion-fixation method enabled the removal of all blood from the animal body, and endovascular fixation. The heart and the whole-long aorta are harvested and placed a plastic tube for ex vivo 11.7T imaging, using 3D FSE sequence, TR=1.5 s, TE=25 ms, ETL=8, four signal averages, 41×24×25mm FOV, 512×256×256 matrix, ≈0.08×0.09×0.1mm3 resolution, and a total imaging time of ≈13.5 h for each animal.

The ascending aorta was selected as the primary region of interest for MRI and histopathological correlation because (i) the ascending aorta is the largest vessel in a mouse, and thus enables optimal imaging; (ii) the root of the ascending aorta is the primary target of atherosclerotic lesions; and (iii) the cardiac base is a useful landmark for allocating the target segment of the ascending aorta for precise correlation between MRI and tissue harvesting. Thus, an approximately 5-mm-long segment of the ascending aorta, measured from the cardiac base, was harvested from all mice. Subsequently the tissues were cryosectioned at 10-μm slice thickness and stained with hematoxylin and eosin (H&E) for histology examination to grade the atherosclerotic lesions. Images were reconstructed using the scanner console (Paravision) and analyzed using Amira software (Mercury Computer Systems Inc).

RESULTS: In vivo 4.7T MRI was successfully performed in all 25 animals. The structures of ascending aortas could be clearly appreciated in 23 animals, while the thoracic structures were unidentifiable, primarily due to unstable heart rates and respiration artifacts, in remaining two animals. Of 23 animals, seventeen cases showed uneven thickening of the ascending aortic walls (Fig. 1A&B). Ex vivo 11.7T MRI was successfully carried out in the 19 animals with visible ascending aortic structures by in vivo MRI. Identification of plaque components was based on the different signal intensities within the plaques. Of the 19 animals, atherosclerotic plaques were detected in 16 cases. Of the 16 cases, fourteen mice showed lipid cores as low signal pools and fibrous caps as high (bright) signal bands covered the lipid cores (Fig. 1C). Remaining three mice did not show low signaled lipid cores, but demonstrated the entire thickened aortic wall with bright signal intensities. Histopathological correlation was performed for all 25 animals, revealing the formation of atherosclerotic plaques in 18 cases and intimal hyperplasia in 7 cases. The visible atherosclerotic plaques by both in vivo and ex vivo MRI were correlated well with histopathological manifestations, demonstrating typical plaque manifestations of lipid cores and fibrous caps (Fig. 1D).



CONCLUSION: This study demonstrated the capability of using 4.7T micro-MRI to detect, in vivo, atherosclerotic plaques in mouse models. Ex vivo 3D, high-spatial resolution 11.7T MRI enabled us to acquire full volume-rendered images of the entire ascending aorta, which thus served to supplement the histology that usually provides limited slices of the target vessel segments at relatively long sectional intervals. The present study also showed that histology could be matched to appropriate ex vivo 11.7T MRI slices to identify existing pathology, and different ex vivo 11.7T MRI slices could be used to match the in vivo 4.7T MR images. These results may encourage the continued efforts to develop high-field MR imaging techniques to characterize atherosclerotic plaques in vivo, so-called "in-vivo plaque pathology," which should become an essential imaging tool for early diagnosis and efficient treatment of atherosclerotic cardiovascular disease.

Acknowledgements: This study was supported by an NIH R01 HL078672 grant.

Reference:

1. Fayad Z. Magn Reson Imaging Clin N Am 2003;11:101-13.
2. Yuan C, Miller Z, Cai J, Hatsukami T. Neuroimaging Clin N Am 2002;12:391-401.
3. Brushett C, Qiu B, Atalar E, Yang X. JMRI 2007(In press).
4. Qiu B, Gao F, Walczak P, Zhang J, Kar S, Bulte J, et al. JMRI 2007;26:339-343.