MR Histology Of Advanced Atherosclerotic Lesions Of ApoE-knockout Mice

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Introduction

Transgenic mice are widely used as animal models for the study of atherosclerosis. Apolipoprotein E–knockout (ApoE-/-) mice produced by gene-targeting technologies spontaneously develop atherosclerotic lesions similar in morphology to those observed in humans [1, 2]. The major challenges for histological MR imaging of atherosclerotic lesions in mice are the very high-resolution requirement, the need to image long vessel segments, and prohibitively long scan time. Several attempts were made to develop MRI protocols for imaging of atherosclerotic plaques in ApoE-/- mice [3-6]. None of these studies have demonstrated the ability of histological MRI to characterize the clinically significant advanced atherosclerotic lesions. The purposes of this study were to examine the feasibility of determining the composition of advanced atherosclerotic plaques in fixed ApoE-/- mice and to develop a time-efficient microimaging protocol for MR histological imaging on mice.

Methods

Specimen preparation: Five transgenic ApoE-/- mice (75 weeks old) were perfusion-fixed at physiologic pressure via the left ventricle (24-g ¾-in Becton Dickinson Angiocath intravenous catheter). The animals were initially perfused with 10mL PBS for 20 seconds followed by a 4-minute perfusion of 10% buffered formalin (Fisher Scientific, Fairlawn, NJ) along with Omniscan (gadodiamide; Amersham Health Inc, NJ), a conventional clinical contrast agent at a volume ratio of 20:1.

MRI: Experiments were performed using a 9.4T Bruker BioSpec MR scanner. 3D spoiled gradient-echo sequence was used with an isotropic FOV of 24 mm^3; 3D matrix of 512x384x256; TR=20.8ms; TE=2.6ms; flip angle=20°. All MR images had a voxel size of 47x63x94μm^3. The total scan time was 1 hour 8 min. These scan parameters allowed coverage of major vasculature spanning from the innominate artery bifurcation to the upper margin of the liver.

Histology: After imaging, the mouse was decalcified in 10% formic acid and then routinely processed en block through graded alcohols, xylene and paraffin. The specimen was embedded in paraffin and 10 micron serial section was collected every 50 microns throughout the whole mouse thorax and stained with Hematoxylin-Eosin. Images obtained with magnetic resonance technique were compared to matched corresponding histology. Anatomic structures such as the aorta, common carotid arteries, spinal cord, myocardium, aortic valve were all used as external fiducial references for alignment of the MR images with the corresponding histology sections. Tissue components in atherosclerotic lesions were identified by histology and then their MR signal properties were characterized.

Results

Advanced atherosclerotic lesions were found by MR examination in the aorta, aortic arch, innominate and carotid arteries of the mice. Atherosclerotic plaque characteristics, such as plaque area, volume, thickness, and major plaque components, such as necrotic core, calcification, and fibrous tissue were determined. Morphologically, the lesions found in the MR images corresponded closely to the histopathological cross-sections taken from the same location. Preliminary results of a pilot study quantifying atherosclerotic lesion components produced a correlation coefficient of 0.98. MR histology, in agreement with prior studies [7, 8], has shown that advanced atherosclerotic lesions of aorta, innominate and carotid arteries in ApoE-/- mice are characterized by high calcification and presence of the large fibrofatty nodules.

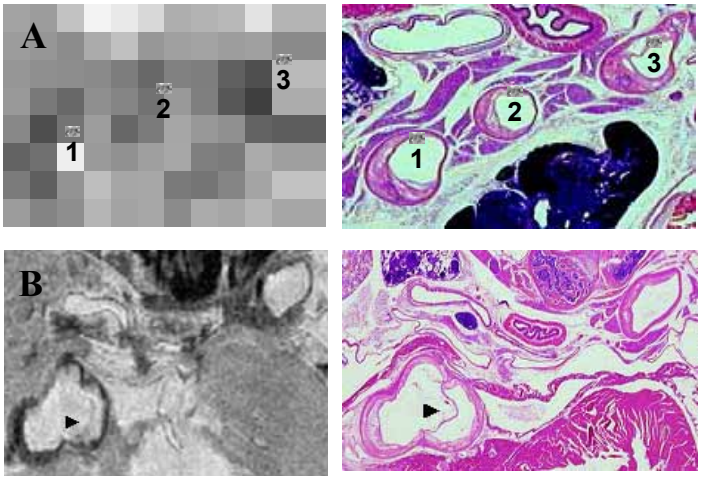

Figure 1. Cross-sectional ^1H MR images and corresponding histological slices through the mouse thorax at the (A) level of ascending from the aortic arch arteries (1 - innominate artery; 2 - left common carotid artery; 3 - left subclavian artery) and (B) at the level of the aortic root showing a clearly defined aortic valve (arrowhead).

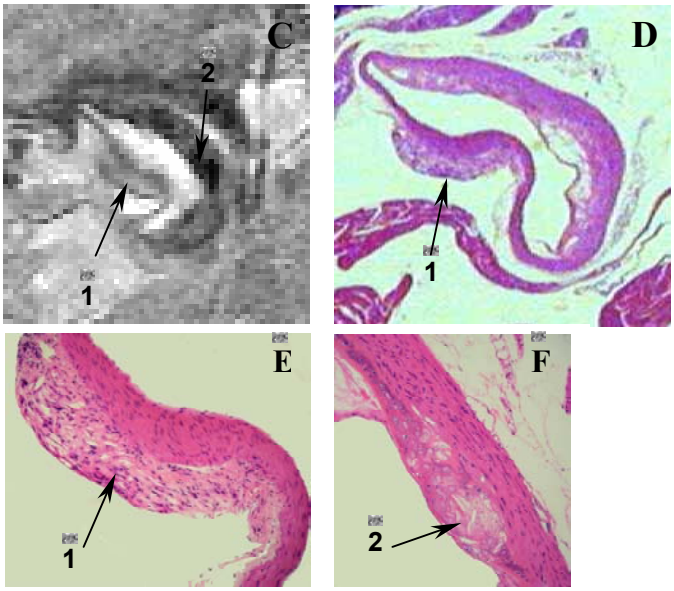

Figure 2. Comparative characterization of atherosclerotic plaque components by MRI (C) and histology (D-F) at the level of aorta on the gadolinium-perfused fixed ApoE-/- mouse. E is a high power view of the soft plaque (1) seen on both MR and low power histology. F is the adjacent histology containing calcification (2) visualized in the MR image.

Conclusion

This study has shown an ability of advanced atherosclerotic lesions characterization by MR histology on the murine model of atherosclerosis. High-field MRI allows non-invasively determination of atherosclerotic plaque composition and precise quantification of the major plaque components in the different vascular beds. A time efficient MRI protocol, based on a single contrast weighting, was developed using fixed ApoE-/- mice and can be used for in vivo studies.

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