Gadofosveset detects endothelial dysfunction associated with atherosclerotic plaque formation and progression in mice

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Introduction: Studies have demonstrated that endothelial dysfunction is one of the earliest manifestations in the pathogenesis of atherosclerosis, even in the absence of angiographic evidence of coronary artery disease in humans [1]. In addition, hyperlipidemia has also been shown to be associated with impaired endothelial function [2]. Dysfunctional endothelium promotes atherosclerosis through vasoconstriction, platelet activation, leukocyte adhesion, thrombogenesis, inflammation, smooth muscle cell proliferation, and collagen breakdown. We sought to examine whether contrast enhanced MRI using gadofosveset (MS-325) could detect endothelial damage associated with atherosclerotic plaque formation and progression in high-fat fed apoE $^{-/-}$ mice. Gadofosveset is a gadolinium based blood pool contrast agent that reversibly binds to albumin and is predominantly present within the vessel lumen but may enter the artery wall through damaged endothelium and/or leaky microvessels.

Materials and Methods: Animal model: Starting at 8 weeks of age, male apoE $^{-/-}$ mice (n=12) were fed a high-fat diet that contained 21% fat from lard and 0.15% (wt/wt) cholesterol. Male C57BL/6J mice (n=2) were fed a normal diet and were used as controls. *In vivo* MRI using a 3T Philips Achieva scanner was performed at 4, and 12 weeks post commencement of the high-fat diet in apoE $^{-/-}$ mice and at 8-weeks in C57BL/6J mice. Images were acquired before and after intravenous administration of 0.03 mmol/kg gadofosveset. Mice were placed prone on a single loop microscopy surface coil (diameter=23mm). Following a 3D GRE scout scan, time-of-flight (TOF) images were acquired for visualization of the aortic arch, the brachiocephalic and carotid arteries with a FOV=20x20x10mm, matrix=160, in-plane resolution=0.3x0.3mm (reconstructed 0.13x0.13mm), slice thickness=0.5mm, TR/TE=37/7.7 ms and flip angle=60°. The maximum intensity projection images were used to plan the subsequent delayed enhancement (DE) and T1 mapping scans. A 2D-Look-Locker sequence planned perpendicular to the ascending aorta, was used to determine the optimal inversion time (TI) for blood signal nulling. Acquisition parameters were: FOV=30mm, matrix=75, in-plane spatial resolution=0.4x0.4mm, slice thickness=2mm, TR/TE=19/8.6 ms, TR between subsequent IR pulses=1000ms, and flip angle=10°. An inversion-recovery-3D-fast-gradient echo sequence was acquired 30 minutes post injection and was used for DE-MRI and visualization of contrast uptake. Imaging parameters were: FOV=30x8x30mm, matrix=300, in-plane resolution=0.1x0.1, measured slice thickness=0.25mm, slices=32, TR/TE=27/8ms, TR between subsequent IR pulses=1000ms, and flip angle=30°. T1 mapping was performed using a sequence that employs two non-selective inversion pulses with inversion times ranging from 20ms to 2000ms, followed by eight segmented readouts for eight individual images. The two imaging trains result in a set of 16 images per slice with increasing inversion times. For T1 mapping the acquisition parameters were: FOV = 22x8x36, matrix = 180x171, in plane resolution= 0.2x0.2, measured slice thickness=0.5mm, slices=16, TR/TE = 9.2/4.7 ms, flip angle = 10°. T1 values were computed on a pixel-by-pixel basis using an in house Matlab algorithm. Histology: Vascular permeability was assessed by visualizing the leakage of Evans blue dye into the vascular wall. Similarly to gadofosveset, Evans blue dye binds to serum albumin and as a result it only enters the vessel wall if there is a dysfunctional endothelium. Evans blue dye (0.1 ml of 4% dye in PBS) was injected intravenously. After 30 minutes the mice were euthanized, the vasculature was perfused through the left ventricle with 10 ml of 4% formaldehyde, excised and viewed with bright field microscopy.

Results and Discussion: The uptake of gadofosveset in atherosclerotic and non-atherosclerotic mice is illustrated in **Fig. 1**. Cross-sectional DE-MR images **(Fig. 1A, F, K)** and DE-MR images fused with the TOF images **(Fig. 1B, G, L)** of the brachiocephalic arteries of control and apoE $^{-/-}$ mice at 4 and12 weeks of HFD show a gradual increase of vessel wall enhancement corresponding to plaque progression. The uptake of gadofosveset within the vessel wall was quantified using the longitudinal relaxivity R1 maps **(Fig. 1C, H, M)** and results are shown in **Fig. 1P**. After 4 weeks on HFD apoE$^{-/-}$ mice showed similar uptake of gadofosveset compared to control mice. However, a significant increase in uptake of gadofosveset was found between apoE$^{-/-}$ mice fed a HFD for 12 weeks and those fed a HFD for 4 weeks as well as control mice. A visual representation of the uptake of gadofosveset in specific locations of the vasculature is illustrated on the fused DE-MR and TOF images reconstructed in a coronal plane **(Fig. 1 D, I, N)**. The color-coding ranges from green to red with red indicating a higher contrast uptake. The uptake of gadofosveset within regions of endothelial dysfunction was corroborated histologically using Evans Blue dye **(Fig. 1 E, J, O)**. The accumulation of the dye was visually similar between the control and apoE$^{-/-}$ mice after 4 weeks of HFD and was in agreement with R1 mapping of gadofosveset (**Fig. 1P**). Conversely, there was a significant and localized increase in the uptake of the dye in the brachiocephalic artery of apoE$^{-/-}$ mice at 12 weeks of HFD associated with plaque formation. This was in agreement with increased gadofosveset uptake on DE-MRI (**Fig. 1 K, P**) and on R1 mapping (**Fig. 1 M, P**).

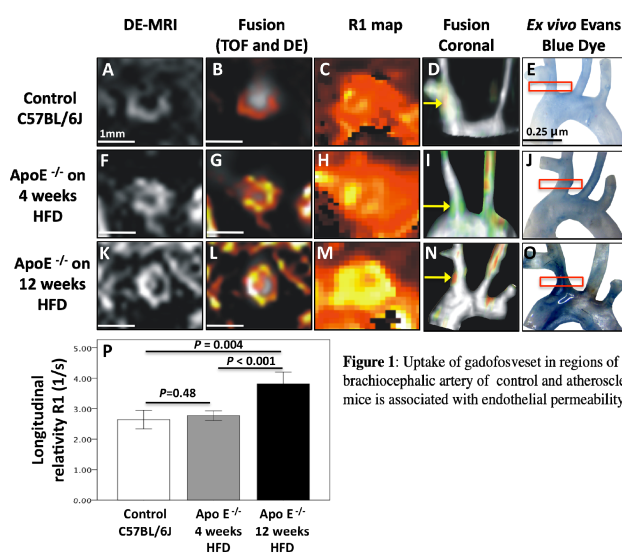

Figure 1: Uptake of gadofosveset in regions of the brachiocephalic artery of control and atherosclerotic mice is associated with endothelial permeability.

Conclusions: Contrast enhanced MRI with gadofosveset appears promising for non-invasive detection of endothelial dysfunction associated with atherosclerotic plaque formation and progression.

References: 1. Werns, S.W., et al., *Evidence of endothelial dysfunction in angiographically normal coronary arteries of patients with coronary artery disease*, in *Circulation*. 1989. p. 287-91. 2. Vogel, R.A., *Cholesterol lowering and endothelial function*, in *The American journal of medicine*. 1999. p. 479-87.