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

A. Phinikaridou\textsuperscript{1}, M. Andia\textsuperscript{1}, and R. Botnar\textsuperscript{1}

\textsuperscript{1}Imaging Sciences, King's College London, London, United Kingdom

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 \cite{1}. In addition, hyperlipidemia has also been shown to be associated with impaired endothelial function \cite{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\textsuperscript{-/-} 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\textsuperscript{-/-} 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\textsuperscript{-/-} 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\degrees. 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=30x30mm, 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=30\degrees. 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\degrees. 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\degrees. 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\textsuperscript{-/-} mice at 4 and 12 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\textsuperscript{-/-} mice showed similar uptake of gadofosveset compared to control mice. However, a significant increase in uptake of gadofosveset was found between apoE\textsuperscript{-/-} 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. 1D, 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. 1E, J, O). The accumulation of the dye was visually similar between the control and apoE\textsuperscript{-/-} 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\textsuperscript{-/-} mice at 12 weeks of HFD associated with plaque formation. This was in agreement with increased gadofosveset uptake on DE-MRI (Fig. 1K, P) and on R1 mapping (Fig. 1M, P).

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