Monitoring vascular permeability and remodelling following endothelial injury in a murine model using an MR albumin binding contrast agent

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Introduction: Endothelial cells play a critical role in maintaining vascular homeostasis. However, vascular damage may lead to endothelial dysfunction characterized by decreased bioavailability of nitric oxide (NO), a signalling molecule involved in vasodilatation, with several anti-atherogenic and anti-inflammatory properties. Previous studies have shown that contrast enhanced MRI using gadofosveset, an albumin binding gadolinium contrast agent, can detect endothelial damage, angiogenesis and vascular permeability in different animal models [1, 2]. In this study, we explored whether imaging with gadofosveset could be used to monitor endothelial cell recovery, permeability and vessel wall remodelling in a murine model of aortic endothelial denudation.

Materials and Methods: Animal model: Surgery was performed under isofluorane anaesthesia using a dissecting microscope in wild-type (WT) and nitric-oxide synthase 3 knock-out (NOS3$^{-/-}$) mice. The abdominal aorta was exposed via a midline incision of the abdomen. The aorta, renal and iliac arteries were exposed and isolated. Subsequently, two ligatures were placed around the aorta, below the renal arteries and above the iliac bifurcation, respectively **(Fig 1A)**. A 30G syringe was introduced into the abdominal aorta via an aortic puncture and allowed by bolus infusion of 1mL saline buffer. This procedure was repeated three times to remove the endothelial cells **(Fig 1B)**. The needle was then removed, the aortic puncture was repaired, and the sutures were removed to restore blood flow. The muscle and skin incisions were sutured and the animals were allowed to recover. *In vivo* MRI was performed using a 3T Philips Achieva system. Images were acquired before and after intravenous administration of 0.03mmol/kg gadofosveset. Mice were placed prone on a single loop microscopy surface coil (diameter=47mm). Following a 3D GRE scout scan, time-of-flight (TOF) images were acquired for visualization of the abdominal aorta with a FOV=35x35x12mm, matrix=233x233, in-plane resolution=0.15x0.15mm (reconstructed 0.10x0.10mm), slice thickness=0.5mm, TR/TE=28/6ms and flip angle=40°. The maximum intensity projection images were used to plan the subsequent delayed enhancement (DE) and T1 mapping scans. 30min post gadofosveset injection, a 2D-Look-Locker sequence planned perpendicular to the aorta, was used to determine the optimal inversion time (TI) for blood signal nulling. Acquisition parameters were: FOV=30x30mm, matrix=76x76, in-plane spatial resolution=0.39x0.39mm, slice thickness=2mm, TR/TE=19/8.2ms, TR between subsequent IR pulses=1000ms, and flip angle=10°. Immediately afterwards, an inversion-recovery 3D fast-gradient echo sequence (DE-MRI) was acquired and used for visualization of contrast uptake. Imaging parameters were: FOV=35x35x12mm, matrix=348x348, in-plane resolution=0.1x0.1x1mm, slices=24, 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=36x22x10mm, matrix=180x102, in-plane resolution=0.2x0.2x0.5mm, slices=16, TR/TE=9/4.6ms, flip angle=10°. T1 values were computed on a pixel-by-pixel basis using an in-house software (Matlab, Natick, MA).

Results and Discussion: Gadofosveset uptake in WT and NOS3$^{-/-}$ mice is illustrated in **Fig.2**. The highest enhancement of the abdominal aortic wall was observed at 7 days **(Fig.2B$_1$ - 2B$_4$)** after endothelial denudation in both WT and NOS3 $^{-/-}$ mice. However, in NOS3 $^{-/-}$ mice **(2C$_3$-2C$_4$, 2D$_3$-2D$_4$)** the enhancement of the vessel wall persisted for 15 and 30 days following endothelial denudation compare with WT mice **(Fig. 2C$_1$, 2C$_2$, 2D$_1$-2D$_2$)**. Quantitation of the contrast enhanced area and the *in situ* vessel wall relaxation rate (R_1) are illustrated in **Fig.3A** and **3B** respectively. Full re-endothelialization of the vessel wall in WT mice significantly reduced the leakage of gadofosveset into the vessel wall reflected by the decreased contrast enhaced area and R1 measurements. Conversely, lack of NOS3 retards and impairs endothelial cell recovery leading to persistent leakage of gadofosvest into the vessel wall.

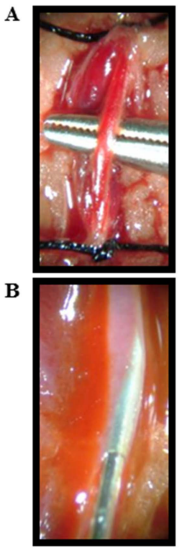

Figure 1: Endothelial denudation protocol.

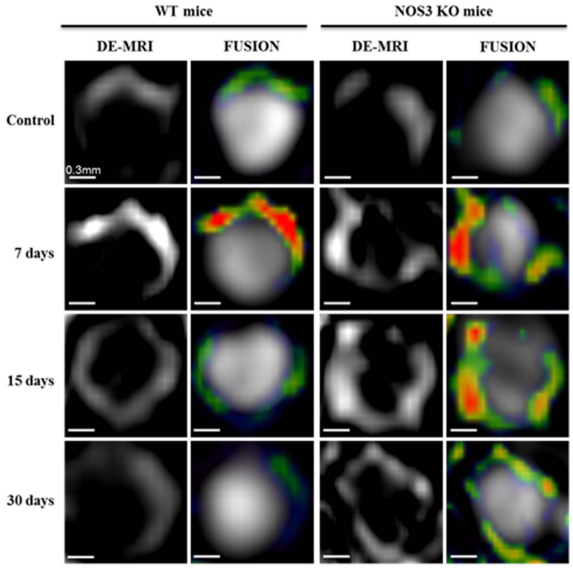

Figure 2: In vivo contrast enhanced MRI with gadofosveset in the abdominal aorta of WT and NOS3 $^{-/-}$ mice after endothelial denudation protocol.

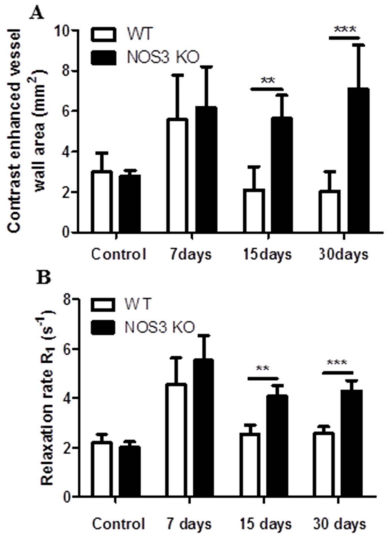

Figure 3: Quantification of the **(A)** contrast enhanced area and **(B)** relaxation rate in the vessel wall of the abdominal aorta of WT and NOS3$^{-/-}$ mice.

Conclusions: We demonstrate that utilization of an albumin-binding contrast agent allows for the non-invasive monitoring of endothelial cell recovery, vascular permeability and remodelling after endothelial denudation protocol.

References:

(1) Pedersen, S.F., et al. J Cardiovasc Magn Reson, **2011**. 13: p. 10. **(2)** Phinikaridou, A., et al. Circulation, **2012**. 126(6): p. 707-19.