

IN VIVO MOLECULAR MRI OF ATHEROSCLEROTIC PLAQUE PROGRESSION IN MICE USING A NOVEL ELASTIN-BINDING CONTRAST AGENT

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Introduction

The extracellular matrix (ECM) plays a pivotal role in the pathogenesis of atherosclerosis and ECM remodeling [1]. Elastin is an essential component of the ECM of the arterial vessel wall. Male ApoE $^{-/-}$ mice have been shown to reproducibly develop progressive atherosclerotic plaques in the innominate artery over a short period on a high fat diet (HFD) [2]. With the advent of a novel elastin binding contrast agent (BMS-753951) imaging of ECM formation in atherosclerosis has become feasible.

Purpose

In this study we sought to investigate whether the use of an elastin-binding contrast agent (BMS-753951) in concert with serial MRI would allow for the detection of atherosclerotic plaque formation in male ApoE $^{-/-}$ in the innominate artery and whether it would facilitate the assessment of plaque progression.

Methods

The time course of plaque development in the innominate artery of male ApoE knockout ($-/-$) mice was assessed. MRI of the innominate vessel wall was performed 4 weeks and 8 weeks after the onset of a high fat diet in a 3T Achieva clinical MR scanner (Philips Healthcare, Best, the Netherlands) equipped with a dedicated software package (R2.5.3), a single loop small animal coil ($\varnothing = 23$ mm) and a clinical gradient system (30mT/m, 200mT/m/ms). For visualization of the innominate artery and planning of the vessel wall scans time-of-flight (TOF) angiography of the innominate arteries was performed. Imaging parameters included TR=31ms, TE=6.7ms, flip angel=60°, spatial resolution=0.2x0.2x0.5 mm. For assessment of ECM remodeling, an inversion recovery (IR) vessel wall sequence was performed prior the injection of the contrast agents (Figure 4 B, G, baseline) and approximately 30-35 minutes post injection of 0.2mmol/kg of Gd-DTPA (Magnevist) (Figure 4 C, H) and of 0.2mmol/kg of BMS-753951 (Lantheus Medical Imaging, Figure 4 D, I), a novel elastin-binding Gd³⁺-based contrast agent. A Look Locker sequence was performed to select the optimal inversion time for blood signal nulling. Imaging parameters included TR=28ms, TE=8.3ms, flip angel=30°, spatial resolution=0.1x0.1x0.5 mm, inversion time approx. 450ms. Contrast-to-noise ratio (CNR) of the innominate artery was determined by manual segmentation of the visually apparent signal of the contrast agent. For absolute quantification of the elastin contrast agent concentration the established T1 mapping sequence MOLLI [3] was modified to a segmented k-space acquisition with two different TIs in order to achieve accurate and reproducible high resolution maps at high heart rates. To verify our MR data, we performed a histological elastica staining in 5 μ m-paraffin slices (Elastica-Van Gieson method). Furthermore elastin content was assessed semi-quantitatively using western immunoblotting.

Results

All animals completed the MR examination without any adverse events. CNR in the vessel walls of the innominate artery were significantly increased ($p<0.05$) at both time points compared to the Gd-DTPA scan. Furthermore CNR after 8 weeks of high fat diet was significantly higher compared to the 4 weeks of high fat diet (CNR: 32 \pm 5. vs. 56.4 \pm 8.4, $p<0.05$, Figure 3). At both time points, the presence and extend of atherosclerotic plaque was confirmed with Elastica Van Gieson staining (Figure 2).

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Histological evaluation of the innominate artery plaque size and elastin content (western blot) were in good agreement with the imaging findings using the IR TFE sequence and suggest an increase in plaque size after 8 weeks of high fat diet compared to 4 weeks.

Conclusions

In this study, we demonstrate the successful non-invasive assessment of alterations in atherosclerotic plaque size in an ApoE mouse model using serial MRI together with a novel elastin specific contrast agent. Molecular alterations, with regard to elastin formation in atherosclerosis can be differentiated using BMS-753951.



Figure 1: Elastica Van Gieson staining of the innominate arteries of ApoE $^{-/-}$ mice after 4 (A) and 8 (B) weeks of HFD.

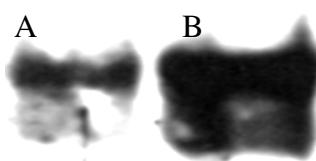


Figure 2: Western blotting shows a robust increase in elastin expression in the mouse brachiocephalic artery after 8 weeks (B) of high fat diet using an elastin specific antibody (anti-elastin 1:1000) compared to 4 weeks of HFD (A).

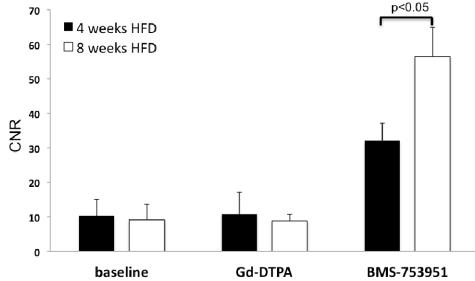


Figure 3: Significant increase of the CNR in the wall of the innominate artery of ApoE-/- mice on HFD for 8 weeks compared to 4 weeks.

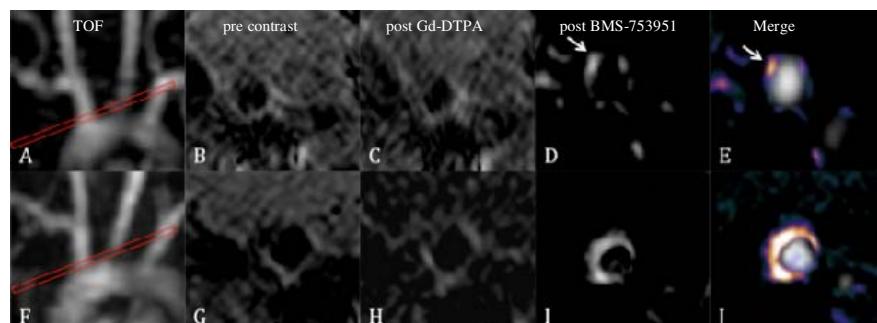


Figure 4: For visualization of the innominate artery and planning of the vessel wall scans time-of-flight (TOF) angiography of the innominate arteries was performed (A, F). For assessment of ECM remodeling, an inversion recovery (IR) vessel wall sequence was performed prior the injection (B, G) and 30-35 minutes p.i. of Gd-DTPA (C, H) and of BMS-753951 (D, I).

References: [1] Nili et al., *Cardiovasc Pathol* 2002; 272-6 [2] Johnson et al., *Circulation* 2005; 1422-30 [3] Messroghli Dr et al., *MRM* 2004; M52-141