NON INVASIVE ASSESSMENT OF PLAQUE PROGRESSION IN APOE-/- MICE USING T2* WEIGHTED AND POSITIVE CONTRAST SGM-MRI

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Introduction

Macrophages have been identified as a contributor to plaque instability in atherosclerosis. Over the past several years macrophage MR imaging with iron oxide particles has been demonstrated in several animal models and in humans. The aim of this study was to noninvasively assess iron oxide uptake at different stages of plaque development in the innominate artery of apoE-/- mice [1] and to evaluate the effect of anti-inflammatory treatment.

Purpose

In this study we investigated whether the use of a iron oxide [2] would allow the serial detection and quantification of macrophage rich atherosclerotic plaque in male apoE-/- mice using T2* weighted and positive contrast susceptibility gradient mapping (SGM) MRI [3].

Methods

The time course of plaque development in the innominate artery of male apoE knockout (-/-) mice was assessed. MRI of the innominate vessel wall of apoE-/- mice on a 3T Achieva MR scanner (Philips Medical Systems) was performed after 4, 8 and 12 weeks of high fat diet with and without statin treatment using a dedicated single loop small animal coil (= 23mm) and a clinical gradient system (30mT/m, 200mT/m/ms). Time-of-flight (TOF) angiography (TR=43, TE=8.1, flip angel=60°, spatial resolution=0.2x0.2x0.5 mm) of the carotid arteries was performed for the visualization of the innominate artery and planning of the subsequent high resolution T2* weighted scan. Imaging parameters of the ECG triggered T2*-weighted 3D gradient echo sequence included flip angle=25°; FOV=16x16x8mm; matrix =176x176x20; TE=6.9ms and TR=21ms. At day 0 and day 1 animals were tail-vein injected with a 300µl/kg dose of VSOP, and imaged on day 2. Negative contrast images were obtained from the T2* weighted 3D gradient echo acquisition. Furthermore complex (real/imaginary) image data was used to calculate a susceptibility gradient map (SGM). In particular, local field inhomogeneities are also created in the presence of iron oxide nanoparticles. Since susceptibility gradients result in a displacement of the associated echoes in k-space, the determination of the shift in k-space allows a parametric map of the regional susceptibility gradient for each pixel [3]. Furthermore, a higher local iron concentration is expected to induce a higher susceptibility gradient and thus a greater echo shift. Areas of signal void were assessed in the T2* images and compared to the areas of positive contrast in SGM images (Figure 1). Areas were determined by manual segmentation of the visually apparent signal loss or positive contrast. Furthermore, the absolute value of the susceptibility gradient map (i.e. echo shift) was evaluated. To verify our MR data, Prussian Blue and Elastica van Gieson staining in 5µm-paraffin slices as well as immunhistochemistry (Mac2) for macrophages was performed.

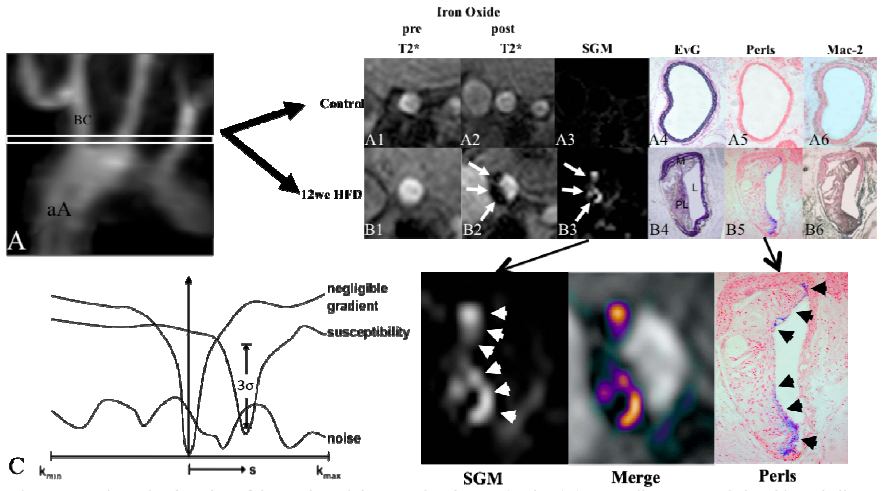

Figure 1: In vivo TOF imaging of the aortic arch in control and apoE-/- mice (aA: ascending aorta, BC: brachiocephalic artery). Bright blood imaging was performed such that a 2D slice was obtained perpendicular through the brachiocephalic artery (A1-3, B1-3). Representative images before and after the injection of the iron oxide agent from control as well as mice on the HFD for 12 weeks. Corresponding Elastica van Giesson, Perl's and immunohistochemical analysis for Mac-2 (macrophage marker) of atherosclerotic plaque associated with the brachiocephalic artery. Perl staining was used to demonstrate colocalization of iron positive area (M: Media, Pl: Plaque, L: Lumen). C: Graphic to illustrate the effect of application of the Lorentzian based filter to the real and imaginary data on the signal intensity in the FT.

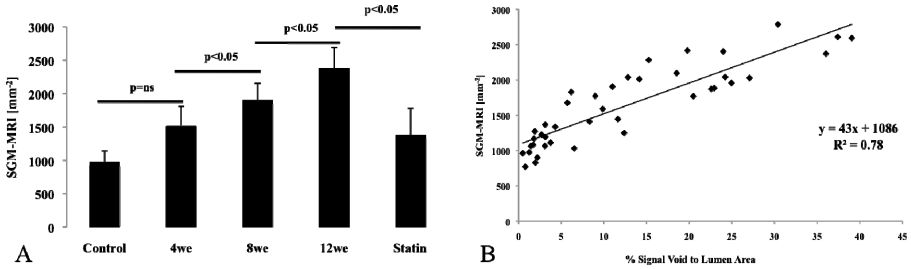

Figure 2: A: SGM-MRI of control as well as after 4, 8, 12 weeks of high fat diet. Data are presented as mean+-SD. B: A good correlation between SGM signal and % signal void to lumen area in atherosclerotic plaques could be found. However for the calculation of % signal void to lumen area the native MRI scan had to be taken into account.

Results

All animals completed the MR examination without any adverse events. No signal void or positive contrast was found in the scans prior to the injection of VSOP iron oxides. Application of SGM to the same slice after VSOP injection produced a positive contrast that coincides with the region of dephasing seen in the negative contrast image. Areas of signal void and positive contrast as well as the echo shift in the parametric maps of the innominate artery significantly increased during plaque development but were reduced in statin treated animals. At all time points, the atherosclerotic plaque was confirmed with Elastica Van Gieson staining as well as immunhistochemistry using Mac2 (1:500) as a marker for macrophages within the plaque (Figure 1). Preliminary evaluation of the innominate plaque size and macrophage content are in good agreement with the MRI findings and suggests an increased plaque size with a higher iron content during plaque development (Figure 2). In addition to the positive contrast produced by SGM, the value of the echo shift correlates with plaque progression and may provide information on the local iron oxide concentration.

Conclusions

In this study, we demonstrate the successful use of iron oxide particles for the non-invasive assessment of alterations in iron content in atherosclerotic plaque in an apoE-/- mouse model of progressive atherosclerosis. Molecular alterations in plaque composition with regard to macrophage content could be detected using iron oxide particles in combination with T2* weighted and SGM MRI. Anti-inflammatory treatment with statins resulted in a smaller SGM signal and smaller signal voids on T2* weighted images.

References: [1] Johnson et al., Circulation 2005;1422-30 [2] Taupitz et al., CMR 2005: 2.03 [3] Dahnke et al., ISMRM 2008 16:1513