Gadolinium mixed micelles containing Apolipoprotein E derived peptide for atherosclerotic plaque detection through interaction with macrophages

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Introduction

Lipid Nanoparticles containing Apolipoprotein E derived peptide (P2A2) have been shown to be effectively incorporated in various endothelial cell types in vitro. P2A2 consists of a 20 Amino Acid tandem dimmer comprising binding sites for the low-density lipoprotein receptor. Untargeted Gadolinium mixed micelles have been shown previously by our group to be an effective magnetic resonance contrast agent for atherosclerotic plaque imaging in ApoE -/- mice through interaction with extracellular matrix. In this study our aim was to target cellular compounds of atherosclerotic plaque through the incorporation of P2A2 into Gadolinium mixed micelles.

Methods

Gadolinium mixed-micelles were prepared through lipid film hydration. P2A2 was added to the lipid lipid film before hdyration. This lead to formation of P2A2 containing Gadolinium mixed micelles (APOE Micelles). Fifteen-month-old ApoE -/- mice (n=5) underwent in vivo MRI of the abdominal aorta using a 9.4T MR system. Lissamine Rhodamine (as a red fluorescent tag) containing APOE Micelles (0.030 mmol Gd/Kg) were injected in the tail vein and pre- and post-contrast enhanced MR at 24h using a T1W black blood sequence was performed. As a control ApoE -/- mice (n=6) were injected with NBD (as a green fluorescent tag) containing untargeted micelles (0.038 mmol Gd/Kg) and were imaged as described. Another control group (n=6) was injected with Gd-DTPA and imaged at 1h and 24h post-injection. After MRI, the aortas were removed and fixed. Frozen sections were obtained and were indicated stained with CD68 for macrophage staining. The sections were then imaged using confocal microscopy for co-localization studies.

Results

Relative to muscle (as described by the %NENH), administration of untargeted and APOE micelles resulted in a significant enhancement of the vessel wall of ApoE-/- mice. % NENH: **62% +/-5** for untargeted micelles, and **113% +/-5** ApoE micelles. Following administration of GdDTPA, transient signal enhancement was observed in the vessel wall of ApoE-/- mice one-hour post injection. No significant enhancement of the vessel wall of ApoE-/- or WT mice was observed 24-hour post injection. Confocal fluorescence imaging demonstrates the localization of untargeted micelles primarily to perivascular areas and areas rich in extracellular matix without significant cellular interaction. Aortic Sections after APOE micelle injection demonstrate localization of ApoE micelles to core areas of atherosclerotic plaque with specific co-localization to macrophages and foam cells.

Conclusion We demonstrate the specific molecular imaging of macrophages using APOE derived peptide containing gadolinium mixed micelles.

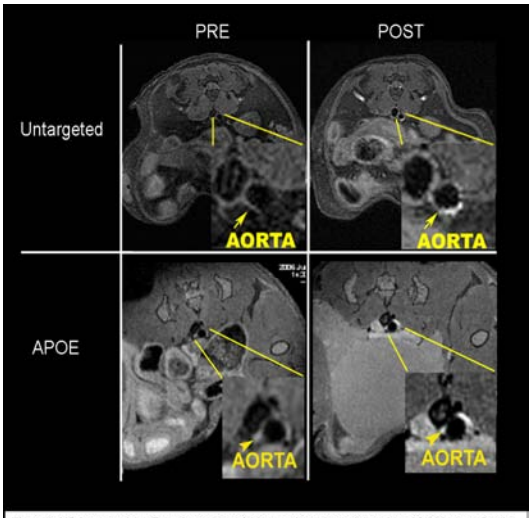

Figure1: MR images of ApoE-/- mice pre and 24 hours after the administration of 0.038 mmol Gd/Kg of untargeted or APOE targeted micelles at 9.4T.

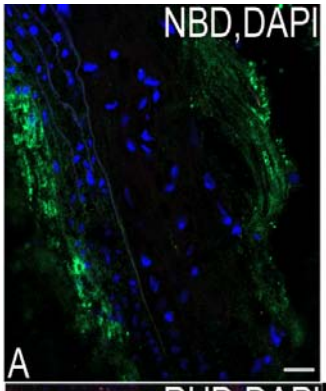

Figure 2 Fluorescence Confocal microscopy of aortic sections 24h post injection of untargeted NBD-tagged micelles (A) and Lissamine Rhodamine-tagged APOE micelles (B-D).

A) Untargeted micelles display diffuse extracellular and perivascular distribution (green areas) with no apparent cellular interaction. Grey bar = 20µm

B) Two channel image overlay shows at low power magnification, an aortic section with APOE micelles (red) **localizing** to core areas of atherosclerotic plaque.

C) RGB and composite image of an adjacent section to B with higher magnification A core plaque area as seen on image B with CD 68 staining (green) for Macrophages. Areas of comparable distribution of APOE micelles (red) and Macrophages (green) on individual channel images. A close association shown between Macrophages and APOE micelles (yellow-orange areas) on composite image.

D) RGB and composite image with magnification of marked area from image B. Both APOE micelle and macrophage are dected in congruent areas of the plaque as seen on individual channel imgages. The composite image reveals punctate ce localization of APOE micelles in tissue macrophages.

DAPI nuclear stain: blue, on individual and composite images.