Delayed gadolinium enhanced MRI reveals nanotherapy-induced normalization of the vessel wall endothelium in atherosclerotic mice

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Target audience: Researchers interested in novel, non-invasive imaging techniques to assess the efficacy of new drugs for cardiovascular disease.

Purpose: Atherosclerosis is the number one killer world-wide. Increased permeability due to inflammation is a hallmark of vulnerable atherosclerotic plaques, at high-risk of causing myocardial infarction or stroke[1]. This knowledge has spurred interest in developing new compounds to lower plaque inflammation, as well as non-invasive techniques to quantify their efficacy. We have previously developed a drug-loaded lipoprotein nanoparticle ([S]-rHDL) with very potent anti-inflammatory properties[2]. As proof-of-concept, we used Gd-DTPA enhanced MRI to measure changes in plaque permeability/inflammation in atherosclerotic mice after intervention with ([S]-rHDL.

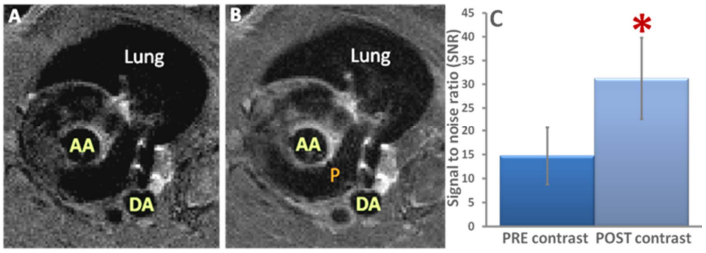

Figure 1: **A** and **B**, axial images at the level of the ascending aorta (**AA**), showing uptake of contrast agent in the vessel wall. **C**, average SNR before and after contrast agent injection ($p<0.001$). **AA**, ascending aorta. **DA**, descending aorta. **P**, pulmonary trunk. Red star indicates a significant difference. Error bars, standard deviation.

Methods: Atherosclerosis was induced in 9 apolipoprotein $E^{-/-}$ (ApoE-KO) mice by feeding them a high fat diet for 16 weeks. Mice were imaged on a 7 Tesla (Bruker Corposation, MS, USA) pre-clinical MR scanner with a custom made coil, before and after treatment. Aortic root images were acquired using an ECG gated, T1 weighted RARE (Rapid Acquisition with Refocused Echoes) sequence (10 axial slices; slice thickness 0.5 mm; spatial resolution 0.117 mm^2; repetition time, 800ms;echo time, 8ms), before (**Fig 1,A**) and immediately after (**Fig 1,B**) the injection of 0.3 mmol/Kg Gd-DTPA. After baseline imaging, mice were randomized into a treatment (n=5) and control (n=4) group. The treatment group received 4 injections of [S]-rHDL in 8 days, a regimen previously proven to have marked anti-inflammatory effects in this model[2]. The enhancement pattern in aortic roots was used as a surrogate measurement marker of plaque permeability, and was quantified by calculating the SNR (signal to noise ratio) of the aortic vessel wall before and after Gd-DTPA injection. After the final imaging session, animals were injected with Evans Blue (EB), a fluorescent dye that binds to albumin and is commonly used to quantify vascular permeability *ex vivo*. Thirty minutes after EB injection, mice were euthanized, the aortas excised and imaged with near infrared fluorescence (NIRF) imaging. Aortic EB uptake was quantified as the radiance in the aortic root (**Fig 2**).

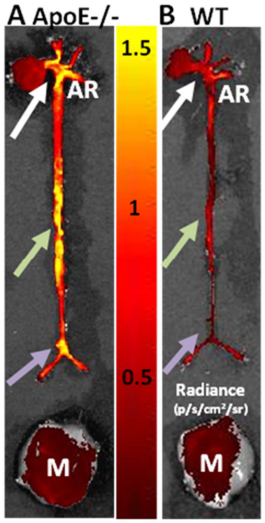

Figure 2: Uptake of Evans Blue shows increased permeability in the aortic root of atherosclerotic (white arrow, **A**) compared to control mice (**B**). White arrow, aortic root (AR). Green arrow, abdominal aorta. Lilac arrow, iliac bifurcation. M, skeletal muscle (low EB uptake).

Results: At baseline and in all mice, aortic root SNR was significantly increased after Gd-DTPA injection ($p< 0.001$), indicating high plaque permeability (**Fig 1,C**). After [S]-rHDL treatment the normalized aortic SNR ($SNR_{post\_contrast}$ / $SNR_{pre\_contrast}$) was significantly lower in the treated mice than the non-treated control group ($p<0.001$, **Fig 3**). In vivo results were corroborated by a significantly lower endothelial permeability in treated mice by as determined by Evans Blue NIRF imaging ($p=0.03$).

Discussion: In the current study we showed that anti-inflammatory [S]-rHDL lowers aortic plaque permeability in atherosclerotic ApoE-KO mice, as determined by in vivo Gd-DTPA enhanced MRI, which was validated by ex vivo EB NIRF imaging. In an ongoing study using histological and gene expression markers we have found these [S]-rHDL to directly lower macrophage inflammation in plaques. Currently, we are further substantiating the biological meaning of our observations and working on translating this technology.

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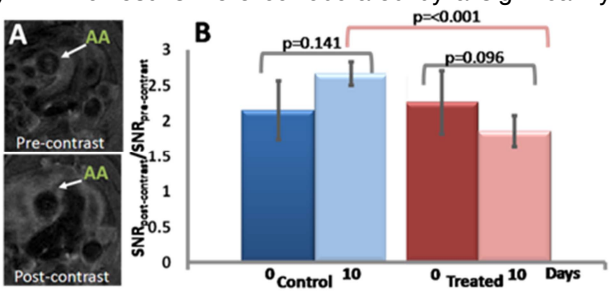

Figure 3. A, pre and post contrast images of the ascending aorta (AA) or a treated mouse. **B**, Bar graph of the SNR after contrast agent injection normalized by the SNR before contrast agent injection in the aortic root of ApoE-/- mice either treated (red) or not treated (blue) with simvastatin nanoparticles. No difference was found between the two groups at day 0 ($p=0.70$).

References: 1. Virmani R, J Am Coll Cardiol, 2006; 2. Tang J, AHA Scientific Sessions, 2011; 3. Phinikaridou A., Circulation, 2012; 4. Greish K, Methods Mol Biol, 2010