In Vivo MR Imaging of Apolipoprotein-E Knockout Mice to Detect Atherosclerosis with Gadolinium-containing Micelles and Immunomicelles Molecularly Targeted to Macrophages.

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Background: The capability to quantify biological activity of atherosclerosis using MR imaging holds great promise in terms of non-invasive risk stratification based on plaque morphology. Macrophages are known to play a central role in the pathogenesis and evolution of atherosclerotic plaque. The ability to detect the uptake of Gadolinium(Gd)-containing compounds in macrophage cells with MR Microscopy (MRM) may enable non-invasive detection of atherosclerotic plaque. Gadolinium-containing immunomicelles (micelles linked to a specific antibody targeting macrophage scavenger receptor) and micelles have been shown to improve *in vitro* and *ex vivo* assessment of macrophages using MRI. The goals of the current study were to evaluate the *in vivo* uptake of immunomicelles, micelles, and standard contrast agents in the murine aorta using *in vivo* MR Microscopy (MRM) and to determine whether immunomicelles and micelles improve *in vivo* imaging of atherosclerotic plaque in Apolipoprotein E knockout (ApoE KO) mice using MR Microscopy.

Methods: Micelles were synthesized using phospholipids, surfactant, and a lipophilic Gadolinium-containing contrast agent. Immunomicelles were made using a biotin-avidin bridge to bind the micelles to a specific antibody targeted to macrophage scavenger receptor. Micelles had an approximate Relaxivity of 30. The size of the micelles could be precisely engineered to be between 20 nm to 100 nm. In previous experiments micelles, immunomicelles, and standard (i.e., Gd-DTPA or Gd-DOTA) paramagnetic contrast agents were tested in the murine RAW 264.7 macrophage cell line. Cells were incubated for 2 hours with different concentrations of Gd-DTPA, micelles, and immunomicelles in culture flasks. The cells were centrifuged into cell pellets and imaged using a 1.5 T MR system with an inversion recovery spin echo sequence to determine the T1 of each cell pellet. *Ex vivo* analysis was performed using a 9.4T MR system with a high-spatial resolution sequence ($70\mu m^3$). In the current *in vivo* study micelles, immunomicelles, and standard (i.e., Gd-DTPA) paramagnetic contrast agents were tested in ApoE KO mice. Mice were imaged at baseline with a 9.4T MR system with a high-spatial resolution sequence (MR Microscopy). The mice were then imaged at intervals following a tail injection of either micelles (n=5 mice), immunomicelles (n=5 mice), or a standard (Gd-DOTA) paramagnetic contrast agent (n=4 mice).

Results: Our experiments have shown that pellets of micelle-treated macrophage cells had a decreased T1 vs. Gd-DTPA-treated cells ($p<0.0001$). Incubation with immunomicelles decreased T1 vs. micelles ($p<0.05$). *Ex vivo* analysis of ApoE KO aortas using confocal microscopy demonstrated uptake of fluorescently-labeled immunomicelles and micelles. Ex vivo data demonstrated a 59% signal intensity increase in aortas incubated with immunomicelles vs. control and a 19% increase in signal for micelles vs. control. In the current *in vivo* work using micelles the ratio of signal intensity in the aortic wall post and pre-contrast normalized to muscle was an average of 1.5 ± 0.3 (an enhancement of 50%) at 1-hour post-contrast, 1.35 ± 0.2 (35% enhancement) at 24-hours post-contrast, 1.22 ± 0.2 (22% enhancement) at 48-hours post, 1.15 ± 0.12 (enhancement of 15%) at 72-hours post and 1.00 (0% enhancement) at 1-week post (See Graph 1). In the control ApoE KO group there was no significant enhancement of the aortic wall using the non-specific Gd-DOTA contrast agent. *In vivo* enhancement of atherosclerotic plaque was also seen in mice following injection of immunomicelle contrast agent (Please see Figure 1). The current *in vivo* study is showing that immunomicelles and micelles cause enhancement of atherosclerotic plaque in the ApoE KO murine aortas.

Conclusions: Immunomicelles and micelles improve *in vitro* and *ex vivo* assessment of macrophages using MRI. The current *in vivo* study shows promising results in the detection of atherosclerotic vascular disease using MR imaging. Immunomicelles may prove quite useful in the detection of high-macrophage density typical of high-risk plaques.

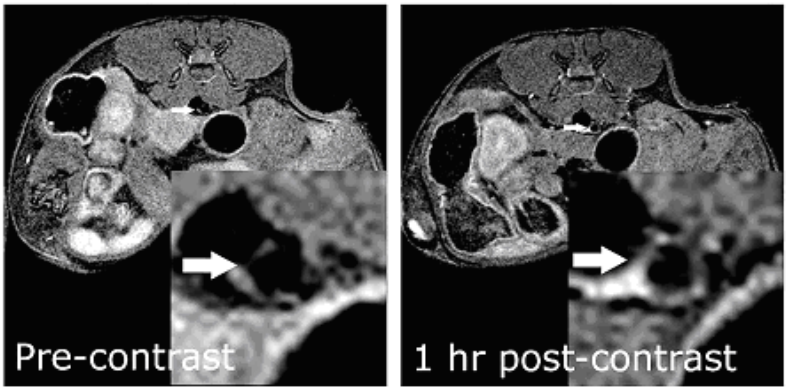
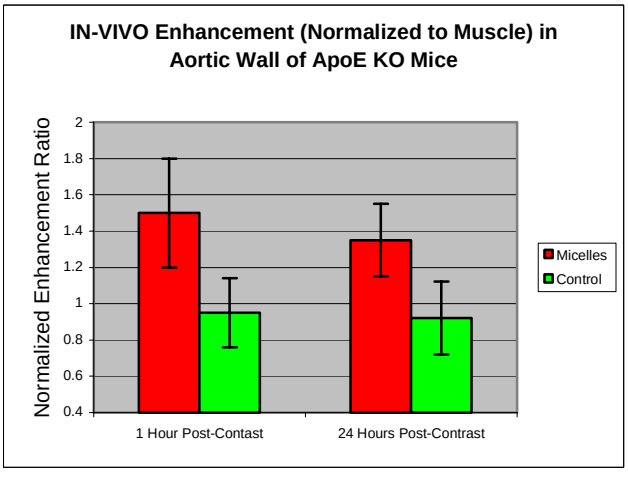


Figure 1:

Enhancement in the Aortic wall of an ApoE KO mouse following injection of Gd-containing immunomicelles targeted to the macrophage scavenger receptor.



Graph 1: In-Vivo Enhancement (Normalized to muscle) of Aortic wall in ApoE KO mice (n=5 experimental, n=4 control).

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