Targeted Iron Oxide Particles For In Vivo MR Detection of Atherosclerotic Lesions Using Antibodies against Oxidized Low Density Lipoprotein: Effect of Particle Size.

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Introduction: Oxidized low-density lipoproteins (OxLDL) play a major role in atherosclerotic plaque progression and destabilization. Reported studies indicate that gadolinium (Gd) micelles labeled with oxidation-specific antibodies allow for in vivo detection of vulnerable plaque using MRI. However, the observed in vivo bio-transformation and retention of Gd may limit the clinical translation of this platform. Iron oxide particles are recognized as safe and effective contrast agents for MRI. However, the efficacy of passively targeted dextran coated iron oxide remains variable. It was, therefore, hypothesized that OxLDL targeted iron oxide particles may allow for the development of a safe and effective platform for the *in vivo* detection of vulnerable plaque. Since reported studies indicate that intraplaque macrophage uptake of dextran coated particles is modulated by particle size, the effect of particle size on the MR efficacy of the OxLDL targeted iron oxide particles was also evaluated in murine models of atherosclerosis.

Methods: Lipid coated ultra-small superparamagnetic iron oxide particles (LUSPIO) and larger superparamagnetic particles (SPIOS) were prepared and conjugated with murine monoclonal antibodies targeted to malondialdehyde (MDA)-lysine (MDA2) or oxidized phospholipids (E06), or single chain human antibody fragments targeted to MDA-like epitopes (IK17). All formulations were characterized with size, relaxation properties, binding specificity, pharmacokinetics, and biodistribution in apolipoprotein deficient mice (ApoE-/-). MRI was performed at 9.4T prior to and 24 hrs after the administration of a 4 mg Fe/kg dose in 10-11 month old ApoE-/-mice. Multiple echo gradient echo and white marker (positive contrast) GRASP sequences were applied. After MR imaging, the aortas were excised and Perl's Prussian blue staining was performed to detect iron deposition within the arterial wall. All histological sections were then matched to the MR images based upon the distance from the renal arteries and/or iliac bifurcation.

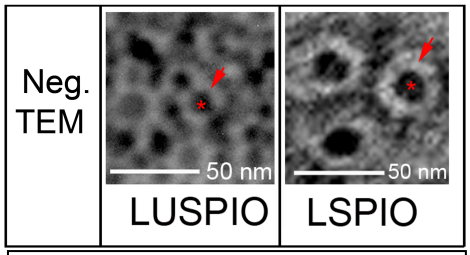

Fig.1: Negative TEM of untargeted formulations. Red asterisk indicates the iron core and red shows the lipid layer.

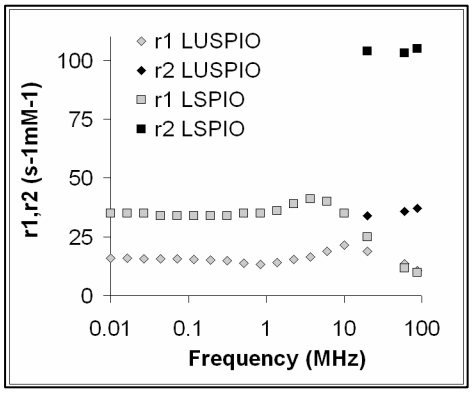

Fig.2: Evolution of r1 and r2 of the untargeted formulation as a function of applied field.

Results and Discussion: The mean particle diameter of the LUSPIO formations were 19, 23, 22 and 20 nm for the untargeted, MDA2, E06, and IK17 formations, respectively. LSPIOs exhibited particle diameters of 45, 48, 49, and 47 nm for the untargeted, MDA2, E06, and IK17 formations, respectively. **Fig. 1** shows the negative transmission microcopy of the untargeted particles. The dark center represents the iron oxide cores and the light ring highlights the presence of lipids. The evolution of the longitudinal and transverse relaxation rates (R1 and R2, respectively) are shown in **Fig. 2**. As anticipated the LSPIOs generate significantly greater R2 effects, when compared to LUSPIOs. Although the iron oxide particles exhibit a significant negative surface change (2-2.2 mV), attachment of the antibodies to the particle did not inhibit the binding specificity to OxLDL. Representative MR images obtained prior to and after administration of the LUSPIO formulations are shown in **Fig. 3**. Good correlation between the MR signal and histology was observed. Limited enhancement was observed, however, following administration of the untargeted materials. Additionally, the LSPIO formulations exhibited significantly lower arterial wall enhancement as reflected by relative changes in the R2* values of the arterial wall of 18±2% and 58±3% for the OxLDL targeted LSPIOs and LUSPIOs, respectively. This data strongly suggests that even though the LSPIOs induce greater R2/R2* effects, the mean diameter of these particles limit luminal diffusion into the arterial wall. The LUSPIOs, on the other hand, exhibit higher luminal penetration thereby allowing for enhanced intraplaque macrophage uptake.

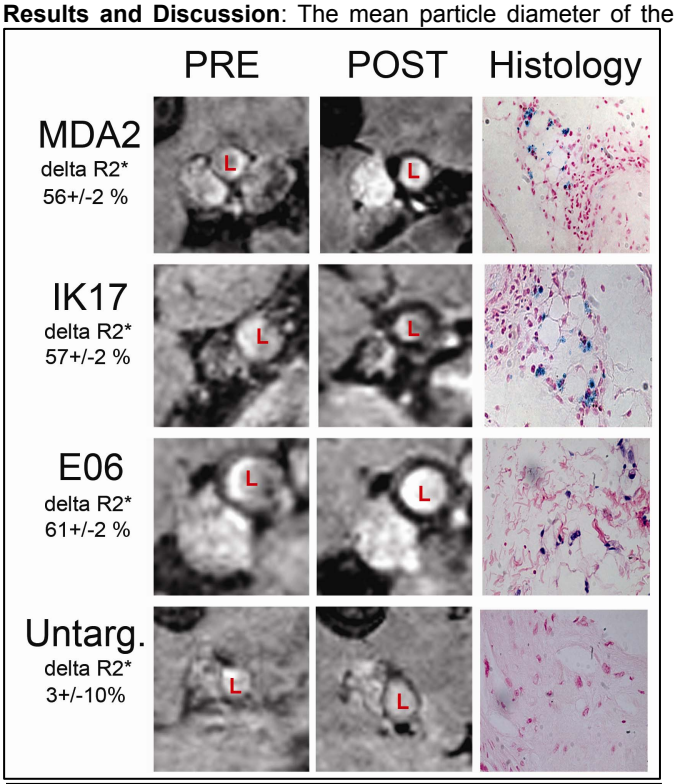

Fig.3: Representative MR GRE images (TE=7 ms) obtained prior to and 24 hours after administration of LUSPIOs (4 mg Fe/Kg). The red L indicates the arterial lumen. Matching histology is shown. Blue indicates iron deposition associated with foam cells.

Conclusions: This study suggests that the formation of small OxLDL targeted iron oxide particles (<25 nm) may provide a clinically translatable platform for the MR detection of OxLDL rich foam cells associated with vulnerable atherosclerotic plaque.