Non-invasive imaging of oxidized LDL using MDA2-labeled gadolinium immunomicelles

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Introduction Oxidation of low-density lipoproteins (LDL) has been linked to the progression of atherosclerosis and plaque vulnerability. Murine monoclonal antibody MDA2 has been shown to bind oxidation-specific epitopes associated with oxidized LDL [1]. Previous studies have shown that radiolabeled MDA2 may be used to target oxidation-rich atherosclerotic lesions. However, the resolution is suboptimal with this approach thereby limiting the diagnostic utility of radiolabeled MDA2[2].

Purpose: The aim of the current study was to synthesize, characterize, and evaluate the *in vivo* MR efficacy of MDA2 labeled gadolinium micelles in ApoE-/- mouse models of atherosclerosis.

Methods: MDA2-labeled immunomicelles and untargeted control micelles were synthesized by dissolving 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[poly(ethylene glycol)](PEG-DSPE), malamide-PEG-DSPE, Rhodamine-DSPE, and GdDTPA-bis(stearyl-amid)) in chloroform:methanol. A thin film was formed and the micelles hydrated in a HEPES buffer. The MDA2 antibody was covalently attached (0.035 mg MDA2/mouse) to the micelles surface following SATA modification[3]. The MDA2 immunomicelles and untargeted control micelles were characterized with respect to size (light scattering) and longitudinal relaxivity (r_1, 60 MHz). The pharmacokinetics and biodistribution were determined in ApoE-/- mice (n=8) following administration of a 0.075 mmol Gd/kg dose. Animals were sacrificed by saline perfusion and tissue removed over a 3 week time period post injection. T1 values of the blood samples were measured and all tissues were sent to ICP-MS for the determination of gadolinium content. The *in vivo* efficacy was evaluated following the administration of a 0.075 mmol Gd/kg dose of either the MDA2 immunomicelles (n=8) or the untargeted control micelles (n=5) in ApoE-/- mice. The normalized signal enhancement (%NENH), relative to muscle, for the vessel wall, liver and kidney was determined at 9.4T using T_1-weighted sequences over a three-week period post injection. All animals were sacrificed via saline perfusion and the aorta and liver removed and fixed for confocal microscopy. Fixed sections were stained for both macrophages (CD68) and cell nuclei (DAPI).

Results: The mean hydrated particle size of the MDA2 immunomicelles and untargeted micelles were 14±2 nm and 22±2 nm, respectively. The r_1 values were 9.3 and 11.6 $s^{-1}mM^{-1}$, for the MDA2 and untargeted micelles respectively. Addition of the antibody generated a significant increase the blood half-life from 1.15 hours for the untargeted micelles to 14.3 hours for the MDA2 immunomicelles. **Fig. 1a and b** summarize the biodistribution of the immunomicelles in ApoE-/- mice. The results suggest that the immunomicelles are partially cleared by the liver. Significant amounts of immunomicelles were observed in the aorta over the 3-week time period tested. **Fig. 1c** illustrates the normalized MR signal enhancement as a function of time post injection. **Fig. 2a** shows representative images obtained following administration of the immunomicelles in ApoE-/- mice. The confocal microscopy confirmed the uptake of the immunomicelles in foam cells associated with the vessel wall (**Fig. 2b**). The prolonged signal enhancement of the vessel wall observed following administration of the immunomicelles may be a result of intracellular uptake of the MDA2 immunomicelles into foam cells that are rich in oxidative epitopes. No significant signal enhancement or uptake into the vessel wall was observed following administration of the untargeted micelles at any of the time points studied.

Conclusions: The results of this study strongly suggest that non-invasive imaging of oxidized LDL epitopes is feasible using MDA2-labeled gadolinium immunomicelles.

References: **1**) Tsimikas S. ATVB 2000;20(3):689-697. **2)** Tsimikas S. J Nucl Cardiol 1999;6:41-53. **3)** Mulder WJ. Nano Lett 2006;6(1):1-6.

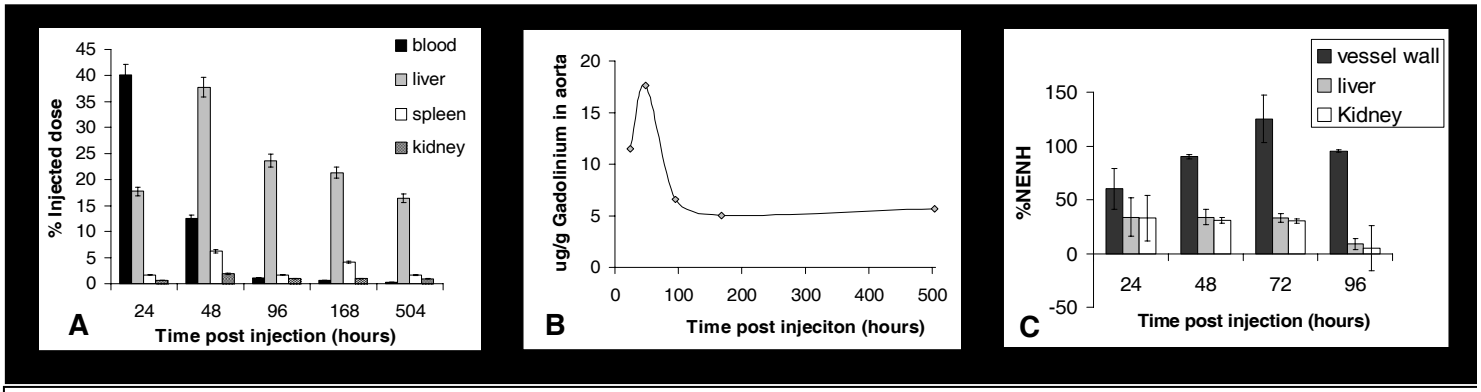

Fig.1a-b: Biodistribution of the MDA2 immunomicelles in ApoE-/- mice (0.075 mmol Gd/kg). Fig.1c: Normalized MR signal enhancement as a function of time post injection of the MDA2 immunomicelles in ApoE-/- mice.

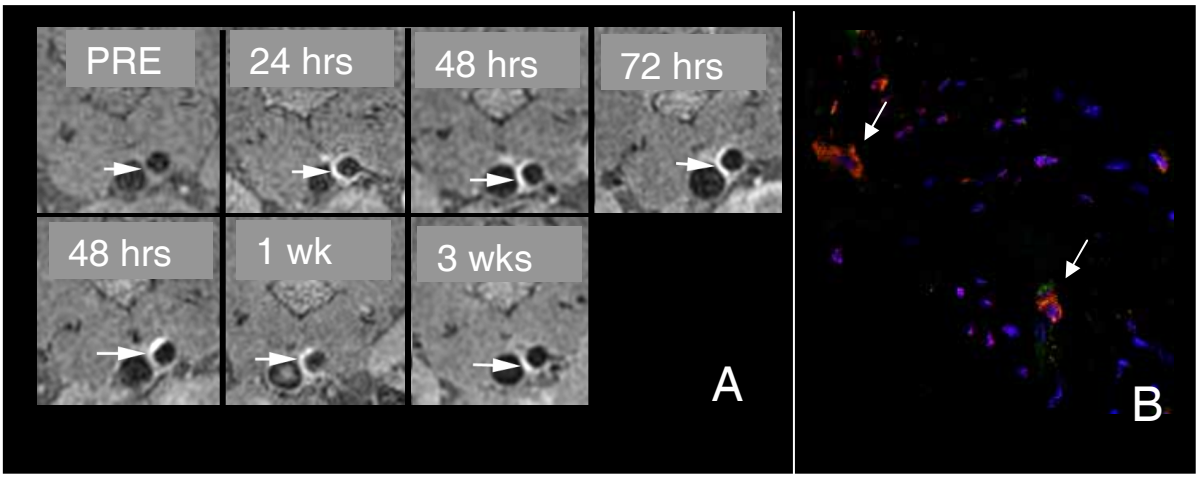

Fig.2a: enhancement of the vessel wall in ApoE-/- mouse as a function of time after injection of 0.075 mmol Gd/kg MDA2 immunomicelles. Arrow shows aorta. **Fig.2b:** Confocal microscopy of aorta 48 hrs after injection of immunomicelles. Red shows micelles, blue is DAPI and green shows macrophages. Arrow points to foam cells.