In-vivo and in-vitro uptake of gadolinium-containing immuno-micelles in a macrophage cell line: detection of atherosclerotic plaque using MRI

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The ability to detect the uptake of gadolinium(Gd)-containing compounds in macrophage cells holds a great deal of promise for the detection of atherosclerotic plaque. Atherosclerotic lesions associated with rupture tend to have large lipid cores composed of foam cells, which are derived from macrophages, and elevated levels of macrophages and other inflammatory cells that degrade the fibrous cap. We set out to determine if Gd-containing compound uptake could be detected in an in-vitro setting using MRI and also determine if we could increase uptake by adding a monoclonal antibody specific to macrophages on the Gd-containing compounds.

Methods: The murine macrophage cell line RAW 264.7 was grown on 75 cm^2 cell culture flasks. The cells were then be incubated for 2 hours with different concentrations of Gd-DTPA and several micelle preparations (see **Table 1**). Mixed micelles were synthesized by sonication of the phospholipids, lipohilic contrast agent and the surfactant at 65 degrees in water[1]. Immunomicelles incorporated 0.5% biotinylated phospholipid.[2] Micelle 1 had a diameter of 152 nm, a relaxivity of 19.2, and 23,000 Gd^{3+} per particle. Micelle 2 had a diameter of 152 nm, a relaxivity of 26.3, and 58 Gd^{3+} per particle. Micelle 3 had a diameter of 21 nm, a relaxivity of 20.7, and 56 Gd^{3+} per particle. The cells were placed in a 0.5 ml tube and imaged using a 1.5 T MR system using an inversion recovery spin echo sequence to determine the T1 of each cell pellet. The immunomicelle was tested on a 6 well cell plate at a concentration of 1 uM. Cells were treated with a 0.27 uM concentration of biotinylated Rat anti-mouse monoclonal antibody to CD204, a RAW 264.7 macrophage scavenger receptor. The antibody was attached to the biotinylated micelle via a biotin-avidin-biotin bridge. Three wells were incubated for two hours with the immunomicelle, the biotinylated micelle and normal media to serve as the control. In-vivo imaging was performed on a mouse using a 9.4T MR microscopy system and T1W black blood imaging. Sixteen contiguous 500 μm thick slices with an in-plane resolution of 93 μm were acquired in 30 minutes. Anti-mouse CD204 (0.1 ml) was injected via the mouse tail vein, 0.05 ml of excess avidin after 15 minutes, and 0.2 ml of biotinylated micelles after another five minutes. MRM images of the matched (pre and post) slices were used for analysis.

Results: Uptake of all contrast agents was observed in-vitro. **Table 1** displays the T1 (ms) of different contrast agents in-vitro at different concentrations. This table reveals that uptake of contrast agent occurs in macrophages, T1 decreases with increased concentrations of contrast agent, and that the addition of an antibody specific to the macrophage increases uptake of the contrast agent by 440% if comparing the relative decreases in T1 (12% decrease compared to 53% decrease). In the in-vivo model, enhancement of atherosclerotic plaque was noted at one hour using the immunomicelle contrast agent.

Contrast Agent	**Control**	**1 uM**	**10 uM**	**50 uM**	**100 uM**
Gd DTPA (Magnevist)	953	886	880	777	698
Micelle **1**	832	713	537	499	440
Micelle **2**	832	793	726	629	564
Micelle **3**	822	726	NA	NA	NA
Anti-CD204 Micelle **3**	822	385	NA	NA	NA

Conclusion: In-vitro uptake of gadolinium-containing contrast agents can be measured in a macrophage cell line using MRI. Cells treated with gadolinium-containing micelles have a lower T1 than cells treated with GdDTPA. The addition of a monoclonal antibody specific to a cellular target further increases uptake, thereby decreasing the T1. The immunomicelle contrast agent allowed enhanced in-vivo imaging of an atherosclerotic plaque of a mouse abdominal aorta. While further work is necessary, the ability to deliver specifically targeted gadolinium-containing contrast agents can not only aid in the detection of high-risk atherosclerotic disease but may also be applicable for the detection and treatment of metastatic cancer.

References:

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