

A Novel Dual MRI-PARACEST/Fluorescent Probe For the Detection of Cathepsin-D Activity in Alzheimer's Disease

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Introduction: A novel dual magnetic resonance imaging (MRI)/fluorescent contrast agent, RW-0505-F6, has been designed that is capable of detecting a biological target. A MRI paramagnetic chemical exchange saturation transfer (PARACEST) contrast agent (2-4) Tm³⁺-DOTA-Glycine coupled to a cell penetrating peptide sequence and a Cathepsin D (CatD) recognition site has been synthesized. CatD is a lysosomal protease found in elevated levels in some cancers and in Alzheimer's disease (1) making it a potential biological marker. A fluorescent probe, Oregon Green, was conjugated to the contrast agent to monitor its molecular behavior optically. The purpose of this study was to demonstrate detection of the agent in an MRI environment and to optically establish the cellular uptake of this novel MRI/fluorescent contrast agent. Image contrast produced by the PARACEST agents was generated by the radiofrequency irradiation of bulk water (on-resonance paramagnetic agent chemical exchange effect (OPARACHEE)) (5,6).

Methods: The OPARACHEE effect of the contrast agent was studied in 5% cross linked bovine serum albumin (BSA), which can simulate tissue relaxation and the endogenous macromolecule magnetization transfer (MT) effect, as function of agent concentration (0, 1, 2, and 4 mM). OPARACHEE contrast was defined as the difference between the bulk water signal intensity change with and without agent present. Images were acquired using a fast spin echo pulse sequence (Field of view = 25.6 × 25.6 mm², data matrix: 256 × 256, repetition time = 4 s, effective echo time = 10 ms, echo train length = 4 and 2 averages), preceded by a 0.24 second 6.0 μT WALTZ-16 pulse train (7) at 23°C. A reference image was also acquired without the WALTZ-16 preparation pulse at 9.4T.

For cell uptake studies, SN56 cells, a neuronal cell line, were cultured and differentiated. To simulate conditions of increased CatD, cells were transfected with a plasmid that drives high level expression of human CatD. Varying concentrations (5, 10, 50, 100 μM) of RW-0505-F6 were added to the media of SN56 cells and confocal microscopy live cell imaging was performed at 30 minutes. Uptake was quantified by counting the proportion of cells, which were labeled with the green fluorescent compound expressed as Mean ± SEM. Finally, cell toxicity studies were performed in triplicate using 0.4% trypan blue.

Results and Discussion: The OPARACHEE contrast observed with different agent concentration in 5% BSA is shown in Figure 1. A 1.5 mM Tm³⁺-DOTA-Gly in 5% BSA produced greater than 1% OPARACHEE contrast. In cellular uptake experiments, SN56 cells took up the agent in a concentration dependent manner from 5 μM to 100 μM range (Figure 2). The agent was observed intracellularly and found within cellular compartments (Figure 3). Cell viability studies yielded no significant difference between cell survival of control cells and those cells treated up to 24 hrs with our contrast agent.

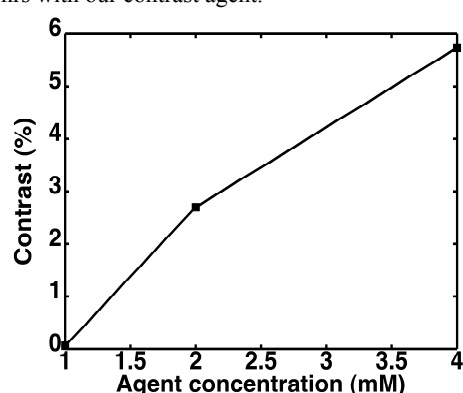


Figure 1. OPARACHEE contrast with varying RW-0505-F6 concentration in 5% BSA generated at 9.4 Tesla using a 0.24 second 6.0 μT WALTZ-16 pulse train as the preparation pulse.

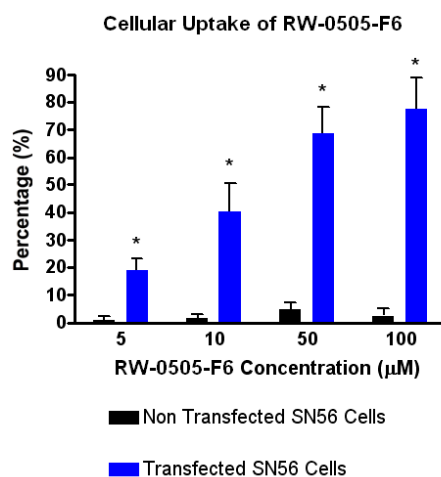


Figure 2. Percentage of cells taking up contrast agent after 30 minutes of incubation with RW-0505-F6.

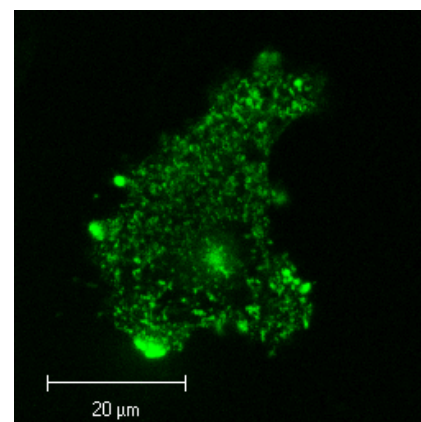


Figure 3. Confocal microscopy image of a SN56 cell showing uptake of 50 μM RW-0505-F6. Note that the contrast agent is being contained in compartments and not diffusely distributed throughout the cell.

Conclusions: Greater than a 2% OPARACHEE contrast was observed in 2 mM Tm³⁺-DOTA-Glycine in a 5% BSA phantom. Furthermore, this contrast agent was detected optically using confocal microscopy and was taken up into neuronal cells. Thus, RW-0505-F6 shows significant potential as a molecular probe in MR and optical imaging.

References: (1). Benes, *et al.*, Critical Reviews in Oncology/Hematology 2008;68:12-28. (2). Sherry AD, *et al.*, Annu Rev Biomed Eng 2008;10:391-411. (3). Terreno E, *et al.*, Invest Radiol 2004;39:235-243. (4). Yoo B, *et al.*, Front Biosci 2008;13:1733-1752. (5). Vinogradov E, *et al.*, J Magn Reson 2005;176:54-63. (6). Vinogradov E, *et al.*, Magn Reson Med 2007;58:650-655. (7). Shaka A, *et al.*, J Magn Reson 1983;52:335-338.