A PARACEST MRI Contrast Agent that Detects Esterase Enzymes

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Introduction:

PARAmagnetic Chemical Exchange Saturation Transfer (PARACEST) MRI contrast agents can reduce the MR signal of water by saturating a unique chemical shift of a chemical group that exchanges protons with water (such as an amine or amide group), and then allowing this saturation to be transferred to water via chemical exchange.[1,2] The chemical shift and exchange rate of the PARACEST effect can be modulated by converting the amine and/or amide group to a new chemical group through enzymatic catalysis, which can be exploited to detect enzyme activity.[3] Esterase enzymes are an attractive objective for molecular imaging because they are predominantly located within live cells, which can be used as a biomarker for intracellular delivery.[4] Unfortunately, ester groups don't possess hydrogens and therefore can't produce a PARACEST effect. A 'trimetyl lock' moiety has been shown to undergo self-immolation following de-esterification, which converts an amide to an imine or amine.[5] Therefore, conjugating this moiety to a PARACEST MRI contrast agent may modulate the PARACEST effect in response to esterase activity.

Methods:

Yb(III)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid o-Aminoanilide (Yb-DO3A-oAA) was synthesized, and the product was confirmed by MS and NMR spectroscopy. The trimethyl lock [TML: 1-(1-dimethylcarboxyethyl)-2,4-methylphenylester] (Sigma Aldrich) was conjugated to the amine of Yb-DO3A-oAA, and this product was incubated with 3 units of porcine liver esterase enzyme (Calbiochem). PARACEST spectra of 25 mM of TML-(Yb-DO3A-oAA) were obtained before and after enzyme catalysis using a modified presaturation pulse sequence with a 600 MHz Varian NMR scanner (Figure 1).[3]

Results and Discussion:

The new PARACEST agent TML-(Yb-DO3A-oAA) was synthesized and characterized (Figure 1A). TML-(Yb-DO3A-oAA) displayed no PARACEST effect prior to the addition of the enzyme, and the product of the reaction displayed a PARACEST effect at +10 ppm (Figure 2). This change in the PARACEST effect can be monitored using MR methods to detect esterase enzyme activities. Because esterases are predominantly located within cells, especially within endosomes and lysosomes, this novel contrast agent may be used to track delivery of payloads into cells.

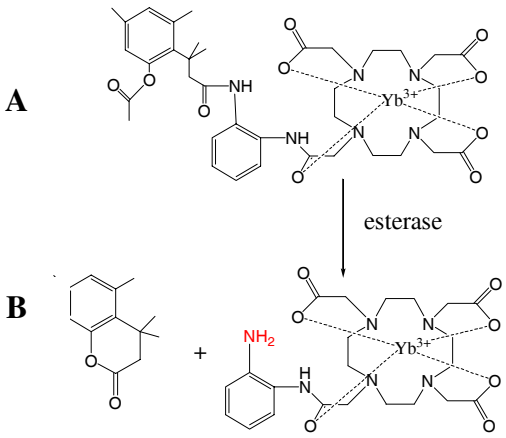

Figure 1. Scheme of the reaction of TML(Yb-DO3A-oAA) and esterase enzyme.

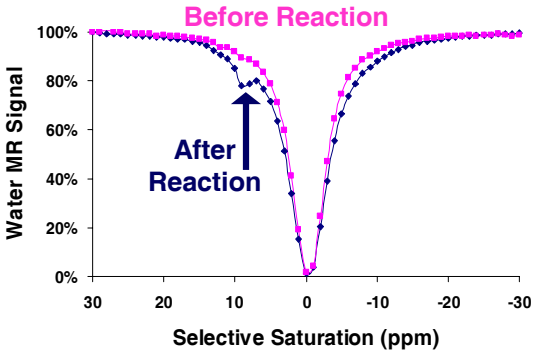

Figure 2. PARACEST spectra of 25 mM of TML(Yb-DO3A-oAA) before and after reaction with 3 units of porcine liver esterase enzyme. Spectra were acquired with a 4.2 μT preseaturation for 3 sec, at 37°C.

Conclusions:

We have developed a new PARACEST MRI contrast agent, TML-(Yb-DO3A-oAA) and demonstrated that it can detect esterase enzymes. This novel contrast agent may be used for tracking intracellular delivery.

References:

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