Enzyme-Responsive PARACEST MRI Contrast Agents That Assess Subsets of the Human Degradome

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Abstract

We have established a new platform technology for detecting multiple protease enzymes with MRI. New PARACEST MRI contrast agents have been developed with a peptidyl ligand that is cleaved by a specific protease, which changes the PARACEST effect of the agent. We've applied this approach to detect Caspase-3, and we are also applying our approach to detect Neutrophil Elastase. The MEROPS peptidase database lists 241 proteases that can be detected using this approach, which indicates that this approach has broad applicability. Multiple PARACEST MRI contrast agents can be selectively detected during the same MRI scan session, so that multiple protease-responsive PARACEST MRI contrast agents may be detected during the same MRI exam. Therefore, this platform technology can be applied to interrogate subsets of the human degradome.

Introduction:

T1 and T2 MRI contrast agents have been developed that detect enzyme activity,[2,3] but only one relaxivity-based MRI contrast agent can be detected during a single MRI scan session. We have recently reported a PARAmagnetic Chemical Exchange Saturation Transfer (PARACEST) MRI contrast agent that selectively detects the caspase-3 protease enzyme.[4] PARACEST agents can be selectively detected, so that multiple PARACEST agents can detect multiple enzymes during a single MRI scan session, and an additional PARACEST agent can be included as a "control" to account for pharmacokinetics within in vivo studies.[5] Therefore, this platform technology can be used to accurately detect multiple proteases.

Methods:

DEVD-(Tm-DOTA) was synthesized using a solid phase peptide synthesizer by following customized methods developed in our laboratory.[6] The CEST spectrum of DEVD-(Tm-DOTA) was obtained with a modified presaturation pulse sequence using a 600 MHz Varian NMR spectrometer (Figure 1). The effects of saturation power & duration, concentration, pH, and temperature were evaluated. 500 U of caspase-3 was added to solutions with varying concentrations of DEVD-(Tm-DOTA), and the PARACEST effect was measured in 5 min increments over 1 hour for each solution. Michaelis-Menten (M-M) kinetics were evaluated for this enzymatic reaction, and compared with M-M kinetics evaluations of caspase-3 cleavage of the fluorescence dye DEVD-AMC and the enzymatically inert Yb-DOTAM-Gly. The PARACEST MRI and fluorescence enzyme kinetics studies were repeated with caspase-8. Molecular modeling of DEVD-(Tm-DOTA) and caspase-3/8 was performed with InsightII (Molecular Simulations Inc). The MEROPS peptidase database was manually pruned to identify other exopeptidases that cleave a non-amino acid residue from the N- or C-terminus of a short peptide sequence, which may also be detected using this same methodology.[7] One of these exopeptidases, Neutrophil Elastase, was selected for PARACEST MRI detection with methylsuccinate-AAPV-(Yb-DOTA) and methylsuccinate-AAP-norvaline-(Yb-DOTA). The M-M kinetics of the cleavage of each of these PARACEST MRI contrast agents with Neutrophil Elastase, and molecular modeling of the agents with the enzyme, are in progress using the same procedure outlined for the DEVD-(Tm-DOTA) kinetics & modeling studies.

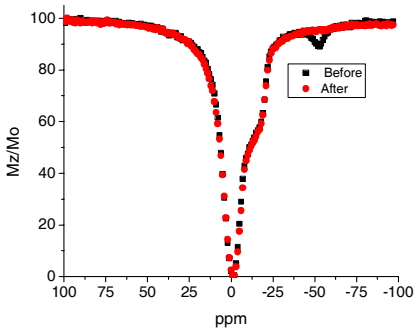

Figure 1. CEST spectrum of 20 mM DEVD-(Tm-DOTA) and Yb-DOTAM-Gly before and after cleavage of the DEVD peptidyl ligand with caspase-3. The PARACEST effect of DEVD-(Tm-DOTA) at -51 ppm is affected by enzyme catalysis, while the PARACEST effect of Yb-DOTAM-Gly at -16ppm remains unchanged.

Results and Discussion:

DEVD-(Tm-DOTA) can be detected at concentrations as low as 5.2 mM (assuming a minimum threshold of a 5% MR signal change), with only minor dependencies on physiological pH and temperature variations. M-M kinetics analysis revealed that caspase-3 selectively cleaves DEVD-(Tm-DOTA) relative to caspase-8. The catalytic efficiency of cleaving DEVD-(Tm-DOTA) by caspase-3 is *greater than* the catalytic efficiency of cleaving DEVD-AMC. Molecular modeling indicated that the greater bulk and polarity of the DOTA relative to the AMC can strain the scissile bond, which may explain this greater catalytic efficiency. Caspase-3 failed to modify Yb-DOTAM-Gly, which has a unique PARACEST chemical shift relative to DEVD-(Tm-DOTA), so that this enzymatically inert PARACEST MRI contrast agent can be used as a control agent to account for pharmacokinetics. The MEROPS database lists 241 exopeptidases that have the same potential for cleaving a peptide ligand that is coupled to a DOTA chelate. The cleavage of two peptidyl-(Yb-DOTA) PARACEST agents with Neutrophil Elastase are under investigation to assess the optimization of peptidyl ligands for enzyme detection through this platform technology.

Conclusions:

This report successfully demonstrates that PARACEST MRI contrast agents can selectively detect protease enzymes, and may be used as a platform technology to accurately detect multiple proteases during a single MRI scan session, in order to interrogate subsets of the human degradome.

References:

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