in vivo CEST imaging using Eu(III)-water molecule exchange system

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Introduction: Chemical exchange saturation transfer (CEST) has drawn considerable attention as a novel mechanism to produce contrast in MRI. Lanthanide(III)-based agents (PARACEST agents) among other exogenous CEST agents have the potential to increase sensitivity and specificity on CEST imaging to provide more detailed physiological and functional information than conventional contrast agents[1]. Such PARACEST agents may be designed to have exchangeable proton site that exchange with water protons at slow-to-intermediate exchange rates and that their frequencies are shifted and separated well away from the bulk water NMR frequency in order to avoid direct saturation. PARACEST agents based on Ln(III)-water molecule exchange have been shown to be highly tunable[2] so that agents with any desired water exchange rate could be designed. Europium(III)-based DOTA-tetraamide complexes are considered good candidates as the coordinated water molecule is well shifted (about +50 ppm) and its exchange rate is slow enough for sensitive detection *in vitro*[1]. Although a number of *in vitro* studies of agents have been reported, studies on *in vivo* CEST imaging with the exogenous agents are sparse and none have been based on a Ln(III)-water molecule exchange system. In the present study, we attempted to apply a newly designed Eu(III)-DOTA-tetraamide complex in *in vivo* imaging. Specifically, we first investigated the CEST properties of this complex *in vitro* in water and in plasma with various presaturation pulses and temperatures. Further the feasibility of detecting this agent *in vivo* by CEST imaging was examined in mice.

Materials and Methods: *Phantom study* Eu(III)-DOTA-$(Me_2Gly)_4^-$ (**Fig. 1**) was prepared and dissolved in either pure water or blood plasma at different concentrations (0.1 to 20 mM). Using the phantoms, we conducted the following series of experiments: [1] Z-spectra (100 to -100 ppm) were collected on the series of MR images to confirm the resonance frequency at 20 °C. A gradient echo sequence following presaturation pulse (B_1=14μT, duration = 5s) was used. Other imaging parameters were: TR/TE = 5006/2.8 ms, 38 × 38 mm FOV, 128 × 128 matrix, 2 mm slice thickness, NEX=1 [2] CEST imaging was performed at 20 °C in order to investigate the saturation time-dependency. A conventional spin echo sequence was used with presaturation pulses varying saturation times (B_1=14μT, duration = 1, 2, 3, 4, 5s) either at 48 ppm (on-resonance) or -48 ppm (off-resonance). Other imaging parameters were: TR/TE = 5023/14 msec, 38 × 38 mm FOV, 128 × 128 matrix, 2 mm slice thickness, NEX=1. [3] To study temperature dependency of the CEST effect of the agent, Z-spectra (80 to -80 ppm) were acquired with a presaturation pulse (B_1=18.8μT, duration=5s) NMR specrtoscopically at 27, 35, 37, 39 °C. *Animal study* Under anesthesia with 1-2% isoflurane, the femoral vein was cannulated with a tube for injection of the PARACEST agent in all mice. Animals were placed supine with the respiratory sensor, respect to the center of a RF coil. On single 1 mm coronal slab delineating both kidneys, CEST images were acquired using a fast spin echo sequence with a presaturation pulse (B_1=7μT, duration=4s) applied either at 45 ppm (on-resonance) or -45 ppm (off-resonance). Other imaging parameters were: TR/TE = 4078/9.4 ms, 30 × 30 mm FOV, echo train = 8, 128 × 128 matrix, 2 mm slice thickness, NEX = 8, resulting in a scan time of 9 min/image. After a set of on/off images was obtained, the agent were injected at a dose of 0.9mmol Eu^{3+}/kg over 2 minutes and the same set of images were collected at immediately, thereafter, every 20 min after the injection. *General remarks* All imaging studies were conducted using a 9.4 Tesla small-bore system (Varian) using a 38 mm birdcage coil and the NMR spectroscopy were performed with a 9.4T NMR system (Bruker). The magnitudes of the CEST effect in the *in vitro* experiments were measured as a % change in water intensity, $(SI_{off}-SI_{on})/SI_{off}$, where SI_{on} and SI_{off} are signal intensities either at "on-resonance" or "off-resonance". CEST images were obtained by subtracting an image collected with the presaturation pulse set "on-resonance" from a second image collected with the presaturation pulse set "off-resonance".

Results and Discussion: CEST effects of the agent reached steady-state with 4-5 seconds of presaturation pulse both in water and blood plasma when the same level of saturation power was applied. No significant differences were seen in the CEST spectra of Eu-DOTA-$(Me_2Gly)_4^-$ dissolved in water versus plasma at 20-27 °C but CEST effect was 18-25% less in the plasma sample compared to water at higher temperatures, 35-37 °C (**Fig. 2**). This suggests the agent interacts more strongly with albumin or other proteins in plasma at higher temperatures but this has not been confirmed by other experiments. Although CEST from the agent increases with higher B_1 saturation powers, so does the inherent tissue magnetization transfer (MT) effects *in vivo* so the effects of the PARACEST agent were obscured[3]. It was determined empirically that lower saturation power (7μT) was best for separating the two effects *in vivo*. A 20-25% reduction in water signal intensity was observed in the kidney and the renal cortex after injection of the agent for 20 minutes (**Fig. 3A**) and an ~20% decrease in SI was evident in the renal medulla between 20-40 minutes (**Fig. 3B**). It was

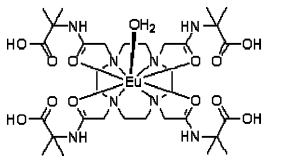

Fig. 1. Eu(III)-DOTA-$(Me_2Gly)_4^-$

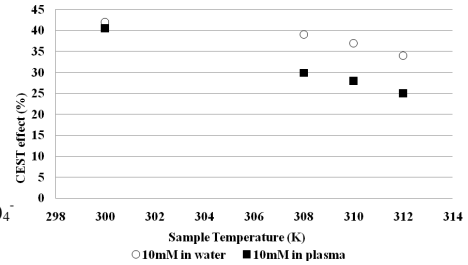

Fig. 2. Temperature dependency of CEST effects in water and blood plasma.

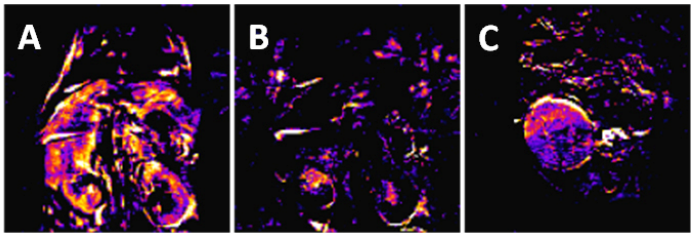

Fig. 3. Off-On MR images. Coronal slices through liver and kidney in A: 0-20min, B: 20-40min. Coronal slice through urinary bladder in C: 120-140min.

confirmed that the agents were cleared from the kidneys into the bladder after the anesthesia was stopped (**Fig. 3C**).

Conclusion: A Eu(III)-based PARACEST agent was successfully detected *in vivo* by CEST imaging even though the competing MT effects from tissue were significant. For optimal application of such agents *in vivo* at reasonable concentrations, agents that can be activated without concomitant activation of the inherent tissue MT effects will need to be developed.

Acknowledgements: Supported in part by grants from the Robert A. Welch Foundation (AT-584) and the National Institutes of Health (EB-04285). **References:** 1. Mark Woods, Donald E. Woessner, and A. Dean Sherry. *Chem. Soc. Rev*., 2006, **35**, 500-511. 2. Shanrong Zhang, Xiuyan Jiang, and A. Dean Sherry. *Helvetica Chimica Acta*, 2005 **88** 923-935. 3. M. T. McMahon, A. A. Gilad, J. Zhaou, P Z. Sun. J. W. M. Bulte, and P C. M. van Zijl. *Magn Reson Med*, 2006, **55**, 836-847.