

## Chemical Exchange Transfer Imaging of Creatine

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## INTRODUCTION

Creatine (Cr) plays an essential role in the storage and transmission of phosphate-bound energy. Because of vital role in the bioenergetics, the brain Cr level has been shown to change almost in all disease conditions. Proton magnetic resonance spectroscopy (<sup>1</sup>H MRS) is most common and widely applicable non-invasive technique used for the quantification of Cr in brain (1, 2) however, is limited to low spatial resolution, currently there are no generally applicable methods available for direct Cr imaging. Here a non-invasive approach for high-resolution mapping of the Cr in rat brain based on the chemical exchange saturation transfer (CEST) technique is demonstrated. The CEST effect from exchangeable amine protons (-NH<sub>2</sub>) of Cr and its role in pH measurement is previously demonstrated through phantom studies (3). In current study, sensitivity of CEST contrast to [Cr] in physiological range is demonstrated using phantom studies, and in addition, [Cr] was modulated in rat brain with tumor for validation of *in-vivo* CrCEST.

## MATERIALS AND METHODS

All *in-vitro* phantom MRI studies were performed on 7T Siemens whole body MRI scanner (Siemens Medical Systems, Erlangen, Germany). Animal experiments were performed in a 9.4T horizontal bore magnet. All *in-vitro* studies were carried out at 37±1°C.

**Phantom Preparation and MR Imaging:** The Cr solution (in PBS) phantoms with different concentrations (2, 4, 6, 8, 10 and 12 mM) at pH=7.0 were imaged using a new pulse sequence designed with an optimized frequency selective saturation pulse (Hanning windowed rectangular) followed by a segmented RF spoiled gradient echo (GRE) readout sequence. The CEST images were collected at saturation pulse peak B<sub>1</sub> of 150Hz and duration 3s over a frequency range of ±4ppm with step size of 0.2ppm. Imaging data for B<sub>0</sub> and B<sub>1</sub> mapping was also collected during the same scan session. The CEST data was collected for different combination of peak B<sub>1</sub> amplitude and saturation pulse durations.

**Rat Tumor Model Preparation and MR Imaging:** To validate the CrCEST *in vivo*, a rat brain tumor with compromised blood brain barrier (BBB) was used. The animal procedures are conducted under the approved institutional animal care and use committee protocol. The rats (n=3) were transferred to a 9.4T imager. Animals were kept under anesthesia and their body temperature maintained. CEST imaging of the rat brain was performed using a custom-programmed segmented RF spoiled gradient echo (GRE) readout pulse sequence with a frequency selective continuous wave (CW) saturation preparation pulse. CEST images were collected using a 3s saturation pulse at peak B<sub>1</sub> of 150Hz and frequencies at ±1.4ppm, ±1.6ppm, ±1.8ppm, ±2.0ppm, ±2.2ppm. The B<sub>0</sub> and B<sub>1</sub> mapping data was also collected for same imaging slice. Single voxel spectroscopy (SVS) was performed with STEAM and voxel was chosen from the center of the brain including the tumor region. After collecting the baseline CEST map and SVS spectra, the animals were injected with 2.5ml, 100 mM Cr solution through the catheter inserted in the tail vein. CEST data was collected for 2 hrs, at multiple time points, after Cr administration. SVS data was gathered at 2 hrs post injection.

**Data Processing:** B<sub>0</sub> map and multiple CEST data, acquired in the neighborhood of ±1.8ppm at steps of 0.2ppm, was used to generate corrected CrCEST images (±1.8ppm) using procedure similar to that described previously. Contrast is computed using equation,  $CEST = 100 * [S(-ppm) - S(+ppm)] / S(-ppm)$ . Normalization by -ve ppm instead of S<sub>0</sub> is essential for CEST in order to minimize contribution from direct water saturation (DS) and magnetization transfer (MT) effect (*in-vivo*). CrCEST maps were also corrected for B<sub>1</sub> inhomogeneities. The SVS spectral region between 0.5 ppm to 3.5ppm of the resultant spectrum was fitted to a sum of 6 Gaussian peaks centered at ~0.9ppm(lipid), ~1.3ppm(lipid+lac), ~2ppm(NAA), ~2.3 ppm(glu), ~3 ppm(Cr) and ~3.2 ppm (Cho). Peak integrals for all metabolites were calculated from the fitted peaks.

## RESULTS AND DISCUSSIONS

**Phantom Studies:** Phantom demonstrating the concentration dependence of CrCEST is shown in Figure 1A. For B<sub>1</sub> of 150Hz and duration of 3s, CrCEST contrast increased linearly with increasing [Cr] with a detection sensitivity of ~1.4% per mM [Cr]. Figure 1C shows that increased B<sub>1</sub> contributes to higher CrCEST contrast and the same is true for duration. CrCEST contrast was maximum at ~pH7.0 and decrease both directions with pulse parameter used.

**In-vivo Animal Imaging and SVS:** Figure 2 shows Cr modulation results of rat brain tumor. CrCEST maps, obtained at multiple time points after injection show that there is a significant increase in CrCEST with a concomitant comparable elevation in the ratio of Cr integral to NAA integral measured with SVS from a voxel containing mainly the tumor region. CrCEST increased by ~36% while -Cr/NAA ratio increased by ~28% two hours after Cr injection. CrCEST contrast increases mostly in the tumor region, demonstrating compromised blood brain barrier in tumor tissue. Similar results were observed from all three animals. The modulation results as such demonstrate the detection feasibility of *in-vivo* Cr using CEST technique, however, contaminations from other exchanging protons to base CEST image at 1.8ppm cannot be ruled out. Studies are underway to optimize saturation pulse parameters for minimizing contamination from other exchanging protons before using this technique for *in-vivo* mapping [Cr] in human.

## REFERENCES:

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## Acknowledgement:

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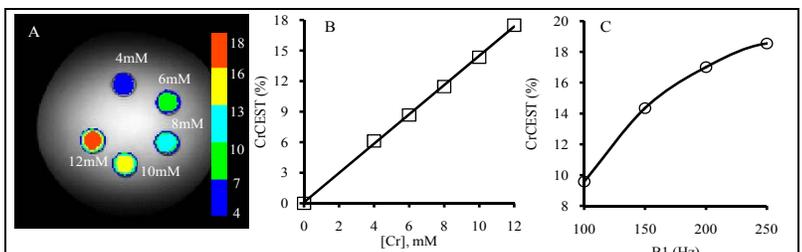


Figure 1. Dependence of CrCEST contrast on concentration is shown in A and B, at B<sub>1</sub>=150Hz and saturation duration =3s. Fig(C) shows CrCEST, from a phantom containing 10mM [Cr], as a function of B<sub>1</sub> for fixed duration of 3s.

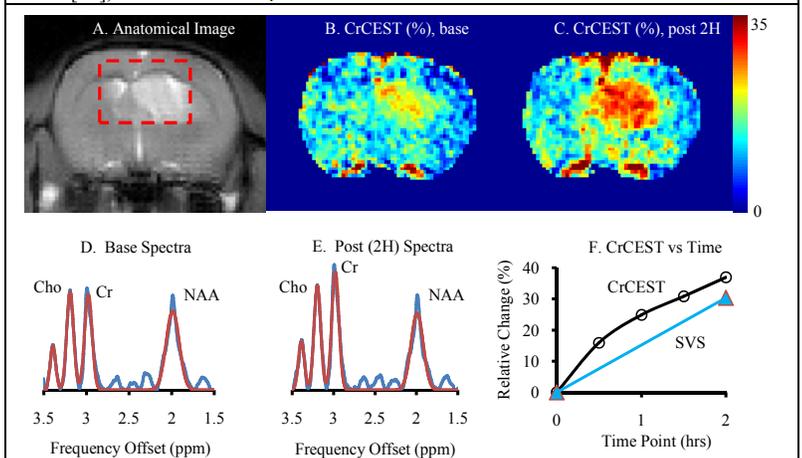


Figure 2. [Cr] modulation results of rat brain tumor using CEST with CW saturation pulse of 150Hz amplitude, 3s duration and SVS.