Dependence of CEST Effect from Amine Protons of Glutamate on pH

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INTRODUCTION

The use of CEST contrast for pH mapping has been demonstrated for labile protons of amide and amine protons of creatine (Cr) (1, 2). Amide proton CEST contrast has been shown to decrease with decrease in pH with a rat brain stroke model. The pH in stroke region of the rat brain decreases with progression of stroke effect. Here we demonstrate the dependence of Glutamate amine protons CEST (GluCEST) contrast on pH and its use in pH calibration. In order to demonstrate GluCEST dependence on pH experiments were performed in phantoms and *in-vivo* on a rat brain with a focal stroke induced via middle cerebral artery occlusion (MCAO).

MATERIALS AND METHODS

Phantom Data: Phantoms with 10mM [Glu] in PBS with different pH over a range 3-8 were prepared and imaged at 37C on 7T clinical scanner. The CEST data (S) at frequencies -6 to +6ppm with step size of 0.2ppm were acquired with flash readout, using *Hanning windowed* saturation pulse with 250Hz amplitude (B_1) and 2s duration. An image without CEST pulse (S_0), B_0 and B_1 map data were also acquired. The data was corrected for B0 in-homogeneities before z-spectra and CEST computation. The CEST contrast at frequency *3ppm* is used to compare pH dependence.

Stroke Data: Sprague-Dawley male rats were anesthetized with isoflurane. Focal cerebral ischemia was induced with the filament technique as described previously (3, 4). MCAO rats (n=3) were transferred to a 9.4T horizontal bore small animal and placed in a 35-mm diameter commercial quadrature proton coil. The animal procedures are conducted under the approved institutional animal care and use committee protocol. Animals were kept under anesthesia and body temperature was maintained using heating device during experiment. CEST imaging of the rat brain was started after 1 hour post MCAO and continued to until 4.5 hours using a frequency selective *continuous wave* (CW) saturation pulse followed by a segmented RF spoiled GRE readout sequence. The sequence parameters were: FOV=35×35mm^2, slice thickness=2 mm, GRE flip angle=15^o, GRE readout TR=6.2ms, TE=2.9ms, matrix size=128×128. CEST images were collected using a 1s saturation pulse at peak B_1 of 250 Hz and frequencies at ±2.4 to ±3.6ppm with step size of 0.2ppm along with image without applying saturation pulse (S_0). The data at frequencies in the neighborhood of ±3ppm along with B_0 map is used for correcting B_0-inhomogenities. Data for B_1 and B_0 maps were also acquired. GluCEST contrast was averaged for regions of interest from both ischemic ipsilateral and contralateral hemispheres of the rat brain.

RESULTS AND DISCUSSIONS

Figure 1 shows z-spectra from Glu phantoms at pH values of 4, 7 and 8. Z-spectra at pH 4 show a clear dip centered around 3ppm and it become broad at pH 7 and disappears completely at pH 8. **Figure 2** shows GluCEST vs. pH curve. The curve shows a clear elevation of CEST contrast with a decrease in pH from 7.4 to 6. At low pH (<6) GluCEST again start decreasing. In fact GluCEST shows non-linear dependence on pH over a range from 1-8. However, over a physiologically relevant small range of pH variations (7.4 to 6) CEST effect is linear. The curve behavior may slightly be different in case of *in-vivo* compared to phantoms due to differences in exchange rates. **Figure 3** shows GluCEST images of ischemic rat brain acquired with CW saturation pulse (250Hz for 1s) at 9.4T. Fig.3A shows rat brain anatomic proton image. Fig. 3B shows the GluCEST map (colorbar represents GluCEST contrast in percentage) of the rat brain acquired at 4.5 hrs following the induction of stroke. Fig. 3C shows the GluCEST contrast vs. time after MCAO at ROI within the rectangular areas shown in Fig. 3A. While gradual elevation in the GluCEST contrast can be seen in the ipsilateral side, there is a fairly constant GluCEST in the contralateral side until 4.5 hours. In the ipsilateral side GluCEST is almost doubled at 4.5 hrs after occlusion. In case of stroke decrease in pH is well known. In this well characterized model, in about 4-5 hours following stoke induction, the pH will decrease to ~6.5 (REF). A clear elevation in GluCEST can be seen with increase in stroke level. In this model, while elevation in Glu, Asp, and GABA concentrations are reported their aggregate change may be well under 1 mM (REF) and their contribution to the observed CEST negligibly small (~1%). While other factors like increase in T_2 values, decrease in MT effect etc, could also contribute to this phenomenon their contributions are expected to be rather small. It is worthwhile to point out that this pH dependence is inverse of the previously reported amide proton results and is consistent with the exchange rate of glutamate being of the order of 1000 – 3000 Hz in the pH range of the stroke model where as the reported exchange rate of amide protons is of the order of 10 to 50 Hz. Linear increase of GluCEST with decreased pH observed in the phantoms potentially could be used in assessing the evolution of stroke in vivo as well as other pathologies such as tumors associated with significant pH changes. However, such studies require correlation between GluCEST and pH changes in vivo. Further work is in progress to quantify changes in pH as well as GluCEST in the stoke model as well as in other pathologies.

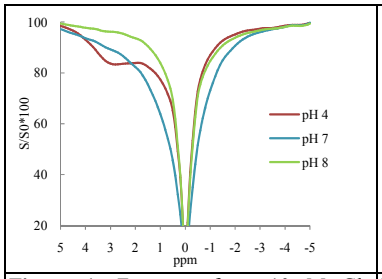

Figure 1. Z-spectra from 10mM Glu solution in pbs at pH4, 7 and 8 on 7T

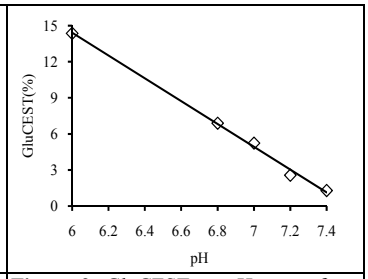

Figure 2. GluCEST vs pH curve from 10mM Glu solution in pbs on 7T

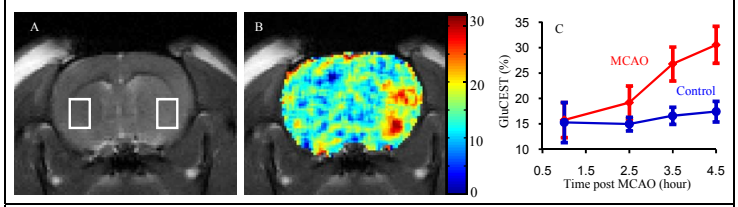

Figure 3. Anatomical Image (A), GluCEST map (B) after 4.5Hrs, and GluCEST vs. time (C) after MCAO at ROI within the rectangular areas shown in Fig. A of ischemic rat brain acquired with CW saturation pulse (250Hz for 1s) at 9.4T.

REFERENCES

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