Detection of carboxypeptidase G2 activity with chemical exchange saturation transfer magnetic resonance.

Yann Jamin1, Thomas R Eykyn1,2, Evon Poon2, Caroline J Springer2, and Simon P Robinson1

1Division of Radiotherapy and Imaging, The Institute of Cancer Research, Sutton, Surrey, United Kingdom, 2Division of Imaging Sciences and Biomedical Engineering, Kings College London, London, United Kingdom, 3Division of Cancer Therapeutics, The Institute of Cancer Research, Sutton, Surrey, United Kingdom

Introduction. The bacterial enzyme carboxypeptidase G2 (CPG2) is a bacterial enzyme which mediates the release of the C-terminal glutamate residue from a wide range of N-acylating moieties including carbonyl, phenol or amine mustards (prodrugs). CPG2 undergoes a promising gene-directed enzyme prodrug therapy (GDEPT) strategy due to enter Phase I clinical evaluation (1). In GDEPT, the gene encoding the prodrug-activating enzyme is targeted selectively to the tumor prior to administration of the prodrug, resulting in the activation of the cytotoxic drug specifically in the tumor. The success of CPG2-based GDEPT relies on the careful optimization of the timing of injection of the prodrug following expression of the transgene and the generation of sufficient concentrations of CPG2 in the tumor. Non-invasive imaging strategies to monitor the prodrug-activating enzyme activity and its bio-distribution would thus be invaluable in guiding the successful translation of this promising therapeutic approach to the clinic. This study demonstrates the potential of CEST-MRI to monitor CPG2 activity in vivo.

Methods. All studies were performed on an 11.7T (Bruker) system equipped with a 5mm BBO probe. CEST-NMR: Z spectra of solutions of 10 mM glutamate, MTX, Folic acid and 3,5-DFBGlu were acquired at 37°C in PBS at pH 5-8. Z spectra were acquired using a series of spectra (NS=4, TR=17 s) with the saturation frequency at a different offset frequency from -5 to 5ppm from the water resonance (increment 0.2ppm). The frequency selective saturation was achieved using CW presaturation for 5sec and followed by a 30Hz offset radio-frequency pulse of DAMPA, measured by conventional 1H-MRS (2), whilst MTX, folic acid and 3,5-DFBGlu did not elicit any detectable CEST effect at 3.5ppm, the frequency of amide protons, at pH 5-8 (Fig 2). The addition of MTX to the extracts of CPG2-expressing WiDr cells caused a time dependent increase of the CEST effect at +3ppm. CPG2 activity was confirmed through the increase in the concentration of 2,4-diamino-N^2-methylpyridic acid (DAMPA), measured by conventional 1H-MRS (Fig 3). Importantly, DAMPA is not expected to elicit a CEST effect at +3 ppm, indicating that the increase in CEST signal correlates with the CPG2-induced increase in glutamate concentration. Figure 4 shows a similar activation of the CEST effect following the addition of 3,5-DFBGlu to extracts of CPG2-expressing WiDr cells. CPG2 activity was also detected by the change in CEST signal at 2.1 ppm with improved sensitivity, which was confirmed by the comparison of the z spectra in the CPG2-expressing and control cell extracts acquired at the end of the experiment.

Discussion and Conclusion. This study demonstrates the potential of CEST-MRI to monitor CPG2 activity through the “activation” of the GluCEST reporter probe 3,5-difluorobenzoyl-L-glutamate. A novel imaging method for metabolic imaging of glutamate, resulting in the “activation” of the GluCEST contrast. We have previously demonstrated the utility of 19F MRS in combination with the imaging reporter 3,5-DFBGlu to monitor CPG2 activity in vivo (3). Although the 19F MRS approach provides invaluable information on the level of CPG2 activity in the tumor, the inherent lack of sensitivity of conventional MRS precluded the assessment of the heterogeneous distribution of the reporter probe. GluCEST MRI is an attractive method for metabolic imaging of glutamate (2), since it utilizes the bulk signal from water protons enabling the indirect detection of glutamate concentration at higher temporal and spatial resolution compared to its conventional direct detection by 1H MRS. GluCEST MRI could thus provide a noninvasive and clinically available method to not just image the biodistribution of CPG2 mediated cleavage of the imaging reporter 3,5-DFBGlu, but also the entire portfolio of prodrugs utilized in GDEPT. This study thus encourages the translation of CEST-MRI to CPG2-based gene therapy in vivo.


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