In vivo ^{19}F MRS detection of Carboxypeptidase G2 activity

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Introduction

Carboxypeptidase G2 (CPG2) is used in Gene Directed Enzyme Prodrug Therapy, a promising gene therapy strategy for cancer treatment to activate non toxic prodrugs into cytotoxic DNA alkylating agents (1). We have previously demonstrated the value of 3,5-DiFluoroBenzoylGlutamic acid (3,5-DFBGlu) as a potential reporter probe for imaging of CPG2 activity *in vivo* (2). 3,5-DFBGlu is cleaved by CPG2 into 3,5-Difluorobenzoic acid (3,5-DFBA) resulting in a detectable 1.4 ppm chemical shift change on a ^{19}F MRS spectrum.

Here we report:

1. *In vivo* non-invasive ^{19}F MRS detection of CPG2 activity following intravenous injection of 3,5-DFBGlu in mice bearing xenografts derived from human colon adenocarcinoma WiDr cells stably expressing CPG2.
2. No acute adverse effects after intravenous injection of 750mg/kg 3,5-DBFGlu.
3. Imaging of the location of CPG2 activity with 3D ^{19}F chemical shift imaging (CSI) due to the excellent signal-to-noise ratio afforded by the two equivalent ^{19}F of the reporter.

Material & Methods

Animal model: Xenografts were established in nude female NCr mice by subcutaneous inoculation (0.1 ml) of a suspension of 8.10^6 cells stCPG2(Q)3 WiDr (stably expressing CPG2, n=3) or LacZ WiDr cells (stably expressing ß-galactosidase, control n=3) in phosphate buffered saline (PBS).

MRS studies were performed on a 7T Bruker Micro-Imaging system using a 2 cm diameter surface coil tuned to either 300 MHz for shimming and ^{1}H reference images or 282.34 MHz for ^{19}F MRS and 3DCSI acquisition.

Unlocalized ^{19}F MRS spectra were acquired using a 60º square pulse-and-acquire sequence (pulse length 70 µs, 16 transients, TR 3s, total scan time: 1min 3s). A ^{19}F MRS baseline measurement was performed followed by the intravenous injection of 750mg/kg 3,5-DFBGlu in PBS via a lateral tail vein line

At 1 hour post injection, a ^{19}F 3D-CSI sequence (8 x 8 x 8 phase encoding steps, 5 cm^2 FOV, 5 cm slice thickness, and 25 kHz bandwidth) was acquired using a 90° adiabatic pulse (TR: 1500 ms, one acquisition, acquisition time: 12 min 43 sec). ^{1}H reference images were acquired using a gradient echo (GE) sequence (TE= 6.2 ms, TR= 101.3 ms, 8 transients, matrix size: 128x128). Images from 8 slices (5x5cm field of view, 1mm thick, 2 mm gap between slices) were acquired through the tumor in 3 different orientations (axial, sagittal, and coronal). ^{19}F 3D CSI datasets were processed using our in-house software developed using the IDL platform.

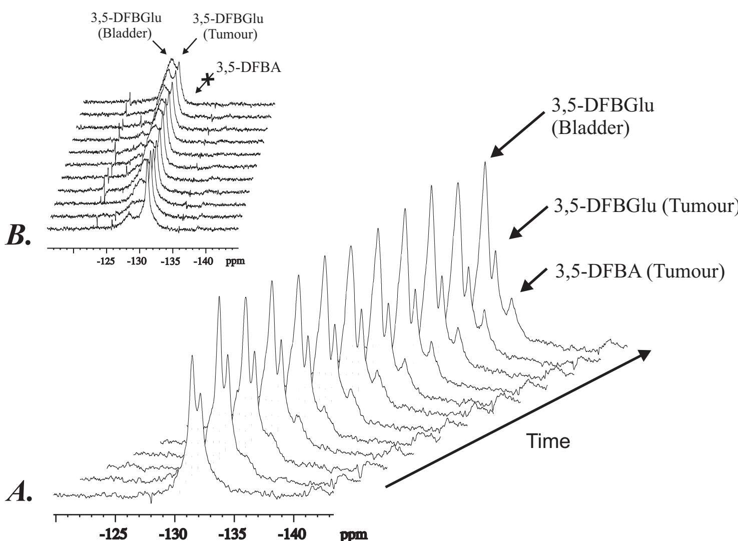

Figure 1. ***A.*** Stack plot of a time series of ^{19}F unlocalized spectra recorded with a 60° pulse a time resolution of 5 min (5 spectra added) that demonstrates conversion of 3,5-DFBGlu into 3,5-DFBA by stCPG2(Q)3 expressing WiDr xenograft after an i.v. injection of 750mg/kg dose. ***B.*** shows the control experiment with ß-Gal expressing WiDr xenograft. No conversion of 3,5-DFBGlu was observed.

Results and Discussion

Figure 1 shows the detection of CPG2-mediated conversion of 3,5-DFBGlu by ^{19}F MRS. Although baseline separation is not achieved, the 1.4 ppm chemical shift is sufficient to observe the 3,5-DFBA resonance. A contaminating signal arises from the region outside the tumour (mainly the bladder). However the high signal to noise ratio (SNR~23 for 1 min measurement) encouraged the use of 3D CSI that offers baseline detection and overcomes the spectral contamination by localising of the spectral signatures (Figure2).

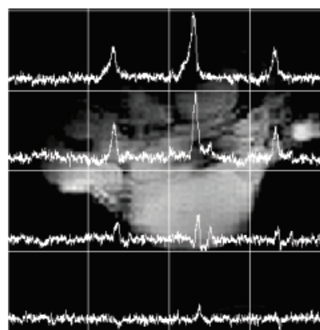

Figure 2 Axial slice through the tumour of a mouse bearing a subcutaneous xenograft that stably expressed stCPG2(Q)3. This was extracted from a ^{19}F 3D CSI acquired 1h post injection.

Conclusion

We demonstrate the value of 3,5-DFBGlu to detect CPG2 activity non-invasively *in vivo* with direct applications to monitor CPG2-based GDEPT as well as the potential to use CPG2 as a gene reporter.

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References (1) Niculescu-Duvaz, I. Mol Biotechnol. 30(1), 71, 2005. (2) Jamin, Y. *Proc. ISMRM* 463, *(2007)*

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