Evaluating Carboxypeptidase G2 (CPG2) based Gene-Directed Enzyme Prodrug Therapy using ^{19}F Magnetic Resonance Spectroscopy: 3,5-Difluorobenzoyl-glutamic acid as a potential *in vivo* reporter of CPG2 activity.

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Introduction

Gene-Directed Enzyme Prodrug Therapy (GDEPT) is a targeted strategy for the treatment of solid tumours. A vector-delivered gene encodes for an enzyme that, once expressed, converts subsequently administered prodrugs into cytotoxic drugs[1]. The carboxypeptidase G2 (CPG2) GDEPT system can activate alkylating agent prodrugs into cytotoxic drugs by hydrolysing the glutamate moiety. The system has shown promising results in preclinical studies[2]. Here we report *in vitro* and preliminary *in vivo* studies using ^{19}F Magnetic Resonance Spectroscopy (MRS) approaches and 3,5-Difluorobenzoylglutamic acid (3,5-DFBGlu) as a potential non toxic *in vivo* reporter probe for CPG2 activity. In particular, we show **(1)** that the ^{19}F chemical shift change (1.4 ppm) upon CPG2-mediated cleavage can be utilised to monitor the dynamic conversion of 3,5-DFBGlu into 3,5-Difluorobenzoic acid (3,5-DFBA). We also demonstrate the utility of ^{19}F MRS to measure the enzyme activity in CPG2 expressing monolayers. **(2)** 3,5-DFBGlu is delivered to a wide panel of tumour xenografts in concentrations sufficient to be MR visible. **(3)** In CPG2 expressing xenograft, 3,5-DFBA is generated and well resolved from 3,5DFBGlu.

Materials and methods

Experiments were carried out on a 7T Bruker Micro-Imaging system using an in-house built 2cm diameter surface coil tunable to either ^{1}H or ^{19}F frequencies. *Cell studies:* 2mM of 3,5-DFBGlu was added to the medium of confluent monolayers with 0, 10, 50, 100 % of stCPG2(Q)3-stably expressing human colon adenocarcinoma WiDr cells (control cells-stably expressing LacZ). ^{19}F NMR spectra were acquired at ~ 37ºC using an adiabatic 90 ° pulse-and-acquire sequence (NA: 128, TR 2s, total scan time: 5min 40s). *In vivo 3,5-DFBGlu tumour pharmacokinetic study*: a pre-established 1500mg/kg dose was injected via an i.p. line into mice bearing a wide panel of subcutaneous xenografts (including U87MG, HCT116, B16, Hepa1-6). A ^{19}F 3D-CSI sequence (8 x 8 x 8 PE steps, 40 mm FOV) was used to interrogate the tumour. Sequence parameters were set as: TR: 1500 ms, flip angle: 90°, one acquisition, acquisition time: 12 min 43 sec. 3D CSI datasets were processed using our in-house software developed using the IDL platform. *Study of stCPG2(Q)3 WiDr xenografts:* ~10 µmol was injected intratumourly into 3 stCPG2(Q)3 WiDr xenografts. ^{19}F NMR spectra were acquired using a 90º adiabatic pulse-and-acquire sequence (NA: 32, TR 2s, total scan time: 1min 42s) between CSI experiments.

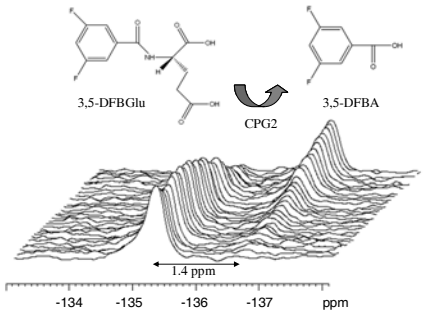

Fig 1. Conversion of 3,5-DFGlu into 3,5DFBA by a stCPG2(Q)3 WiDr mono-layer.(22.7 min time resolution)

Results

Cell studies: Fig. 1 shows that CPG2 cleaves 3,5-DFBGlu into 3,5-Difluorobenzoic acid (3,5-DFBA), with a consequent change in frequency of 1.4 ppm in ^{19}F MR resonance at physiological pH. Fig. 1 and Fig. 2a also show that we can utilise this chemical shift change to monitor the dynamic conversion of 3,5-DFBGlu in stCPG2(Q)3 expressing WiDr mono-layers. Fig. 2b shows that the MR-determined CPG2 activity linearly correlates with the proportion of stCPG2(Q)3 WiDr cells in the monolayer. The good SNR ~18.5 for a 2mM starting concentration of 3,5-DFBGlu monolayer experiment encouraged translation to *in vivo* studies.

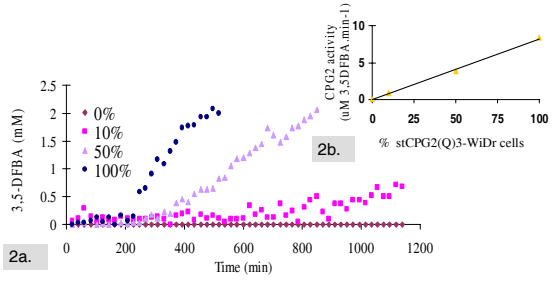

Fig 2. MR determined CPG2 activity in WiDr monolayers with 0, 10, 50, 100% stCPG2(Q)3 WiDr cells.

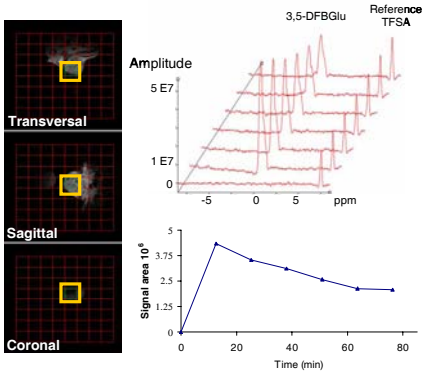

Fig 3. *In vivo* pharmacokinetics of 3,5-DFBGlu in a B16 tumour using ^{19}F 3D CSI. Stacked Spectra extracted from the highlighted voxel. Time resolution:12 min 43 sec

In vivo 3,5-DFBGlu tumour pharmacokinetic study: 3,5-DFBGlu was detected in all the tumours studied. Fig 3 shows that after reaching a maximum at the first acquisition post injection, the concentration of 3,5-DFBGlu remained relatively stable (50-40% of maximum after 1h) with good SNR in the ^{19}F spectrum over the experimental time course.

In vivo conversion of 3,5DFBGlu in stCPG2(Q)3 WiDr xenografts: Fig. 4a and 4b shows two resonances separated by 1.4 ppm at 20 minutes post intratumoural injection and then a visible increase of 3,5DFBA concentration over time. The rate of conversion appears to be compatible with the scanning time of localised MRS approaches.

Discussion and Conclusion

This study shows that 3,5-DFBGlu is a ^{19}F MRS reporter probe of CPG2 activity as a consequence of the 1.4ppm chemical shift change upon CPG2 mediated cleavage. It also demonstrates that ^{19}F MRS is a reliable and accurate approach to assess CPG2 activity as shown in the stCPG2(Q)3 expressing WiDr mono-layers. Moreover 3,5-DFBGlu has not shown any toxic effects and is offering appropriate *in vivo* tumour pharmacokinetics as well as MR compatible intra-tumoural concentration and is therefore a potential *in vivo* reporter of CPG2 activity. Finally the overall good SNR and the rate of conversion that CSI protocols are appropriate techniques to non invasively assess *in vivo* the CPG2 activity.

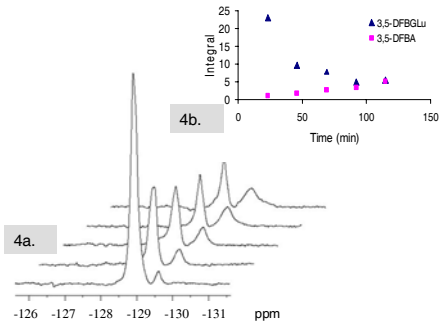

Fig 4. *In vivo* conversion of 3,5DFBGlu in a stCPG2(Q)3 WiDr xenograft. First data point was obtained 20 minutes post intra-tumoural injection. Each point is separated by 21 minutes.

Acknowledgements

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References

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