Noninvasive detection of Carboxypeptidase G2 activity *in vivo* using ^{19}F 3D CSI

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INTRODUCTION

Carboxypeptidase G2 is a bacterial enzyme that is currently employed in a range of cancer chemotherapy strategies, such as gene-directed enzyme prodrug therapy (GDEPT), designed to selectively activate nontoxic prodrugs into potent cytotoxics in the tumor (1). These therapeutic strategies would benefit from appropriate imaging techniques to assess the biodistribution of CPG2 activity. We have previously demonstrated the non-invasive detection of CPG2 activity using ^{19}F MRS, through the CPG2-mediated cleavage of the reporter probe 3,5-difluorobenzoyl-L-glutamic acid (3,5-DFBGlu) into 3,5-Difluorobenzoic acid (3,5-DFBA) which occurs with a detectable 1.4 ppm chemical shift change (2). Here we report on the use of ^{19}F 3D CSI to dynamically and quantitatively monitor CPG2 activity in xenograft tumors engineered to stably express CPG2.

MATERIALS & METHODS

- *In vivo* MRS studies Serial ^{19}F 3D CSI datasets were acquired immediately following i.v. injection of 750mg/kg 3,5-DFBGlu to mice bearing human colon WiDr xenografts that stably express stCPG2(Q)3 (n=6) or β-Galactosidase (control, n=3) positioned in a ^{1}H/^{19}F surface coil in a 7T Bruker MicroImaging system. (*^{19}F 3D CSI*: 90° adiabatic pulse, TR = 1500 ms, NS = 1, BW = 25 kHz, 8 x 8 x 8 phase encoding steps, 5 x 5 x 5 cm FOV, AQ = 12 min 43s). One ^{1}H 3D CSI dataset was acquired with the same parameters to obtain signal from tissue water for quantification of the ^{19}F metabolites (3).

- *Ex vivo* validation Tumors and normal tissues were excised and immediately snap-frozen and homogenized as described in (4). CPG2 activity was assessed by recording 100 serial ^{1}H-decoupled ^{19}F spectra at 470 MHz and 30 °C following addition of 6 µmol 3,5-DFBGlu to the homogenates (11.7 T Bruker Avance system, 90° pulse-and-acquire sequence, 8 transients, TR= 4.6 s, AQ= 35s). CPG2 activity was determined by linear regression on the decrease of the 3,5-DFBGlu resonance over time. 3,5-DFBGlu and 3,5-DFBA concentrations in tissues were determined with ^{19}F MRS on the remaining homogenates (+ iced cold methanol) at 470 MHz using the tetrafluorosuccinic acid as a concentration reference.

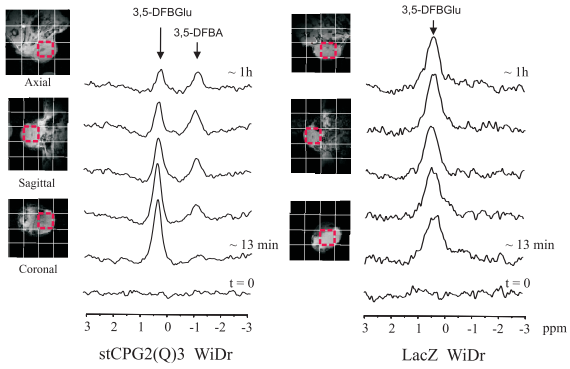

Figure 1. Non invasive detection of CPG2 activity in WiDr models stably expressing stCPG2(Q)3 using serial ^{19}F spectra from a voxel (---) within the tumor extracted from ^{19}F 3D CSI datasets. No CPG2 activity was detected in the control cohort.

RESULTS AND DISCUSSION

- *In vivo* MRS: Figure 1 shows that the high SNR afforded by the two ^{19}F of 3,5-DFBGlu allows the use of ^{19}F 3D CSI to detect dynamically CPG2 activity in WiDr tumors, which stably express CPG2. No 3,5-DFBA was detected in any of the control xenografts. 3,5-DFBGlu presents very stable tumor pharmacokinetics over 40 min (Fig. 2), as shown in the control cohort, which allows the measurement of the increasing concentration of 3,5-DFBA (12.3 ± 2.1 µM.min^{-1}) resulting from CPG2 activity in CPG2-expressing xenografts.

- *Ex vivo* validation:
The *ex vivo* ^{19}F MRS assay of CPG2 activity confirms that no CPG2 activity was present in control tumors whereas stCPG2(Q)3 xenografts demonstrate an activity of 2.1 ± 0.4 µmol 3,5-DFBGlu.min^{-1}. g^{-1} (= 1.1 unit CPG2, n=3, SD) which is similar to the activity found in xenograft models undergoing CPG2-based therapy (5).
3,5-DFBA was detected in homogenates of control tumors and normal tissues one hour post injection. The concentration of 3,5-DFBA (1.8 ± 0.6 µmol.g^{-1} of wet tissue (n=3, SE) = ~ 2.8 % of the injected dose of 3,5-DFBGlu) was found to be ~ 10 times higher in stCPG2(Q)3 tumours compared with control tumours (p = 0.09). Normalising the concentration of 3,5-DFBA to the total amount of 3,5-DFBGlu and 3,5-DFBA should correct for the bias due to differences in the delivery of the reporter probe to the tumour and different organs across the cohort. The ratio $^{3,5\text{-DFBA}}/_{3,5\text{-DFBA+3,5-DFBGlu}}$ in stCPG2(Q)3 tumour xenografts was ~ 4 and significantly ($p < 0.001$) higher compared with control tumours (Fig. 3). No significant difference was found in this ratio across the tumour and the normal tissues of the control mice. A significant difference ($p < 0.001$) was found in the concentration of 3,5-DFBA across the tumour and the normal tissues of mice bearing stCPG2(Q)3 WiDr xenografts. The presence of 3,5-DFBA in tissues of the control cohort may be due to its presence as an impurity in the injected solution (3,5-DFBGlu >99% pure) or by the cleavage of 3,5-DFBGlu by bacteria of the intestinal flora.

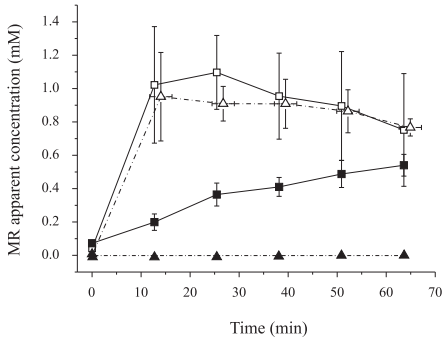

Figure 2. *In vivo* pharmacokinetics of 3,5-DFBGlu derived from ^{19}F MR signals in control (–△–)WiDr tumors (n=3) and in stCPG2(Q)3 tumors (–□–) (n=6) where it is converted by CPG2 into 3,5-DFBA (–■–) (not corrected for T_1 partial saturation effects) . No 3,5-DFBA (-▲-) was detected in control WiDr tumors.

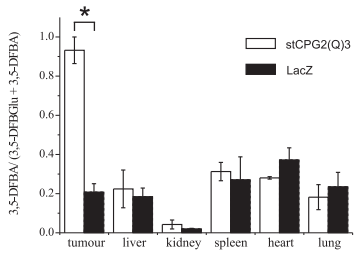

Figure 3. Ratio $^{3,5\text{-DFBA}}/_{(3,5\text{-DFBGlu+3,5-DFBA})}$ in homogenates of tumor and normal tissues excised from mice bearing stCPG2(Q)3 and LacZ WiDr tumor xenografts excised one hour following intravenous injection of 750 mg/kg 3,5-DFBGlu. Data points represent mean values (±SE) from 3 mice.

CONCLUSION

We demonstrate that ^{19}F 3D CSI in combination with a ^{1}H/^{19}F surface coil can quantitatively and noninvasively assess the CPG2-mediated conversion of 3,5-DFBGlu to 3,5-DFBA in WiDr xenograft models that express relevant levels of CPG2 activity for CPG2-based therapy. The measured rate of production of 3,5-DFBA could provide a surrogate marker of CPG2 activity levels in the tumor.

ACKNOWLEDGEMENTS

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