Imaging Cancer Gene Therapy using ^{13}C Hyperpolarised MR Technology

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INTRODUCTION

Within the last 3 years Dynamic Nuclear Polarization has been shown to be a highly versatile technique for hyperpolarizing ^{13}C or ^{15}N in a variety of small molecules in solution state and has been shown to be capable of enhancing the MR signal of ^{13}C NMR by more than a factor 10,000 (1). Golman *et al* (2) demonstrated the feasibility of carrying out fast metabolic imaging of hyperpolarized substrates *in vivo* with ^{13}C labelled pyruvate to probe enhanced metabolic activity in tumours due to lactate dehydrogenase (LDH). This work yields both diagnostic information as well as the potential to probe response to novel therapeutic agents.

Here we report hyperpolarisation of 3,5-Difluorobenzoylglutamic acid (3,5-DFBGlu), an *in vivo* reporter of the enzyme Carboxypeptidase G2 (CPG2). 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 (3). 3,5-DFBGlu is rapidly cleaved by CPG2 into 3,5-Difluorobenzoic acid (3,5-DFBA) and glutamic acid.

We have previously reported the potential of. 3,5-DFBGlu as an *in vivo* reporter of CPG2 activity (4) . Here we report:

1. the dynamic monitoring of CPG2-mediated conversion of 3,5-DFBGlu in solution using (natural abundance) ^{13}C MRS.
2. the ^{13}C chemical shifts resulting from CPG2-mediated cleavage and the ^{13}C spin-lattice relaxation time of three ^{13}C nuclei, potential candidates for hyperpolarization and *in vivo* application.
3. the successful hyperpolarization of these three (natural abundance) ^{13}C nuclei of interest.

MATERIAL & METHODS

^{13}C MRS. 100 µL of a solution of CPG2 (1 unit.mL^{-1}) were added to 100 mM of 3,5-DFBGlu solutions prepared in the enzyme buffer (100 mM TrisHCl pH 7.3, 260 µM $ZnCl_2$). Studies were performed on an 11.7T Bruker Avance system at ~ 37ºC using a 90º pulse-and-acquire sequence (16 transients, repetition time 30s, total scan time: 8min 30s) with Waltz decoupling. Serial spectra were acquired over time. Spin lattice relaxation time measurements were performed on a 100mM solution of 3,5-DFBGlu and 3,5-DFBA in 100mM Tris 100 mM TrisHCl pH 7.3, 260 µM $ZnCl_2$ using an inversion recovery sequences with Waltz ^{1}H decoupling.

Hyperpolarizing 3,5-DFBGlu. 4.5mg of 3,5-DFBGlu (natural abundance 1% ^{13}C) was dissolved in a solution containing 3.18mg of the free radical OX63 with a glassing agent: 2:1 DMSO:H_2O. The sample was polarized for 3 hours in a HyperSense® DNP polarizer at low temperature (1.4K) in a superconducting magnet (3.35T) with microwave irradiation at 94 GHz. The polarized sample was dissolved in 4ml EDTA solution (~4 mM 3,5-DFBGlu) and transferred to a high field NMR system (9.4T) prior to detection of ^{13}C.

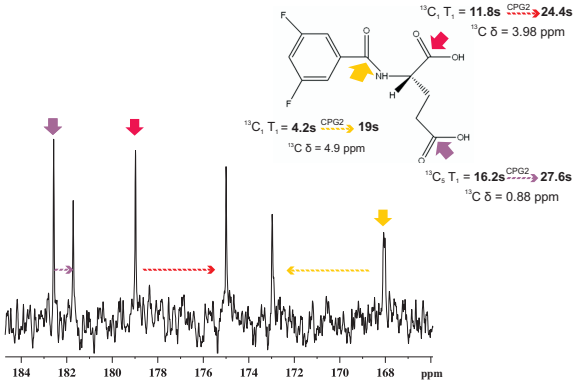

Figure 1. : 3,5-DFBGlu: a potential *in vivo* ^{13}C MR reporter molecule for CPG2 activity. The ^{13}C (natural abundance) spectrum (NS 8, TR=30s) was acquired 1h after adding 100mM of 3,5-DFBGlu to a solution of CPG2 and show the chemical shift change upon cleavage (---▶) of three ^{13}C from carbonyl groups (⇩). Top shows the structure of 3,5-DFBGlu and summarizes the changes in the ^{13}C chemical shift and T_1 upon cleavage by CPG2 into 3,5-DFBA.

RESULTS

Figure 1 shows that 3,5-DFBGlu presents three ^{13}C (C_1 of benzoyl core, C_1 and C_5 of glutamate moiety) with relatively long T_1. The C_1 of benzoyl core and C_1 glutamate moiety also present sufficient chemical shift difference upon CPG2-mediated cleavage to translate this approach *in vivo*. The bottom panel of figure 2 shows the polarized ^{13}C spectrum. The three carbonyl resonances can be clearly observed at high chemical shift. The top panel shows a time series with spectra recorded every 3.2s and employing a 30 degree flip angle. At least 10 spectra can be resolved before the signal decays to thermal equilibrium yielding a maximum time resolution of about 30s.

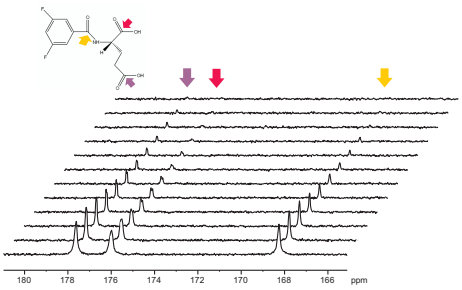

Figure 2. Hyperpolarization of ^{13}C in 3,5-DFBGlu (4mM). Top stack plot shows a time series recorded with a 30 degree pulse every 3.2s . Bottom shows the spectrum immediately after transfer to the NMR system.

DISCUSSION AND CONCLUSION

We report the hyperpolarisation of three carbonyl ^{13}C of the *in vivo* reporter probe of CPG2: 3,5-DFBGlu. They demonstrate sufficiently long T_1 and chemical shift difference upon CPG2-mediated cleavage to be translated to *in vivo* application. Isotopically labelling one of the candidate ^{13}C will provide a further 100-fold enhancement of the signal and the potential to develop real-time metabolic strategies for imaging hyperpolarized ^{13}C 3,5-DFBGlu and thereby yield metabolic maps of CPG2 activity and transgene expression.

ACKNOWLEDGEMENTS This work was supported by Cancer Research UK [CUK] C1060/A5117 and C309/A8274, The Royal Society and Basic Technology Programme GR/S23612/01.

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