Hyperpolarised ^{13}C MRS on a 3T Clinical Platform: Kinetic Modelling of Pyruvate-Lactate Exchange Provides a Sensitive Measure of Response to Dichloroacetate in HT29 Xenografts

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Introduction: The majority of pre-clinical cancer imaging studies *in vivo* utilise rodent tumours or human tumour xenografts propagated subcutaneously in the flank of immunodeficient mice. The exploitation of clinical MR platforms for pre-clinical studies is attractive, given their superior hardware advances, larger available field of view and clinically relevant field strengths. The lower field strengths are particularly advantageous for Dynamic Nuclear Polarisation (DNP) studies, where hyperpolarised signals decay more slowly, increasing the time available for data acquisition. Signal enhancement from hyperpolarised ^{13}C MRS by DNP facilitates real-time metabolic imaging of tumours *in vivo* (1). In this study we have developed and acquired hyperpolarised ^{13}C MRS data from subcutaneous xenografts in mice on a 3T clinical platform. Hyperpolarised [1-^{13}C]pyruvate, and its exchange with [1-^{13}C]lactate by the enzyme lactate dehydrogenase (LDH), was used to interrogate changes in tumour metabolism induced by dichloroacetate (DCA), a pyruvate dehydrogenase kinase (PDK) inhibitor currently under clinical investigation (2).

Methods: *In vivo* **tumour propagation and DCA treatment:** Human HT29 colon carcinoma cells (5×10^6) were injected subcutaneously in NCr nude mice. Tumours were scanned once a tumour volume of ~500 mm^3 was established (day one), then mice were treated on days two and three with 200mg/kg DCA (n=3), or with vehicle (saline, n=3) p.o.. A final dose was given on day four, one hour before the post-treatment scan. **MRI:** Mice bearing HT29 xenografts were positioned with their tumour suspended into a custom made 2 cm ^{13}C transmit/receive surface coil at the isocentre of a 3T Philips Achieva clinical scanner (Fig. 1). ^{1}H imaging was performed using a prostate endorectal coil, designed for clinical use, placed next to the mouse. Total tumour volumes were calculated from transverse ^{1}H images using OsiriX (Pixmeo). **Hyperpolarised ^{13}C:** [1-^{13}C]pyruvic acid (99% isotopically enriched, Sigma Aldrich) was prepared and polarised in a HyperSense® DNP polariser (Oxford Instruments Molecular Biotools Ltd.) according to (3). **MRS:** 175 μl 80 mM hyperpolarised [1-^{13}C]pyruvate was administered *in situ* via a lateral tail vein over 5 s. A series of ^{13}C spectra were recorded at 32 MHz every 3 s using a 20° slice selective pulse-and-acquire sequence (10 mm slice thickness, 1 transient, 2048 time domain points, 8 kHz spectral width). **Kinetic Modelling:** Spectra were zero filled to 4096 points and line broadened by 60 Hz. Metabolite peak areas were calculated by peak fitting using the Amares fitting tool in jMRUI. Kinetic modelling was carried out in Matlab (Mathworks) by fitting the modified Bloch equations to the time series of peak areas. The kinetic model employed maximum likelihood estimation and a raised cosine input function for the pyruvate bolus. Apparent rate constants obtained from the model were normalised to the tumour volume included within the spectroscopy acquisition slice (measured using OsiriX).

Results: ^{1}H imaging was used to define the ^{13}C slice selective spectroscopy geometry and obtain reference images to calculate tumour volume (Fig. 2A). Apparent rate constants obtained from kinetic modelling were normalised to the tumour volume included in the acquisition slice (Fig. 2B). Treatment with DCA induced a significant 49% reduction in the apparent forward rate constant of pyruvate-lactate conversion compared with the pre-treatment value (Pre-DCA mean k_{PL}=0.048±0.006; Post-DCA mean k_{PL}=0.025±0.003pmol/s/mm^3 tissue volume, p=0.01, Student's two-tailed paired t-test). The vehicle group showed no significant change in k_{PL} between scans (Fig. 3). A significant 24% tumour growth inhibition (p=0.02, Student's two-tailed unpaired t-test) was observed in the DCA treated cohort compared with vehicle treated controls after three days of treatment.

Discussion & Conclusions: We have developed a platform to interrogate tumour metabolism using hyperpolarised [1-^{13}C]pyruvate in subcutaneous murine xenograft models on a clinical scanner. The ^{13}C surface coil is optimised for monitoring subcutaneous tumours. Signal enhancements from DNP enabled apparent rate constants of hyperpolarised [1-^{13}C]pyruvate-[1-^{13}C]lactate exchange to be measured *in vivo*. Quantification of the hyperpolarised signals by full kinetic modelling provided a sensitive assay, which exemplifies this technique as a superior analysis method when handling data from small animal cohorts. We have shown that DCA treatment significantly reduced the apparent forward rate constant in HT29 xenografts in mice after three days of treatment. This observation is consistent with the action of DCA (4) and is complementary to the steady state findings reported previously (5).
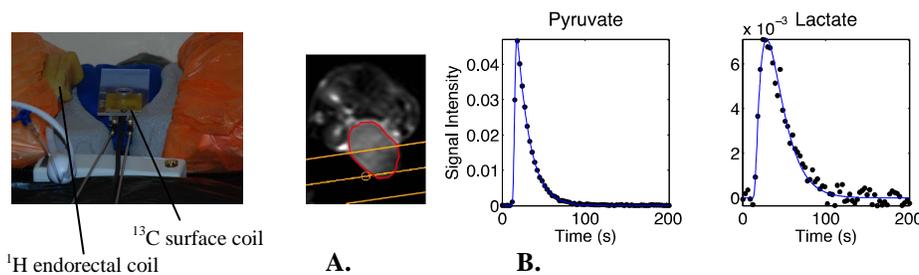

Figure 1. Coil set-up. When in use, the mouse was positioned on top of the coil platform with the tumour at the centre of the ^{13}C surface coil; the centre of the endorectal coil was aligned with the tumour and secured directly to the mouse.

Figure 2. A: Transverse image of a mouse with a subcutaneous HT29 xenograft (outlined in red). The acquisition slice for ^{13}C spectroscopy is depicted in orange. **B:** *In vivo* pyruvate and lactate signal integrals (black dots) and Matlab kinetic model fitting (blue line) were used to obtain the apparent rate constants of pyruvate-lactate exchange.
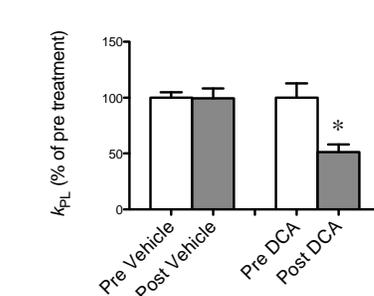
Figure 3. Apparent forward rate constant of pyruvate-lactate exchange (k_{PL}) for vehicle (saline, n=3) and DCA treated (n=3) mice bearing subcutaneous HT29 xenografts. DCA treatment induced a significant 49% reduction in k_{PL} (*p=0.01). There was no significant change between pre and post scans in the control group.

References. (1) Ardenkjaer-Larsen J.H. *et al.* Proc. Natl. Acad. Sci. 100, 10158-10163 (2003). (2) Michelakis E.D. *et al.* Sci. Transl. Med. 31, 1-8 (2010). (3) Hill D.K. *et al*. Proc. Intl. Soc. Mag. Reson. Med. 18, Abstract 1738 (2012). (4) Seth P. *et al.* Neoplasia 13, 60-71 (2011). (5) Chung Y-L. *et al*.,Proc. Intl. Soc. Mag. Reson. Med. 18, Abstract 2699 (2010).