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

Deborah K. Hill\(^1\), Jessica K. R. Boult\(^1\), Ralfal Panek\(^1\), Harold G. Parkes\(^1\), Matthew R. Orton\(^1\), Anne-Christine LF. Wong Te Fong\(^1\), Maysam Jafar\(^1\), Simon P. Robinson\(^1\), Martin O. Leach\(^1\), Thomas R. Eykyn\(^1,2\), and Yuen-Li Chung\(^1\)

\(^{1}\text{CR-UK and EPSRC Cancer Imaging Centre, The Institute of Cancer Research and Royal Marsden NHS Trust, Sutton, Surrey, United Kingdom,}\(^{2}\text{Division of Imaging Sciences and Biomedical Engineering, Kings College London, St Thomas Hospital London, United Kingdom}\)

**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 \(^{13}\text{C}\) MRS by DNP facilitates real-time metabolic imaging of tumours in vivo (1). In this study we have developed and acquired hyperpolarised \(^{13}\text{C}\) MRS data from subcutaneous xenografts in mice on a 3T clinical platform. Hyperpolarised \([^{13}\text{C}]\)pyruvate, and its exchange with \([^{13}\text{C}]\)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 (5x10\(^5\)) were injected subcutaneously in NCr nude mice. Tumours were scanned once a tumour volume of ~500 mm\(^3\) was measured (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 \(^{13}\text{C}\) transmit/receive surface coil at the isocentre of a 3T Philips Achieva clinical scanner (Fig. 1). \(^{1}\text{H}\) imaging was performed using a prostate endorectal coil, designed for clinical use, placed next to the mouse. Total tumour volumes were calculated from transverse \(^{1}\text{H}\) images using OsiriX (Pixmeo).

Hyperpolarised \(^{13}\text{C}\)\([^{13}\text{C}]\)pyruvate acid (99% isotopically enriched, Sigma Aldrich) was prepared and polarised in a HyperSense® DNP polariser (Oxford Instruments Molecular Biotechs Ltd.) according to (3). MRS: 175 \(\mu\)l 80 mM hyperpolarised \([^{13}\text{C}]\)pyruvate was administered in situ via a lateral tail vein over 5 s. A series of \(^{13}\text{C}\) 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 to 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 \([^{13}\text{C}]\)pyruvate signal on a time domain basis (4).

**Results:** \(^{1}\text{H}\) imaging was used to define the \(^{13}\text{C}\) 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 within the spectroscopy acquisition slice (measured using OsiriX).

Discussion & Conclusions: We have developed a platform to interrogate tumour metabolism using hyperpolarised \([^{13}\text{C}]\)pyruvate in subcutaneous murine xenograft models on a clinical scanner. The \(^{13}\text{C}\) surface coil is optimised for monitoring subcutaneous tumours. Signal enhancements from DNP enabled apparent rate constants of hyperpolarised \([^{13}\text{C}]\)pyruvate-[\([^{13}\text{C}]\)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 investigations. 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).


**Acknowledgments:** We acknowledge the support received for the CRUK and EPSRC Cancer Imaging Centre in association with the MRC and Department of Health (England) (grants C1060/A10334 and C16412/A6269), NHS funding to the NIHR Biomedical Research Centre.

**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 \(^{13}\text{C}\) 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 \(^{13}\text{C}\) 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.