Model Free Approach to Kinetic Analysis of Real-Time Hyperpolarised ^{13}C MRS Data

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Introduction: Dynamic Nuclear Polarisation (DNP) is revolutionising the use of ^{13}C MRS, allowing real-time metabolic imaging both *in vitro* and *in vivo*[1]. A major application of hyperpolarised ^{13}C MRS is to determine the apparent reaction rate constants governing pyruvate-lactate exchange (forward rate constant k_1), which reflects lactate dehydrogenase activity, a key metabolic enzyme that is commonly upregulated in cancer cells. Therapy-induced changes in k_1 have been suggested as an early biomarker of treatment response in cancer[2]. These apparent reaction rate constants are typically determined by kinetic modelling of the dynamic data using the modified Bloch equations[2], a method that requires expertise in computer modelling and knowledge of the reaction mechanism. Seth et al.[3] use a model-free approach by reporting the ratios of maximum lactate/pyruvate signals to detect response to treatment *in vivo*. Here, we propose a potentially more robust model-free analysis method in which the ratio of the total integrated areas under the dynamic lactate to pyruvate curves is calculated. The utilisation of the entire data set should generate a metric with less associated error than using maximum recorded signal alone. In this study we compare k_1, calculated using an in-house kinetic model, to the ratio of the total areas under the lactate/pyruvate dynamic curves across a wide variety of cancer cell types (PC3, HCT116, SF188) and also in response to drug treatments (PI3K/mTOR inhibitor PI-103 and PDK inhibitor dichloroacetate (DCA)) *in vitro*. This comparison of analysis methods has also been applied to *in vivo* data sets of mice bearing subcutaneous HT29 or SW1222 colon cancer xenografts treated with DCA.

Methods: MRS was performed at 37°C on a Bruker 500MHz (*in vitro*) or Bruker 300MHz (*in vivo*) spectrometer. **Hyperpolarised ^{13}C**: 18mg/26mg (*in vitro*/*in vivo*) [1-^{13}C] pyruvic acid (99% isotopically enriched containing 15mM trityl free radical OX63) was polarised in a HyperSense® DNP polariser for 1h. The polarised sample was dissolved in 4ml aqueous buffer (*in vitro*: 50mM sodium lactate, 50mM NaOH, 1mM EDTA; *in vivo*: Trizma buffer containing 80mM NaOH, 1mM EDTA) resulting in a 50mM/80mM (*in vitro*/*in vivo*) pyruvate solution at pH 7, 37°C. *In vitro*, 100µl of this solution was mixed with a 500µl cell suspension, during which ^{13}C spectra were acquired every 2s using a single scan and a 10° flip angle. *In vivo,* 150µl 80mM hyperpolarised [1-^{13}C] pyruvate was administered *in situ* via a lateral tail vein over approximately 5s and a series of ^{13}C spectra were recorded at 75MHz every 2s using a 20° slice selective pulse-and-acquire sequence (1 transient, 32k time domain points, 15kHz spectral width). ***In vivo* tumour implantation and DCA treatment:** Human HT29 adenocarcinoma or SW1222 colorectal carcinoma cells (5×10^6) were propagated subcutaneously in NCr nude mice. Tumours were scanned (day one), then mice were treated on days two and three with 200mg/kg DCA p.o. and a final dose was given on day four, one hour before the post-treatment scan. **Data analysis:** Spectra were phase and baseline corrected, and peaks of interest selected and integrated over the time course of the experiment. **Kinetic modelling:** The integrals of the hyperpolarised [1-^{13}C] resonances of pyruvate and lactate were fitted to the modified Bloch Equations for two-site exchange[2] and normalised to pyruvate signal intensity at time t=0. Kinetic modelling was performed using Matlab. **Dynamic Curve Area:** The time courses of integrals from each hyperpolarised metabolite signal were summed and the ratios of the lactate/pyruvate curves were calculated.

Results and Discussion: Fig. 1 shows that there is no significant difference between the ratio of k_1 vs dynamic lactate/pyruvate curve areas *in vitro*, indicating that the methods measure equivalent changes upon drug treatment and are therefore of comparable sensitivity. Figures 1b and 2 show the dynamic profiles of MRS signals derived from the injection of hyperpolarised [1-^{13}C] pyruvate *in vitro* and *in vivo*, with kinetic model fits overlaid; k_1 is determined from the kinetic model and can be compared to the relative areas under these dynamic curves. Figures 1b and 2 show that changes in the ratio of lactate/pyruvate dynamic peak area is proportional to the changes in forward apparent rate constant, k_1. The Pearson correlation coefficient (r) for *in vitro* data is r= 0.97 and P<0.0001 and for *in vivo* data r=0.993 and P<0.0001, showing that in both cases the correlation between data sets is highly significant.

Conclusions: We have shown that a model-free approach of measuring the ratio of the lactate/pyruvate dynamic curve areas can detect therapy-induced changes across different cancer cell lines and drug treatments both *in vitro* and *in vivo*, with equivalent sensitivity to changes in k_1 determined by kinetic modelling. This model-free approach is found to be proportional to the measured kinetics, with highly significant Pearson correlation coefficient (r=0.97, P<0.0001 *in vitro* and r=0.993, P<0.0001 *in vivo*), offers a simple alternative for the analysis of hyperpolarised metabolic data, and will prove useful for assessing drug induced metabolic changes measured by hyperpolarised ^{13}C.

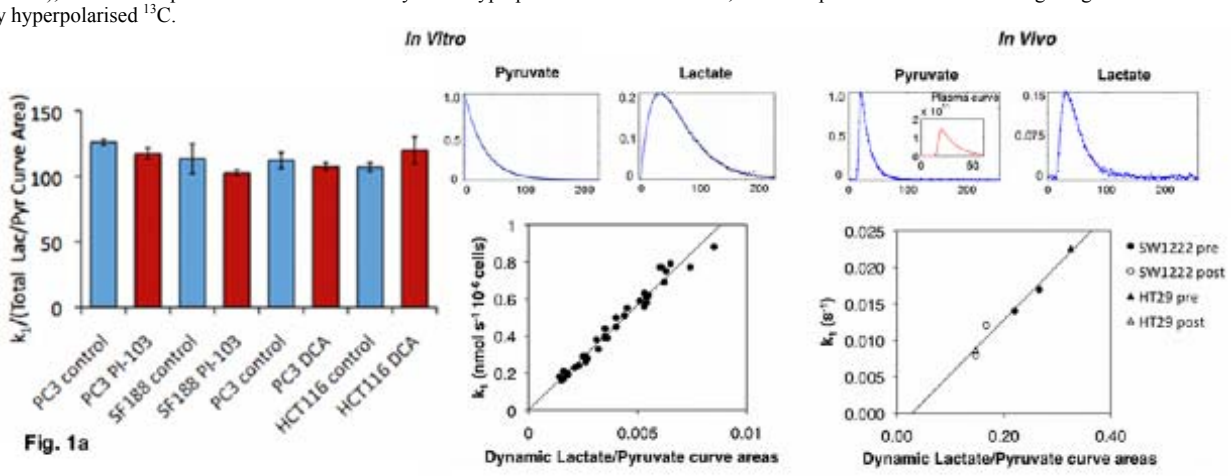

Fig. 1a

Fig. 1b

Fig. 2

Figure 1. *In vitro*: a. The ratio of k_1 vs. lactate/pyruvate dynamic peak area is consistent between cell types (n=3 per group) and upon various drug treatments *in vitro*; the model-free approach and kinetic model display equivalent sensitivity to treatment effects. **b. Top:** Peak integrals (dots) and kinetic modelling (line) of *in vitro* dynamic ^{13}C spectra from pyruvate and lactate. **Bottom:** Apparent rate constant of pyruvate-lactate exchange (k_1) plotted against the ratio of total dynamic curve areas of lactate/pyruvate for *in vitro* studies shows the methods detected comparable response across all the cell lines; Pearson correlation coefficient is r=0.97 and P<0.0001.

Figure 2. *In vivo*: Top: Peak integrals (dots) and kinetic modelling (line) of *in vivo* dynamic ^{13}C spectra from pyruvate and lactate. **Bottom:** Apparent rate constant of pyruvate-lactate exchange (k_1) plotted against the ratio of total dynamic curve areas of lactate/pyruvate for *in vivo* studies. HT29 or SW1222 cancer xenografts are indicated, and pre and post DCA treatment data points are distinguished. The Pearson correlation coefficient is r=0.99 and P<0.0001.

References. (1) Ardenkjaer-Larsen, J.H., *et al. Proc Natl Acad Sci* (2003) **(2)** Day, S.E., *et al. Nat Med* (2007) **(3)** Seth, P., *et al. Neoplasia* (2011)

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.