

¹H MRS and Hyperpolarised ¹³C MRS Assays of Pyruvate-Lactate Exchange in SW1222 Cancer Cells *In Vitro*

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Introduction

Signal enhancement by Dynamic Nuclear Polarisation (DNP) is revolutionising the use of ¹³C MRS, allowing real-time metabolic imaging both *in vitro* and *in vivo*¹. A major application of hyperpolarised ¹³C MRS is to determine the rate of pyruvate-to-lactate exchange, which has been linked with lactate dehydrogenase activity (LDH)², a key metabolic enzyme that is commonly upregulated in cancer cells. We have performed a comparative study of the *in vitro* kinetics of this reaction in SW1222 colorectal cancer cells, employing either thermally polarised ¹H MRS, as proposed by Day *et al*², or hyperpolarised ¹³C MRS under the same physiological conditions. These kinetics were also determined from Paclitaxel treated cells to assess the sensitivity of the two assays to drug mediated change in pyruvate-to-lactate exchange rates.

Methods

All MRS was performed on a Bruker 500MHz spectrometer at 37°C.

¹H MRS-based assay: 100µl D₂O and 100µl of a solution of 50mM [3-¹³C] pyruvate and 50mM unlabelled lactate (in PBS, pH 7) was mixed with a 500µl cell suspension of SW1222 cells. Proton spectra were recorded with a single scan and data acquired every 5s using a 60° flip angle and NOESY presat for water suppression.

Hyperpolarised ¹³C MRS-based assay: 18mg [1-¹³C] 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 (50mM sodium lactate, NaOH, 1mM EDTA) resulting in a 46±3mM pyruvate solution at pH 7. 100µl of this solution was mixed with a 500µl cell suspension of SW1222 cells, during which ¹³C spectra were acquired every 2s using a single scan and a 10° flip angle.

Paclitaxel Treatment: Cells were incubated for 24h prior to treatment with 1µM Paclitaxel or vehicle (ethanol). NMR assays were performed 48h later.

Data analysis: Spectra were phase and base line corrected, and peaks of interest selected and integrated over the time course of the experiment. The integrals of the ¹H resonances of ¹²C and [3-¹³C] pyruvate were fitted according to a two way chemical exchange model to determine forward (k₁, loss of [3-¹³C] pyruvate) and backward (k₂, gain of ¹²C pyruvate) rate constants. Integrals of the hyperpolarised [1-¹³C] resonances of pyruvate and lactate were fitted to the modified Bloch Equations for two-site exchange² and normalised to pyruvate signal intensity at time t=0. Kinetic modelling was performed using Matlab.

Results and Discussion

Despite the differences in SNR (¹³C signal was 270×¹H), the ¹H assay had adequate sensitivity to dynamically monitor the decrease in [3-¹³C] pyruvate and increase in ¹²C pyruvate resonances due to pyruvate-lactate exchange (Figure 1). Forward rates (k₁) obtained from both assays were highly reproducible (Table 1). The ¹H assay also allowed reproducible measurement of k₂ as the lactate and pyruvate curves are only influenced by reaction kinetics, and not by relaxation processes. In contrast, accurate estimation of k₂ is more difficult with the hyperpolarised ¹³C assay because the initial condition of an empty ¹³C lactate pool coupled with the decay of all the signals due to relaxation, and the relative speeds of the various reaction and relaxation rates, lead to greatest uncertainty in k₂ estimates.

¹H and ¹³C assays detected comparable and significant increases in k₁ when normalised to cell number following Paclitaxel treatment (Figure 2A, B). However, when normalised to protein content, there was no significant change in k₁ (Figure 2C, D). This observation is consistent with the mechanism of action of Paclitaxel, which inhibits mitosis causing G₂/M arrest (confirmed by cell cycle analysis), resulting in a doubling of cell protein concentration and volume with respect to controls (data not shown). Therefore, in Paclitaxel-treated cells there is likely to be an increase in the amount of LDH enzyme per cell, causing an apparent increase in k₁ when normalising to cell number.

Conclusions

In this study we present a ¹H MRS-based assay which enables a measurement of pyruvate-lactate exchange with results comparable to DNP, but with the advantage of also accurately probing the backward reaction. This assay may provide a more convenient alternative to the DNP assay to measure pyruvate-lactate exchange *in vitro*. These data also emphasise that caution should be exercised when interpreting kinetics depending on how the reaction is normalised.

References. (1) Ardenjaer-Larsen, J.H., *et al. Proc Natl Acad Sci* (2003) (2) Day, S.E., *et al. Nat Med* (2007).

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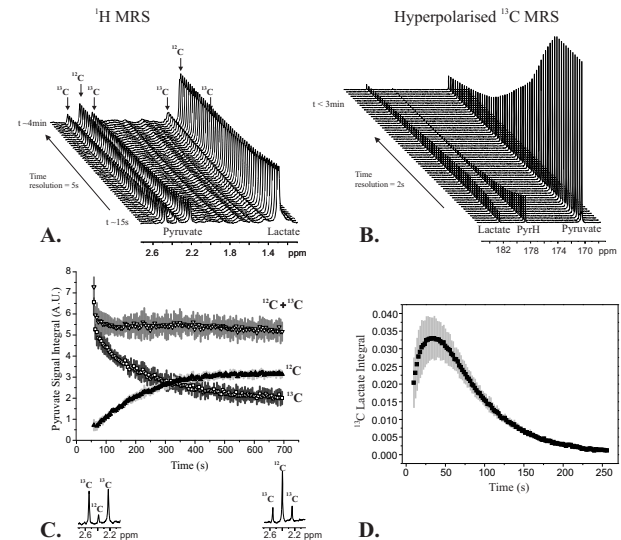


Figure 1. Real-time ¹H MRS (A) and hyperpolarised ¹³C MRS (B) based assay of LDH activity in SW1222 cancer cells, and the corresponding time evolution of the spectral integrals (C and D). The hyperpolarised lactate signal was normalised to the initial pyruvate signal at time t=0.

Assay	k ₁	k ₂
¹ H	0.50±0.07	0.31±0.07
¹³ C	0.30±0.06	0.3±0.2

Table 1. Forward (k₁) and backward (k₂) rate constants of pyruvate-lactate exchange. Data are in nmol s⁻¹ 10⁶ cells and represent mean±SD (n=3). Cell numbers: 37±5×10⁶, 35±2×10⁶ (¹H, ¹³C respectively).

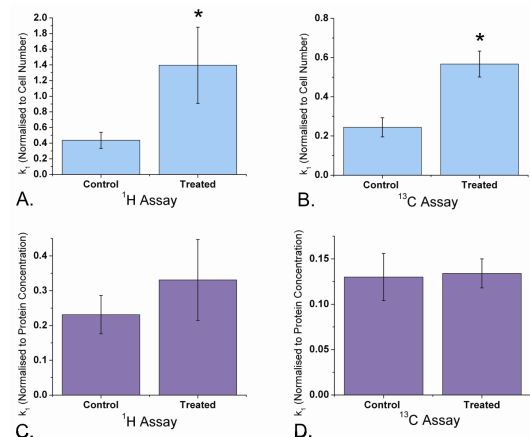


Figure 2. Forward (k₁) reaction rates of pyruvate-to-lactate exchange from ¹H (A, C) or ¹³C (B, D) MRS-based assays in control and 48h Paclitaxel treated cells. A, B: k₁ is normalised to cell number (Data are in nmol s⁻¹ 10⁶ cells). C, D: k₁ is normalised to protein concentration (Data are in nmol s⁻¹ mg⁻¹ protein). Data represent mean±SD (n=3), Cell numbers: 25±7×10⁶, 7±1×10⁶ (¹H control, treated), 31±3×10⁶, 8±1×10⁶ (¹³C control, treated). (*p<0.05, unpaired two-tailed Student's t-test).