Compartmentation of MCF-7 tumour cell metabolites characterised by hyperpolarised ^{13}C diffusion-weighted spectroscopy

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Introduction:

Quantifying and understanding tumour metabolism is a central issue in diagnosis and treatment response analysis of tumours. Using kinetic modelling, the uptake and metabolism of hyperpolarised ^{13}C-labelled agents, such as [1-^{13}C]pyruvate, in tumours can be analysed. The signal of each metabolite emanates from both extra- and intracellular compartments, which currently cannot be separated in experiments. The diffusion coefficient between different cellular compartments can vary up to 10-fold, therefore diffusion-weighted spectroscopy is well suited to differentiate them (1,2,3). Many efforts have been made to overcome the low *SNR* of ^{13}C metabolites *in-vivo* using indirect detection techniques like *ACED-STEAM* (3). In this study, hyperpolarisation is used to overcome the low *SNR* limitations (4). For the first time, we show using real-time direct detection, that diffusion coefficients of [1-^{13}C]pyruvate and its metabolites in tumour cell spheroids can be determined.

Methods:

All experiments were performed with spheroids of *MCF-7* human breast cancer cells serving as a model system. [1-^{13}C]pyruvate was hyperpolarised using a *HyperSense* DNP polarizer (*Oxford Instruments, Oxfordshire, UK*). Pyruvate was rapidly dissolved in a D_2O buffer solution (10 mM final concentration). Then, the sample was attached to a preheated (45°C) 0.5 T permanent magnet, while it was being transported 0.5 km distance by bike to a 14.1 T *Bruker* microimaging system (*Bruker, Rheinstetten, Germany*). The transport time was 120 s, which led to a signal decay of around 50%. 0.2 ml pyruvate-D_2O solution was injected into an 8 mm susceptibility corrected NMR glass tube containing 0.8 ml with 40 million *MCF-7* cells. Data acquisition was performed with a modified pulsed gradient spin echo (PGSE) sequence using low flip angle (α) excitation (Fig. 1). We set the diffusion time to $\Delta = 40$ ms, the gradient time to $\delta = 2$ ms, and varied the gradient amplitudes up to a maximum of $g = 2.7$ T/m for a half-sine gradient pulse along z-direction. The sequence was repeated in a loop structure starting with a reference scan for $b = 0$, continuing with the largest b-value, and then decreasing the b-value by reducing g. The signal equation is given by the *Stejskal-Tanner* expression modified with an exponential damping with T_{eff}, which consists of T_1 relaxation and loss of M_z due to repeated excitation:

$$I_g = I_0 \times exp\left(\frac{-t}{T_{eff}}\right) \times exp(-bD) = I_0 \times exp\left(\frac{-t}{T_{eff}}\right) \times exp\left(-\left(\Delta - \frac{\delta}{4}\right)\left(\frac{2\gamma\, g\delta}{\pi}\right)^2 D\right)$$

A correction based on two-side kinetic modelling (5) was performed before data were fitted to the signal equation (data not shown).

Results and Discussion:

Twenty seconds after injection of hyperpolarized pyruvate, 16 diffusion weighted spectra were collected (Fig. 2 a,c) at pH 7.3 and 6.7. We observed lactate, pyruvate hydrate and pyruvate signals (line width = 10 Hz), showing significantly different diffusion behaviour. Fitting the data to the *Stejskal-Tanner* equation showed monoexponential decay for all metabolites with a high fit correlation in all cases ($R^2 > 0.98$, see Fig. 2 b,d). At pH 7.3, we found $D_{Lactate} = 1.25$, $D_{Pyr.Hydr.} = 2.95$, $D_{Pyruvate} = 3.41$ (D [μm^2/ms]). The apparent diffusion coefficient of lactate is lower compared to that of pyruvate. This indicates that pyruvate was taken up and converted into lactate within the cells, where diffusion is restricted. At pH 6.7, diffusion was markedly reduced with $D_{Lactate} = 0.71$, $D_{Pyr.Hydr.} = 0.81$, $D_{Pyruvate} = 0.91$ (D [μm^2/ms]), indicating very slow diffusion in the sample. This may be caused by membrane disruption and cell leakage, which could lead to slower diffusion throughout the whole sample by exposing inner compartments of the cell to all metabolites.

We have thus demonstrated that hyperpolarized ^{13}C diffusion-weighted spectroscopy in vitro is feasible. Future work will focus on using this new technique to separate intra- and extracellular compartments in spectroscopic images of tumours, to determine uptake and conversion rates of each metabolite, and to separate metabolic conversion from perfusion by large diffusion weightings.

References: [1] van Zijl, P. C. M. et al. PNAS 88:3228-32(1991); [2] Malveau, C. et al. J Magn Reson 130(1):131-4(1998); [3] Pfeuffer, J. C. et al. J Magn Reson 177:129-138(2005); [4]Ardenkjaer-Larsen, J. H. et al, PNAS 100:10158(2003); [5] Khegai, O. et al. Proc. ISMRM 19(2011).

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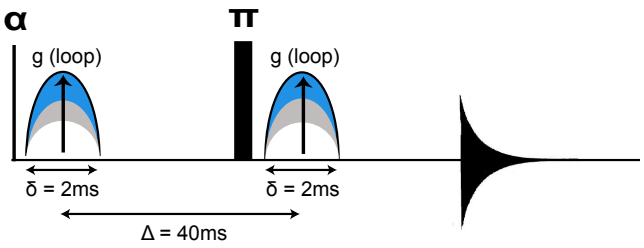
Fig. 1: diffusion-weighted low flip-angle pulsed gradient spin echo (*PGSE*)



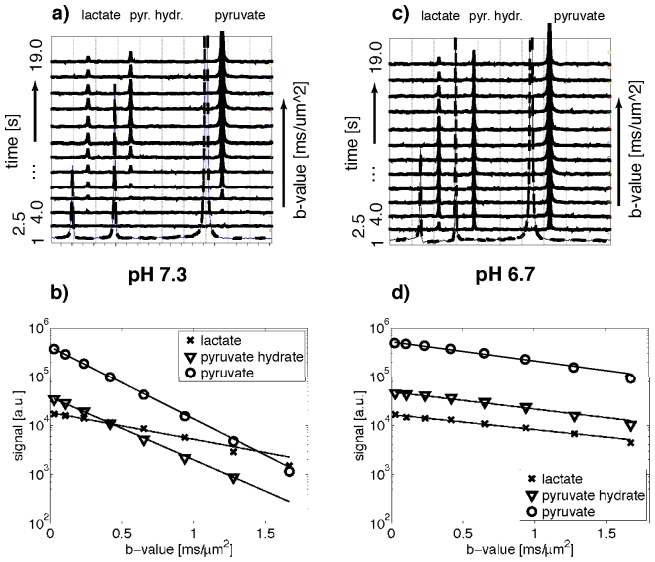


Fig. 2: ^{13}C diffusion in MCF-7 cells at pH 7.3 (a,b) and pH 6.7 (c,d) measured with the sequence described above. 16 diffusion weighted spectra are shown (a,c). The first one (dashed line) was collected as a reference for $b = 0$. Then, after starting with the maximum b-value, b-values were decreased incrementally. At pH 7.3 the diffusion coefficient for lactate is clearly lower than for the two other metabolites (b). At pH 6.7 all metabolites show a low diffusion coefficient (d).