Towards a more quantitative physiological analysis: comprehensive kinetic modeling of pyruvate metabolism in tumors via co-injection of hyperpolarized ^{13}C pyruvate and urea in combination with measurement of arterial input functions

Samira Kazan[1], Steven Reynolds[2], Tooba Alizadeh[1], Adriana Bucur[2], Aneurin Kennerley[3], Vincent Cunningham[4], Martyn Paley[2], and Gillian Tozer[1]

[1]CR-UK/YCR Sheffield Cancer Research Centre, University of Sheffield, Sheffield, South Yorkshire, United Kingdom, [2]Academic Unit of Radiology, University of Sheffield, Sheffield, South Yorkshire, United Kingdom, [3]Signal Processing in Neuroimaging and Systems Neuroscience, University of Sheffield, Sheffield, South Yorkshire, United Kingdom, [4]Aberdeen Biomedical Imaging Centre, University of Aberdeen, Aberdeen, Scotland, United Kingdom

Target audience: Dynamic NMR Spectroscopy and hyperpolarized MR researchers, mathematical modelers, Perfusion and MR for Cancer researchers.

Introduction: Mathematical models are required to estimate kinetic parameters of [1-^{13}C] pyruvate-lactate interconversion from magnetic resonance spectroscopy data. It has been shown previously that the use of a measured arterial input function (AIF) provides a robust and more comprehensive analysis of the kinetics compared to using a hypothetical box-car input function [1]. A measured AIF reduces the number of free parameters for fitting and provides physiologically meaningful values not only for the rate constant of conversion of pyruvate to lactate (k_{pl}), but also for clearance of pyruvate from the blood to the tissue (*Kip*). This parameter would be of particular relevance if blood flow effects were of interest in a study. Urea has been used previously as a perfusion marker to track the perfusion of pyruvate to the site of interest [2]. In this study, by simultaneous injection of hyperpolarized ^{13}C pyruvate and urea we measured both AIFs in order to compare the clearance/perfusion rates obtained using both methods at a tumor site.

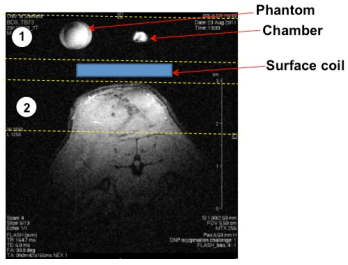

Figure 1: slice selection for spectroscopy through the phantom and chamber (1), through the tumor (2); parallel lines show the localised planes superimposed on a ^{1}H FLASH structural image.

Methods: BDIX rats, with subcutaneously transplanted P22 sarcomas, were anaesthetised and femoral vein and artery cannulations were performed for drug administration, AIF measurements and blood pressure monitoring. ^{13}C urea was dissolved in ~3:2 ^{12}C-DMSO:D_2O with 15mM OX63 trityl radical and 1.5mM DOTAREM. 15mM OX063 trityl radical and 1.5mM DOTAREM were added to $^{13}C_1$-pyruvic acid (PA) for the PA sample. 45mg of PA sample was flash frozen in a sample cup with liquid nitrogen, then 35 µl of urea sample was frozen on top of the PA sample. The total sample was hyperpolarized using a HyperSense DNP polariser and dissolved in HEPES buffer solution. To measure pyruvate and urea AIF simultaneously, 1 ml of arterial blood was continuously withdrawn through a small chamber at 1ml/min starting simultaneously with the intravenous injection of the hyperpolarized solution at 5ml/kg over 13s via a femoral vein cannula. A fiber optic temperature probe was placed inside the chamber to measure the temperature of the blood as it was withdrawn. The bleed chamber was positioned above the surface coil located over the P22 tumor, so that MRS signals from both tumor and bleed chamber could be acquired simultaneously. The ^{13}C signals were localized using a 20mm ^{13}C/^{1}H surface coil and slice selection (Figure 1). ^{13}C spectroscopic data were acquired using a Gaussian pulse (20deg flip angle, TR=1s) on a Bruker 7T small animal MRI system. A complementary co-injection of ^{13}C-pyruvate and ^{13}C urea and scan (TR=4s) was performed for the measurement of T_1 of pyruvate and urea (T_{1py} and T_{1urea}) in blood. Blood flow was halted and the decaying portion of the signal was fitted to obtain the T_1 using a measured flip angle via a separate injection using a short TR of 0.2 s. To allow for the estimation of the direct AIF, the measured MRS signals in the bleed chamber (pyruvate and urea) were corrected for T_1 relaxation effects, RF pulses, withdrawal rates, and dispersion in the chamber. The relative clearance rate was determined for 2 cases: 1- direct pyruvate AIF (K_{ip}); 2- urea AIF (K_{iurea}). Taking tumor data for three animals following co-injection of pyruvate and urea, relative clearance rates were determined using the two-way exchange model as per [1].

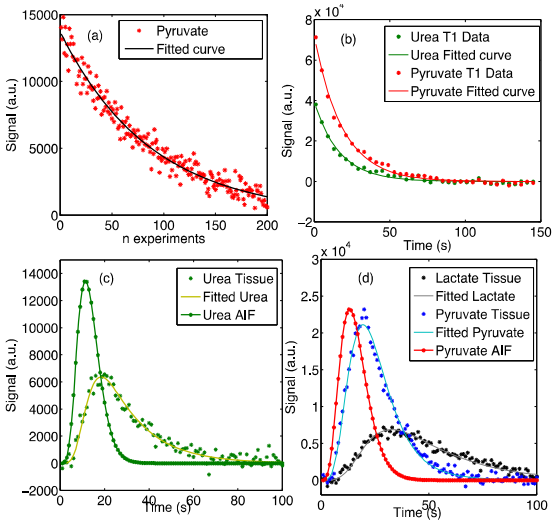

Figure 2: (a) Fitted curves for flip angle, θ, in the chamber; (b) Fitted curves of T_{1py} and T_{1urea} of blood in the chamber; (c) Fitted curves for ^{13}C urea in the tumor using a direct urea AIF; (d) Fitted curves for pyruvate and lactate in the tumor using a direct pyruvate AIF.

Results and discussion: Figure 2a shows the fit to the temporal curve of the pyruvate signal acquired at a small TR of 0.2s to predict the flip angle, which was then used to estimate the T_{1py} and T_{1urea} (Figure 2b) at the given temperature of blood in the chamber (Table 1). The results of the fits for one representative example are presented in Figure 2c for urea and Figure 2d for pyruvate together with the direct AIF for each case. Table 1 shows kinetic estimates using urea AIF and pyruvate AIF together with the measured parameters. The clearance of urea is probably heterogeneous within tumours, as a convolution of the input function with 2 exponentials gives a better fit to the tissue response than with one, although the overall clearance is similar with both models. The clearance of pyruvate from the blood was very similar in the 3 animals, whereas the clearance of urea is very variable. It remains to be established whether the clearance of either pyruvate or urea is a good quantitative index of the absolute rate of blood flow as opposed to relative perfusion. A simple precursor-product relationship between pyruvate and lactate can be estimated independently of blood flow and clearance rates.

Table 1: Fitted and measured parameters

	Animal1	Animal2	Animal3
K_{iurea} (s^{-1}) – one exp	0.019	0.041	0.067
K_{iurea} (s^{-1}) - two exp	0.022	0.044	0.064
K_{ip} (s^{-1})	0.038	0.040	0.039
k_{pl} (s^{-1})	0.061	0.046	0.029
θ (°)	16	15	12
T_{1py} (s)	21	21	18
T_{1urea} (s)	24	14	16
Temp (°C)	29	28	29

References: [1]. Kazan, S. *et* al. (2012) Magnetic Resonance in Medicine; In Press.
[2]. von Morze, *et al*. (2011) J Magn Reson Imaging (33), No. 3, p. 692-697.