Direct measurement of pyruvate T1 in vivo and the effect of blood oxygenation

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Introduction: Dynamic nuclear polarization (DNP) is a novel technique for increasing the sensitivity of magnetic resonance spectroscopy and imaging (MRS/MRSI). Intravenous (i.v.) administration of hyperpolarised pyruvate provides a means for quantifying pyruvate-lactate interconversion in living tissues via MRS/MRSI. In oncology, this approach has potential for monitoring tumour cell viability and oxygenation following treatment. Mathematical models are required to extract kinetic parameters from the spectroscopy data. The rate constant of conversion of pyruvate to lactate (k_{pl}), for example, is a potential marker for the efficacy of drugs. The quantification of this parameter depends on the total number of fitted parameters and on the assumptions regarding the T_1 estimates. Here, we developed a novel technique for the direct measurement of apparent rate of relaxation of hyperpolarized substrate online in a living animal. We have also investigated the influence of different systemic oxygenation states on the T_1 of pyruvate (T_{1pyr}) in blood. Knowledge of the T_1 could be used as a robust input parameter for the kinetic modelling. In addition, we investigate the influence of the systemic blood oxygenation on $T_{1pyr.}$ In separate experiments we also investigate the influence of temperature on $T_{1pyr.}$

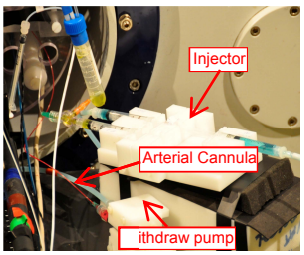
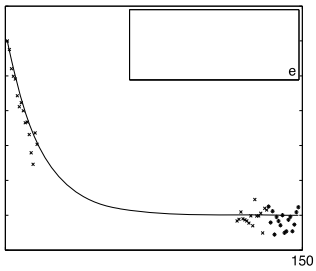


Figure 1: Injection and withdrawal

Methods

Preparation: Male, tumour-bearing BDIX rats (250-350g, n=8) were placed on a temperature-controlled heat mat and anaesthetised with isoflurane (5% induction; 1.5-3% maintenance in 1:1 O_2:N_2O at 2 l/min). Rat body temperature was monitored via a rectal probe and maintained at 37°C. Three blood vessel cannulations were performed: one femoral vein for delivery of hyperpolarized ^{13}C-pyruvate, one femoral artery for blood pressure monitoring and for blood withdrawal, one tail vein for propofol anaesthetic infusion (Rapinovet). Animals were dosed with atropine (0.1 ml s.c.) and then tracheotomised for artificial ventilation delivered at 1 l/min.

MRS: Once prepared the animal was transferred into a cradle containing a temperature-controlled heat mat which was then placed at the center of a 310 mm bore, 7T small animal magnet connected to a Bruker BioSpec, Avance II spectrometer. Full anaesthesia was then maintained by an i.v. propofol infusion (30-36 mg kg^{-1} h^{-1}). Heparin (0.1ml i.v.) was administered following the insertion into the tumour of two oxygen-sensing probes (OxyLite system). Blood pressure, respiration rate, body temperature and tumour pO_2 were continually monitored. Pyruvate was hyperpolarized using a HyperSense system (Oxford Instruments) and ^{13}C spectroscopic slice localised data was acquired using a Gaussian pulse (20° flip angle (θ), repetition time, TR=1s) and a 20mm ^{13}C/^{1}H surface coil. An aqueous 13.6 mM ^{13}C1-pyruvate solution (10% D_2O) was separately prepared and placed in a 9.4T Bruker Avance III spectrometer to determine T_1 versus temperature. An inversion recovery pulse sequence and Topspin were used to measure T_1 at each temperature point.



Experiment: 5ml/kg of hyperpolarised ^{13}C-pyruvate (1.40 ml of PA, ~150 mM) was administered over 13s using a custom MR compatible automated injector. To measure the T_1 of hyperpolarized pyruvate, a 0.4 ml aliquot of blood was withdrawn from the femoral artery into a chamber embedded in the cannula using a blood withdrawal pump (Figure 1). Blood flow was halted once the chamber was filled and slice select MRS was performed in the chamber where it was assumed that no observable lactate production was taking place (thus any MR signal decay is a product of RF saturation and T_1 relaxation). The decaying portion of the signal, $\boldsymbol{M_z}$, was fitted to obtain the T_{1pyr} and the applied flip angle at that location using Equation 1. This was performed under three respiratory challenges, (1) normal air (21% O_2; 79% N_2), (2) a hypoxic mixture (10% O_2; 4% CO_2; 86% N_2) and (3) carbogen (95% O_2; 5% CO_2).

$$\boldsymbol{M_z = M_0(\cos\theta)^n e^{-\frac{TR}{T_{1pyr}}}} \qquad (1)$$

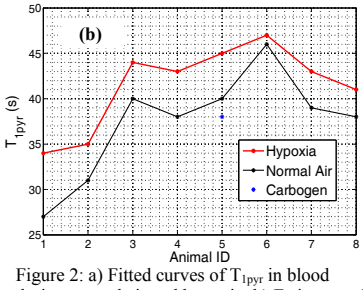


Figure 2: a) Fitted curves of T_{1pyr} in blood during normal air and hypoxia; b) Estimates of T_{1pyr} during normal air and hypoxia.

Table 1: Physiological paramters under normal air and hypoxia.

n=8	Normal Air	Hypoxia
pO_2*(mmHg)	72 ± 9	46 ± 6
BP (mmHg)	93 ± 18	80 ± 13
Resp (bpm)	75 ± 18	76 ± 14

*In arterial blood

Results

The MRS signal decay of blood in the cannula chamber for two injections in a single animal under normal air and hypoxic conditions, together with the fits, are shown in Figure 2a. The signal takes longer to decay under hypoxia indicating that T_1 has lengthened. Significant differences were found in T_1 between the normal air and hypoxia conditions across all animals, Figure 2b (paired t-test p=0.00026). The mean values of T_{1pyr} for hypoxia and normal air were 41.5±1.6s and 37.3±2.1s respectively (± standard error). Figure 3 shows a strong positive correlation (r=0.72) between temperature and T_{1pyr} at 9.4T. A linear fit shows ~ 11s T_{1pyr} change between 20 °C and 40 °C. In our experiments, we expect temperature losses during blood withdrawal resulting in an absolute decrease in the measured T_1, however, this should not affect the observed trend during oxygenation. The measured physiological parameters under normoxia and hypoxia respectively are listed in Table 1. Moreover, tumor pO_2 decreased by approximately 93% during hypoxia, as measured by luminescence decay of oxygen-sensitive probes.

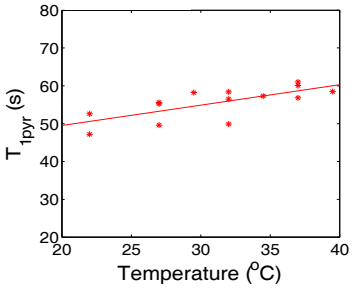


Figure 3: ^{13}C1-pyruvate T_1 versus temperature.

Discussion:

We have established a methodology to allow the direct estimation T_1 of pyruvate in an arterial cannula following intravenous injection of hyperpolarised ^{13}C pyruvate/lactate in rats. We found a significant increase in T_{1pyr} under hypoxic conditions (n=8) and a decrease under carbogen (n=1). This is possibly due to an increase in the paramagnetic gaseous oxygen within the blood. Tadamura *et al.* [1] reported a T_1 shortening during carbogen (100%) in arterial blood using ^{1}H spectroscopy in the heart. From our measurements we conclude that there was a considerable variation in the value of T_1 for pyruvate. T_1 measurements show a statistically significant variation with systemic oxygenation under hypoxia which is most likely due to the paramagnetic properties of oxygen within the blood. This could be used to determine local tisssue oxygenation state.

References: Tadamura *et al.* "Effect of oxygen inhalation on relaxation times in various tisses", JMRI, 7(1), 221.