Spin echo measurements of the extravasation and tumor cell uptake of hyperpolarized [1-^{13}C]lactate and [1-^{13}C]pyruvate

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Target audience This work is aimed at basic scientists and radiologists who have an interest in the application of hyperpolarized ^{13}C-labelled cell substrates to investigate tissue metabolism *in vivo*.

Purpose In a recent study where lactate dehydrogenase activity was determined by measuring ^{1}H/^{2}H exchange between injected L-[1-^{13}C,U-^{2}H]lactate and endogenous protonated lactate in a heteronuclear spin-echo experiment [1], a time-dependent increase in the ratio of the echo signal to the FID signal obtained immediately after the excitation pulse was observed. This implied redistribution of lactate between the vascular and extravascular spaces. In this study we investigated whether this change in echo/FID ratio could report on lactate and pyruvate transport into the tumor cells *in vivo*.

Methods Female C57BL/6 mice bearing EL-4 lymphomas (n = 60) were imaged at 9.4 T using a ^{13}C-surface coil (24mm). Two hundred µl of hyperpolarized 45-90 mM L-[1-^{13}C]lactate [1], 75 mM [1-^{13}C]pyruvate or 45 mM [^{13}C]HP001 [2] were injected i.v. and data acquired using a spin-echo pulse sequence, with simultaneous FID and echo sampling, was started 8 seconds after the beginning of injection. For lactate a single-refocus sequence was used (TR 4s, TE 310ms) [1] while for pyruvate a double-refocus sequence was used (TR 3 s, TE either 310ms or varying combinations of TE/b, see figure 2) [3]. In some experiments, transport into cells was inhibited by injecting either a monocarboxylate transport (MCT) inhibitor (α-cyano-4-hydroxycinnamate [4], 4-CIN, 150 mg/kg i.p.) or non-labeled L-lactate (200 µl of 2M, i.p. or 200µl of 3x the injected hyperpolarized concentration i.v.) prior to injection of the hyperpolarized L-[1-^{13}C]lactate or by injection of 4-CIN before injection of pyruvate or HP001. Additional diffusion (ADC, b-values 8-1130 s/mm^2, Δ=24 ms, TE=170ms or b-value of 730 s/mm^2, Δ=23-74 ms, TE=240ms) and relaxation time (TE=100-600ms) measurements were performed using the same metabolites. Echo/FID ratios were calculated and the change in ratio between time points 8 s and 40 s were estimated. Apparent exchange rates were also estimated from the pyruvate data [5].

Results A longer T_2 relaxation time and lower ADC was observed for lactate labeled by exchange after injection of [1-^{13}C]pyruvate, which was assumed to be mostly intracellular, when compared to injected [1-^{13}C]lactate, [1-^{13}C]pyruvate and HP001, which were mainly extracellular (Fig. 1, Table 1). The different relaxation and diffusion behavior of the intra- and extravascular signals affected measurements of the apparent label exchange rate constants using spin-echo data (Fig. 2D).

An increasing tumor echo/FID ratio was observed for all three molecules, which could be explained by their extravasation into the tumor interstitial space. Inhibition of the monocarboxylate transporter, which decreased by 40% the label exchange between pyruvate and lactate, reduced the increase in the echo/FID ratio for lactate (Fig. 2A&B) and pyruvate (Fig. 2C), but not for HP001, demonstrating that some of the increase in the echo/FID ratio was due to cell uptake of lactate and pyruvate.

Discussion This study suggests that by acquiring the FID and echo signals in a spin echo experiment we can obtain information about lactate and pyruvate uptake into the cell. However, in this tumor model, movement of metabolites between the vascular and interstitial pools would appear to have the biggest effect. Further optimization of acquisition parameters, based on the measured relaxation and diffusion properties, may allow us to minimize the vascular component and thus measure directly lactate transport into the tumor cell.

Conclusion Simultaneous collection of both FID and echo signals can provide information on cell uptake thus giving further insight into the kinetics of hyperpolarized ^{13}C label exchange. Care is needed when comparing exchange rate constants determined in spin-echo-based studies.

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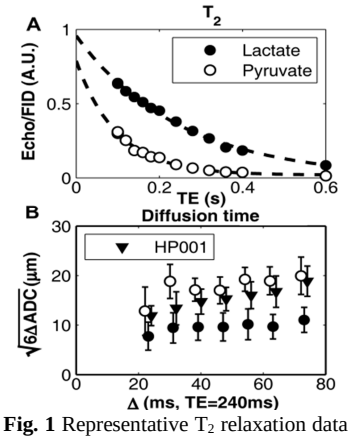

Fig. 1 Representative T_2 relaxation data (A) and the effect of diffusion time on apparent restriction (B) following injection of pyruvate and HP001.

Table 1 Apparent *in vivo* relaxation times and diffusion coefficients

	Pyruvate Injected	Lactate Exchange	Lactate Injected	HP001 Injected
T_2 (s)	0.17 ± 0.04	0.27 ± 0.02	0.21 ± 0.07*	0.40 ± 0.04
S_0	0.68 ± 0.08	1.00 ± 0.09	0.92 ± 0.10	0.99 ± 0.10
ADC (x10^{-3} mm^2/s)	1.7 ± 0.6	0.8 ± 0.2	1.4 ± 0.5*	1.4 ± 0.5

n>3 in all groups. * p<0.05 between injected lactate and lactate labelled from pyruvate

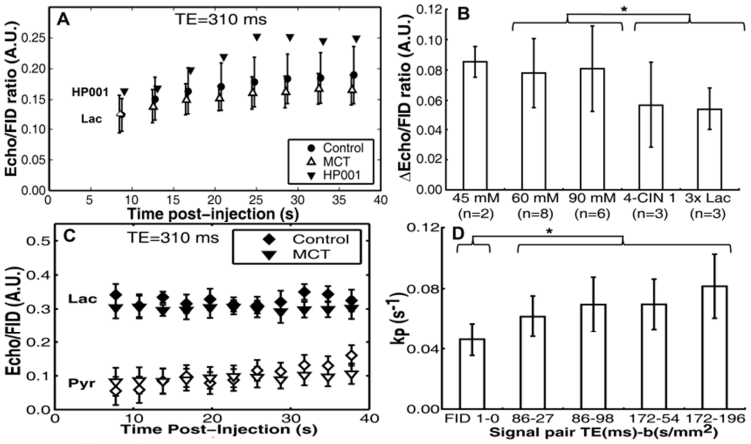

Fig. 2 Effect of MCT inhibition on lactate (A,B) and pyruvate (C) signal kinetics. A decrease in echo/FID ratio of the injected metabolite is observed following MCT inhibition while little change is seen in lactate labelled by exchange (C). Increased exchange rates were estimated for spin-echo data compared to FID data (D). * p<0.05

References

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