Investigating Lactate Pool-Size Effects in Cancer using Metabolic Activity Decomposition and Hyperpolarized Carbon-13 MR

Christine M Leon[1,2], Larson EZ Peder[1], Ralph E Hurd[3], Robert Bok[1], Kristen R Scott[1], John Kurhanewicz[1], and Daniel B Vigneron[1]

[1]*Department of Radiology and Biomedical Imaging, University of California, San Francisco, San Francisco, CA, United States,* [2]*UC Berkeley | UCSF Graduate Group in Bioengineering, University of California, Berkeley and University of California, San Francisco, San Francisco, CA, United States,* [3]*GE Healthcare, Menlo Park, CA, United States*

Introduction

Recently, it has been shown, in normal tissue and under typical 80 mM [1-^{13}C] pyruvate dose conditions, that the steady-state lactate pool-size can be the limit of detection for HP [1-^{13}C] lactate[1]. With the addition of unlabeled lactate to the bolus, the signal is no longer limited by low steady-state lactate pool-size (e.g. no longer limited by lactate pool-size restricted [NADH] availability). It has been shown that the conversion between lactate and pyruvate may alter the NAD+/NADH ratio in cells, and this exchange may coordinate metabolism of groups of cells[2]. Using this approach, Exchange-Linked Dissolution Agents (ELDA), with sufficient unlabeled lactate, the rate of [1-^{13}C] lactate should become dependent on the pyruvate dose and/or LDH enzyme activity[1]. In this study, MAD-STEAM was used to determine if the TRAMP tumor model demonstrated a pool-size limit under these dose conditions, and to investigate the relationship between any pool-size effects, on the isotopic exchange process. Recent work by our group has shown that the MAD-STEAM technique can been used to directly observe real-time conversion of metabolites[3] due to the activity of the enzyme and provide a robust and novel tool for calculation of multiple T_1s and rates of conversion simultaneously. Meanwhile, STEAM reduces the potential for confounding vascular effects[5].

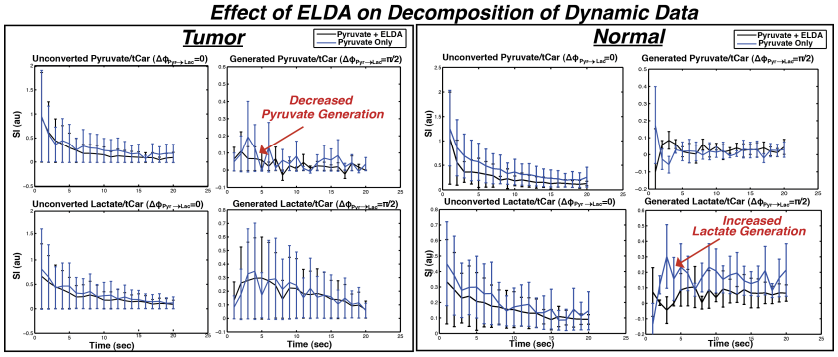

Figure 1: Dynamic curves from Metabolic Acitivty Decomposition showing decreased generation of pyruvate normalized to total carbon in tumors with the addition of ELDA versus normal tissue which has increased lactate generation.

Methods

To investigate the effect of ELDA (1) 80mM HP [1-^{13}C] pyruvate n=3 and (2) 80mM HP [1-^{13}C]-pyruvate and 40mM ^{12}C-lactate n=3 in both TRAMP tumor mouse models (n=3) and in normal mice (n=3). All experiments were co-polarized with HP ^{13}C-urea for phase reference. Data was acquired with stimulated echo acquisition mode (STEAM) (TE=14ms, $\Delta\phi_{Pyr\to Lac}=\pi/2$, TMs=1sec, 10 acquisitions, progressive flip angle, adiabatic double spin echo, slab 20cm)[3]. Because STEAM suppresses flowing spins[4], spins in the vasculature are suppressed and the acquisition started at 25sec after injection, such that perfusion of the bolus was negligible. T_2-weighted anatomical images were acquired for localization.

Results and Discussion

Unlike normal tissue under these dose conditions, the addition of unlabeled lactate to the pyruvate bolus (ELDA) did not appreciably increase HP [1-^{13}C] lactate levels in the TRAMP tumors (i.e. TRAMP tumors do not appear to be significantly lactate pool-size limited under these conditions). However, there was an increase in the ratio of lactate/total carbon, and concomitant decrease in pyruvate/total carbon signal, also reflected as a significant rise in lac/pyr with time (Data not shown). Unlike the previous studies of comparing tumor versus normal tissue, the effect was not seen in the phase shifted lactate signal, which is known to show real-time lactate generation[3]. However, there was a difference between the generated pyruvate signal (Figure 1), which decreases with the addition of ELDA.

Consistent with the absence of a pool-size limit in TRAMP tumors, the conversion of pyruvate to lactate ($K_{Pyr\to Lac}$) was not different with the addition of ELDA unlike normal tissues (Table 1). Conventional two-site exchange also shows no differences between the two groups. Because Metabolic Activity Decomposition is able to measure both the forward and the back reaction, it was used to investigate the conversion of lactate back to pyruvate ($K_{Lac\to Pyr}$), which decreased with the addition of ELDA (Table 1).

Effect of Exchange-Linked Dissolution Agents

	Pyruvate + ELDA	Pyruvate Only	P-value
Tumor			
$K_{Pyr\to Lac}$			
Metabolic Activity Decomposition:	0.137 ± 0.0069	0.123 ± 0.0084	0.257
Conventional Two-site Exchange:	0.095 ± 0.0090	0.074 ± 0.0129	0.436
↓ $K_{Lac\to Pyr}$			
Metabolic Activity Decomposition:	0.0042 ± 0.0022	0.0098 ± 0.0023	0.042
Normal			
↑ $K_{Pyr\to Lac}$			
Metabolic Activity Decomposition:	0.037 ± 0.0136	0.017 ± 0.0167	0.009
Conventional Two-site Exchange:	0.047 ± 0.0151	0.033 ± 0.0063	0.313
$K_{Lac\to Pyr}$			
Metabolic Activity Decomposition:	0.037 ± 0.0394	0.0145 ± 0.0024	0.204

*Two-sided, paried t-test(n=3 ,α=0.05). Data is reported as *mean ± mse (P-values).*

Table 1: Effect of Exchange-Linked Dissolution Agents on rates on conversion with Metabolic Activity Decomposititon and Conventional Two-site Exchange in tumor and normal.

Conclusions

Using a technique that is not confounded by vascular effects, we show that *lactate enters the cell,* which was demonstrated by its ability to effect the amount of conversion. This conversion back to pyruvate decreases with the addition of ELDA in tumors. We hypothesis that with a larger pool-size of unlabeled lactate (high co-factor availability), HP [1-^{13}C] lactate is less likely to be converted back to HP [1-^{13}C] pyruvate since there are more ^{12}C-lactate molecules that the enzyme can interact (Figure 2). Thus, even in a case where the rate of conversion of pyruvate to lactate ($K_{Pyr\to Lac}$) does not change, the back-reaction rate ($K_{Lac\to Pyr}$) appears lower in tumor tissue where there is a substantial population of lactate (Figure 2) available for conversion to [1-^{13}C] pyruvate. Therefore, the lactate-to-pyruvate ratio increases in tumors even without a lactate pool-size limit. In the case, where there is a lactate pool-size limit (limited NADH availability), as observed in normal tissue under these conditions, the observed isotope exchange as measured by $K_{Pyr\to Lac}$ increased with unlabeled lactate.

Although there is a relationship between $K_{Pyr\to Lac}$ and $K_{Lac\to Pyr}$. Only using the forward reaction underestimates $K_{Pyr\to Lac}$, when there is a significant population of lactate, which is known to be the case in the TRAMP model at these doses. Because MAD-STEAM can be used to calculate both the forward and the reverse reaction, we were better able to describe and understand the exchange kinetics resulting from pool-size changes. As hyperpolarized carbon-13 MR progresses to different disease applications and to clinical research, this study demonstrates the necessity to consider the pool-size of metabolites for accurate interpretation of hyperpolarized MR dynamics.

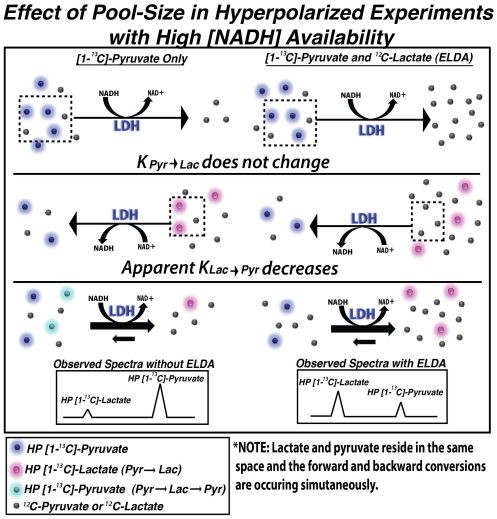

Figure 2: Hypothesized effect of Pool-Size on observed HP Cabon-13 spectra and conversion rates in tumors.

References: [1] Hurd et al. ISMRM. 2011: 19, #654. [2] Hsu and Sabatini. Cell 2008; 5;134(5):703-7. [3]Larson et al. ISMRM. 2011: 19, #655. [4] Frahm et al. JMR 1985; 64: 81-93.

Acknowledgments: We would like to acknowledge Galen Reed, Simon Hu, Cornelius Von Morze, Hikari Yoshihara, and Peter Shin for technical assistance, as well as, funding from NIH P41-EB01359, NIGMS-IMSD R25-GM56847 and NIH R00-EB012064.