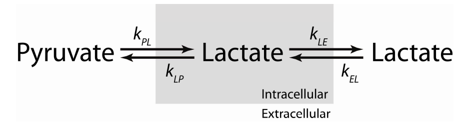
Comparison of Models for Analysis of Flux Through Lactate Dehydrogenase in Glioblastoma Cells Using Hyperpolarized [1-^{13}C]Pyruvate

C. Harrison[1], R. J. DeBerardinis[2,3], C. Yang[2], A. K. Jindal[1], A. D. Sherry[1,4], and C. R. Malloy[1,5]

[1]Advanced Imaging Research Center, UT Southwestern, Dallas, TX, United States, [2]Pediatrics, UT Southwestern, Dallas, TX, United States, [3]McDermott Center for Human Growth and Development, UT Southwestern, Dallas, TX, United States, [4]Chemistry, UT Dallas, Richardson, TX, United States, [5]Veterans Affairs, North Texas Health Care System, Dallas, TX, United States

Introduction

Recent advances in fast dissolution hyperpolarization provide the opportunity to measure fluxes in specific metabolic pathways in cancer. For example, excess conversion of pyruvate to lactate may occur as a result of hypoxia or of intrinsic metabolic reprogramming during malignant transformation, and the detection of excess lactate may therefore be a useful biomarker of malignancy. Hyperpolarized (HP) ^{13}C NMR offers the ability to monitor lactate signal after the administration of HP pyruvate. However a key problem is to assess initial flux into the lactate pool. The loss of signal over time inherent in the technique requires precise models to extract flux measurements. Multiple models have been proposed for analysis of the lactate appearance curve. In this study, [1-$^{13}C_1$]lactate was monitored by NMR and mass spectrometry (MS) following the addition of [1-$^{13}C_1$]pyruvate to cultured glioblastoma cells. Direct measurements of the amount and fractional enrichment of lactate by mass spectrometry allowed us to distinguish among kinetic models.



$$\frac{d}{dt}\begin{bmatrix} Pyr \\ Lac \\ ExLac \end{bmatrix} = \begin{bmatrix} -k_{PL}-\rho_P & k_{LP} & 0 \\ k_{PL} & -k_{LE}-k_{LP}-\rho_L & k_{EL} \\ 0 & k_{LE} & -k_E-\rho_L \end{bmatrix}\begin{bmatrix} Pyr \\ Lac \\ ExLac \end{bmatrix}$$

Figure 1: Total pyruvate is modeled in exchange with lactate such that it is converted instantly upon entering the cytosol and the intracellular lactate pool is in exchange with the extracellular lactate pool. k_{LP}, k_{LE}, and k_{EL} can be set to zero in different combinations to create the various models.

Methods

SF188 glioblastoma cells were grown in DMEM in 15-cm culture dishes as described.[1] On the day of the experiment, four dishes of cells (~100 million) were trypsinized, rinsed and resuspended in 1.5 ml DMEM lacking glucose and pyruvate. 10.9 mg of [1-^{13}C]pyruvic acid containing 15 mM trityl radical was hyperpolarized for 120 minutes prior to dissolution with 4 ml of 15.3 mM sodium bicarbonate. 200 µl of solution was placed in the bottom of a 10 mm NMR tube and inserted into the center of a Varian VNMRS 14.1 T system. The cell suspension was added via syringe at the start of acquisition resulting in a total volume of 1 ml inside the NMR coil with 6 mM HP pyruvate. Serial 18° doubly-selective Gaussian pulses designed to excite [1-^{13}C]lactate and the natural abundance pyruvate C2 doublet simultaneously were applied every 2 seconds over 200 seconds. For MS, cells were supplemented with 6 mM Na-[3-^{13}C]pyruvate and incubated for 0, 1, 3, and 10 minutes. Both the medium and cell pellets were analyzed for total lactate abundance and ^{13}C enrichment.

Six first-order models were constructed for data fitting. The two-pool model of total lactate and total pyruvate allowed exchange or forward flux from pyruvate to lactate. The three-pool model incorporated pyruvate, intracellular lactate, and extracellular lactate. This model allowed unidirectional flux from pyruvate to lactate to extracellular lactate, bidirectional flux between pyruvate and intracellular lactate, between intracellular lactate and extracellular lactate, or bidirectional flux among all three compartments (Figure 1).

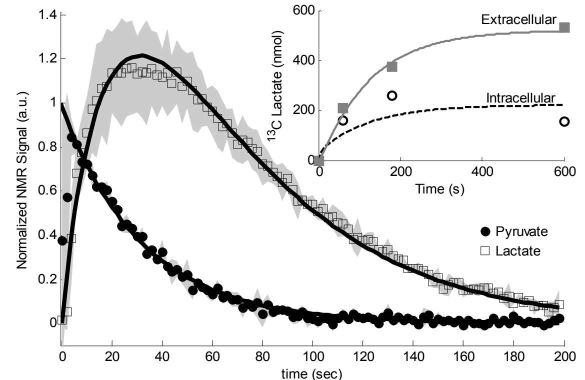


Figure 2: Hyperpolarized data collected with a double-Gaussian excitation and ^{13}C lactate detected by MS (inset) fit simultaneously to determine kinetic rates for a three-pool exchange model (fitted curves). Shaded areas denote the standard deviations of the HP lactate and pyruvate C2 data.

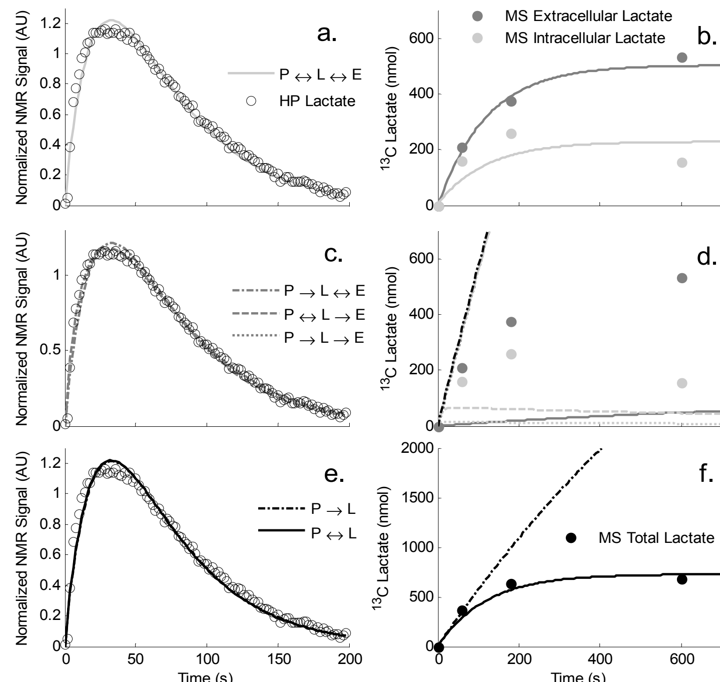


Figure 3: (a & b) Data fits for HP and MS for the bidirectional three-pool model. (c & d) Fits for HP and modeled ^{13}C lactate concentrations for intracellular and extracellular pools for the additional three-pool models. (e & f) Fits for HP and MS total lactate for two-pool models.

Results

After examining a variety of first-order kinetic models, it was determined that a three-pool bidirectional model most accurately describes pyruvate metabolism in these cells (Figure 2). The abundance of ^{13}C lactate measured quantitatively by MS reached equilibrium inside and outside the cell within 10 minutes (Figure 2 inset), while the total lactate pool sizes did not change over this time period. The flux of [1-$^{13}C_1$]pyruvate into [1-$^{13}C_1$]lactate was determined to be 224 µmol/billion cells/hr for a three-pool exchange model, a figure that agrees closely with measurements of lactate labeling using conventional NMR over much longer time scales.[2] The various models produced indistinguishable HP fits even though the MS data can only be fit by fully bidirectional exchange models (Figure 3). However, the resulting initial flux values for pyruvate to lactate conversion are identical for all models investigated because the C2 pyruvate data provides a measure of the absolute signal to concentration ratio.

Conclusions

Conversion of pyruvate to lactate is a classic hallmark of tumor metabolism and a precise measurement of lactate dehydrogenase activity is a major goal for advanced metabolic imaging of cancer. With the signal-to-concentration ratio provided by the pyruvate C2 signal, the initial flux rate of pyruvate into lactate can be precisely determined regardless of the model chosen for *in vitro* experiments. Therefore, by applying a combination of selective pulses and MS-based pool size measurements, HP-NMR can be used to determine quantitative fluxes in cancer cells. This is a first step towards definitive flux measurements in live tumors.

REFERENCES

[1] Yang et al. *Cancer Res*. 2009;69:7986-7993

[2] DeBerardinis et al. *Proc Natl Acad Sci U S A*. 2007;104:19345-19350