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Kinetic modeling of hyperpolarized $^{13}C_1$-pyruvate metabolism in normal rats and TRAMP mice

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ABSTRACT

Purpose: To investigate metabolic exchange between $^{13}C_1$-pyruvate, $^{13}C_1$-lactate, and $^{13}C_1$-alanine in preclinical model systems using kinetic modeling of dynamic hyperpolarized ^{13}C spectroscopic data and to examine the relationship between fitted parameters and dose–response.

Materials and methods: Dynamic ^{13}C spectroscopy data were acquired in normal rats, wild type mice, and mice with transgenic prostate tumors (TRAMP) either within a single slice or using a one-dimensional echo-planar spectroscopic imaging (1D-EPSI) encoding technique. Rate constants were estimated by fitting a set of exponential equations to the dynamic data. Variations in fitted parameters were used to determine model robustness in 15 mm slices centered on normal rat kidneys. Parameter values were used to investigate differences in metabolism between and within TRAMP and wild type mice.

Results: The kinetic model was shown here to be robust when fitting data from a rat given similar doses. In normal rats, Michaelis–Menten kinetics were able to describe the dose–response of the fitted exchange rate constants with a 13.65% and 16.75% scaled fitting error (SFE) for $k_{pyr\rightarrow lac}$ and $k_{pyr\rightarrow ala}$, respectively. In TRAMP mice, $k_{pyr\rightarrow lac}$ increased an average of 94% after up to 23 days of disease progression, whether the mice were untreated or treated with casodex. Parameters estimated from dynamic ^{13}C 1D-EPSI data were able to differentiate anatomical structures within both wild type and TRAMP mice.

Conclusions: The metabolic parameters estimated using this approach may be useful for *in vivo* monitoring of tumor progression and treatment efficacy, as well as to distinguish between various tissues based on metabolic activity.

1. Introduction

The biochemical processes observed in cancerous tissue may be markedly different than those in anatomically similar normal tissue [1,2]. A number of such processes have been investigated using *in vivo* ^{13}C magnetic resonance spectroscopy (MRS) and mathematical modeling, but have suffered from relatively low signal to noise ratios [3,4]. With the recent development of dynamic nuclear polarization (DNP) it has been possible to increase the ^{13}C MR signal intensity by more than 10,000-fold [5] and to provide dramatic improvements in the ability to study *in vivo* ^{13}C metabolism [6–10].

Pyruvate is an important end product of glycolysis that is preferentially converted in the mitochondria of healthy cells to acetyl-CoA and carbon dioxide via pyruvate dehydrogenase (PDH). Acetyl-CoA enters the tricarboxylic acid (TCA) cycle and eventually provides cellular energy as ATP. Without oxygen, or if the TCA cycle is running at full capacity, healthy tissue is forced to maintain energy needs through an alternate pathway that converts pyruvate to lactate via lactate dehydrogenase (LDH) and oxidizes NADH to NAD^+. The NAD^+ is then available to aid in further glycolytic conversion of glucose to pyruvate, providing cellular energy as ATP. Another common pathway for pyruvate is conversion to alanine via alanine transaminase (ALT), which is known to occur in healthy liver and sometimes muscle. Fig. 1 shows a simplified diagram of these biochemical pathways that follows the C_1 carbon of pyruvate.

There are a number of changes in the metabolic products of pyruvate that may be of interest for the evaluation of tumors. One key finding is that all tumors preferentially undergo conversion of pyruvate to lactic acid, even in the presence of oxygen. This observation is known as aerobic glycolysis, or the Warburg effect [11,12]. Some cancer cells also show changes in transaminase

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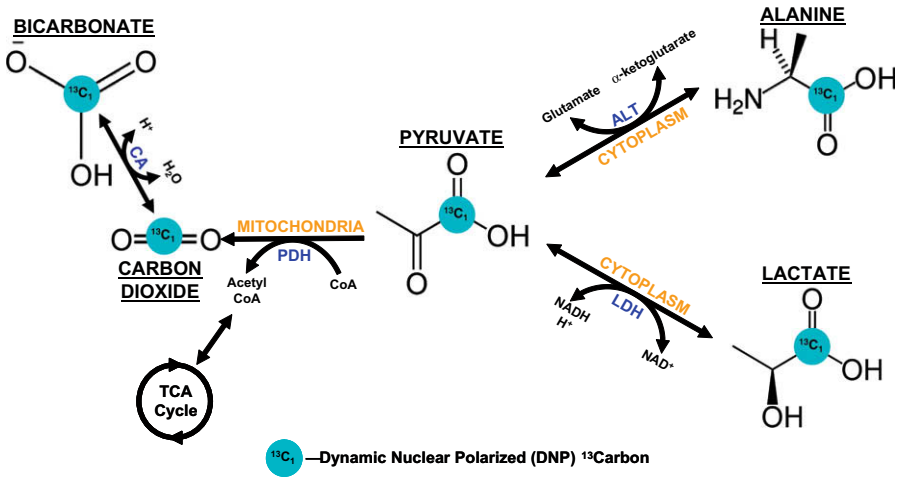

Fig. 1. Simplified diagram of the biochemical pathways taken by $^{13}C_1$-pyruvate that are visible with the hyperpolarized MR spectroscopy techniques discussed here. The position of the hyperpolarized ^{13}C nucleus is shown in all metabolites with the $^{13}C_1$ symbol. LDH = lactate dehydrogenase. PDH, pyruvate dehydrogenase complex. ALT, alanine transaminase. CA, carbonic anhydrase.

activity, suggesting pyruvate-to-alanine exchange may be enhanced or suppressed, depending on tumor type [13–16]. Therefore, it may be possible to discern healthy tissue from cancerous tissue or to monitor cancer progression by injecting ^{13}C-pyruvate and monitoring the changes in ^{13}C-lactate, ^{13}C-alanine, and/or the MR signals from other metabolic products.

Prostate cancer is the most prevalent form of cancer in men, making up approximately 25% of all new male cancer diagnoses each year in the US [17]. A transgenic model of prostate cancer in mice (TRAMP) has been developed for its similarities to human prostate cancer, in that the tumor occurs spontaneously and follows a similar pattern of progression [18]. By studying the TRAMP model using hyperpolarized ^{13}C techniques, important details about prostate cancer may be learned, with future studies aimed at investigating other tumor types and cancer in general.

This study uses dynamic MRS data to investigate $^{13}C_1$-pyruvate metabolism in healthy rats and mice, and in TRAMP mice. This agent was chosen because of the relatively long T_1 value of the ^{13}C nucleus in the C_1 position. Simple exponential equations were used to model the dynamic changes in $^{13}C_1$-pyruvate, $^{13}C_1$-lactate, and $^{13}C_1$-alanine levels following a bolus of the hyperpolarized agent. The goal of the study was to use a range of different doses of hyperpolarized $^{13}C_1$-pyruvate in order to assess the dose–response and to investigate whether it could be modeled with Michaelis–Menten-like kinetics.

2. Methods

Twelve healthy male Sprague–Dawley rats (median mass of 330 g) were injected with hyperpolarized $^{13}C_1$-pyruvate through a tail vein catheter. Two male wild type and ten TRAMP mice (median mass of 35.5 g) were injected with hyperpolarized $^{13}C_1$-pyruvate through a jugular vein catheter. The TRAMP mice were serially studied to monitor tumor progression and treatment response, whereas healthy rats and mice were sacrificed immediately after imaging. The TRAMP mice received up to 12 $^{13}C_1$-pyruvate injections over multiple studies and the healthy mice and rats received a maximum of three $^{13}C_1$-pyruvate injections. Imaging and spectroscopy were performed on a GE 3T MR scanner (GE Healthcare, Waukesha, WI) with a dual-tuned ($^1H/^{13}C$) quadrature coil, either customized for rats (8 cm inner diameter, 9 cm length, sensitive volume: 75 mm diameter and 90 mm length) or customized for mice (5 cm inner diameter,

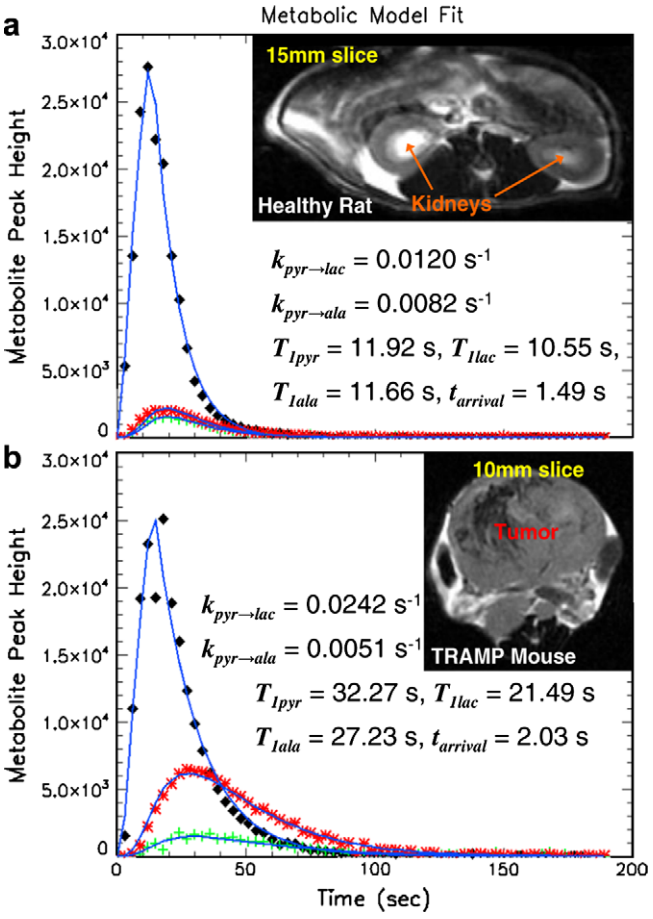

Fig. 2. Examples of dynamic curves of metabolite levels in a 15 mm slice centered on healthy rat kidney (a) and a 10 mm slice centered on TRAMP tumor (b). Pyruvate data are represented by black diamonds (♦), lactate data are represented by red asterisks (∗), alanine data are represented by green plus signs (+), and the estimated best fit lines are represented by the blue lines. Metabolic parameters, estimated from the model in equations 1 and 2 are also listed inside their respective plots, along with a T_2 fast spin-echo (FSE) ^1H image of the slice used to acquire ^{13}C data. Both animals received a dose of approximately 725 μmol/kg of hyperpolarized $^{13}C_1$-pyruvate injected over 12 s.