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Original contributions

Feasibility of using hyperpolarized [1-^{13}C]lactate as a substrate for in vivo metabolic ^{13}C MRSI studies

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Abstract

The development of dynamic nuclear polarization in solution has enabled in vivo ^{13}C MR studies at high signal-to-noise ratio following injection of prepolarized ^{13}C substrates. While prior studies have demonstrated the ability to observe metabolism following injection of hyperpolarized ^{13}C pyruvate, the goal of this study was to develop and test a new hyperpolarized agent for investigating in vivo metabolism, [1-^{13}C]lactate. A preparation for prepolarized ^{13}C lactate and the requisite dissolution media were developed to investigate the feasibility for in vivo ^{13}C MRS/MRSI studies following injection of this hyperpolarized agent. This study demonstrated, for the first time, not only the ability to detect hyperpolarized [1-^{13}C]lactate in vivo but also the metabolic products ^{13}C pyruvate, ^{13}C alanine and ^{13}C bicarbonate following injection in normal rats. The use of ^{13}C lactate as a substrate provided the opportunity to study the conversion of lactate to pyruvate in vivo and to detect the secondary conversions to alanine and bicarbonate through pyruvate. This study also demonstrated the potential value of this hyperpolarized agent to investigate in vivo lactate uptake and metabolism in preclinical animal models.

Keywords: DNP; C13; MRSI

1. Introduction

Recent studies have demonstrated that signal enhancements from dynamic nuclear polarization (DNP) can be retained in solution [1]. This development has enabled in vivo ^{13}C MR studies at high signal-to-noise ratio (SNR) following injection of prepolarized ^{13}C substrates [2]. It has been shown that by using [1-^{13}C]pyruvate as the substrate for hyperpolarized ^{13}C MRS/MRSI experiment, real-time cellular metabolism can be studied in normal and diseased tissues in vivo [3–5]. These studies have shown the ability to detect the metabolic conversion of the hyperpolarized [1-^{13}C]pyruvate into [1-^{13}C]lactate, [1-^{13}C]alanine and ^{13}C bicarbonate after in vivo injection in animal models. In these studies using prepolarized ^{13}C pyruvate as the substrate, the concentration of pyruvate in the injected solution was much higher than the endogenous concentration [3–5]. Thus, one of the processes that can be probed in these conditions is the cellular conversion of the ^{13}C pyruvate bolus to ^{13}C lactate [6].

All these prior studies [2–6] have used ^{13}C pyruvic acid; however, if ^{13}C lactate could be used as the substrate in prepolarized ^{13}C MRS/MRSI study, it then may be possible to probe the reverse enzymatic conversion of hyperpolarized ^{13}C lactate into the unlabeled pyruvate pool. This could allow better understanding of the lactate dehydrogenase (LDH) activity in vivo in different tissue types or diseases at different stages. The development of ^{13}C lactate as a new hyperpolarized agent for in vivo studies requires overcoming several obstacles. [1-^{13}C]Pyruvic acid is an especially good compound for hyperpolarized studies since, as a neat solution, it naturally forms an amorphous solid at 1 K, polarizes rapidly to a high percentage in less than 1 h and retains polarization well due to its long T_1 in solution. For [1-^{13}C]lactate, a different preparation is required with a high substrate concentration, a glassing agent/solvent to insure an amorphous solid at 1 K, a short DNP enhancement rate constant and a high degree of polarization. The goal of this

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study was to develop a hyperpolarized MR probe based on [1-^{13}C]lactate as the substrate for in vivo ^{13}C MRS/MRSI studies and to determine if metabolic products could be detected following injection in animal models.

2. Methods

2.1. Polarizer and compound

A HyperSense DNP polarizer (Oxford Instruments, Abingdon, UK) was used in this study. The preparation for prepolarization developed in this project was a mixture of [1-^{13}C]lactate (Isotec, Miamisburg, OH), water, DMSO and OX63 trityl radical (Oxford Instruments). The mixture contained 38.5% $^{13}C_1$ lactate and 30% DMSO. The trityl radical concentration was 15 mM. The [1-^{13}C]lactate mixture was polarized in a field of 3.35 T at approximately 1.4 K by irradiation of 94.116-GHz microwaves similarly to what was described previously for [1-^{13}C]pyruvate [1]. Each sample used in the animal experiments was polarized for ~100 min prior to dissolution.

A normal saline and 100 mg/L sodium EDTA solution was used as the dissolution medium. The ^{13}C lactate concentration in the hyperpolarized lactate solution was 44 mM. Immediately after the dissolution, an aliquot of the hyperpolarized lactate solution was used to measure the percentage of polarization (ranging from 6.9% to 12.4%; the average for these experiments was 10.0%). The duration for the dissolution process and transferring of the solution was approximately 10–15 s. In each rat studied, ~2.5 ml (ranging from 2.2 to 3 ml; average, 2.6 ml) of the final lactate solution was injected into the rat over a 12-s period followed by a normal saline flush.

2.2. Animal handling

All animal studies were carried out under a protocol approved by the UCSF Institutional Animal Care and Use Committee. Male Sprague–Dawley rats weighing 250–350 g were placed on a heated pad and anesthetized with isoflurane (2–3%). A catheter was introduced into the tail vein for the eventual intravenous administration of hyperpolarized lactate solution, and the rat was transferred into a heated pad in the RF coil in the MR scanner. While in the scanner, anesthesia was maintained by a continual delivery of isoflurane (1–2%) via a long tube to a cone placed over the rat's nose and mouth. The rat's vital signs (heart rate and oxygen saturation) were continually monitored. Care was taken to ensure that body temperature was maintained at 37°C throughout the imaging procedures by maintaining a flow of heated water through the pad underneath the rat. At the end of the study, the rat was euthanized by a combination of overanesthesia with isoflurane and bilateral thoracotomy following an approved protocol.

2.3. MRI, MRS and MRSI studies

All studies were performed using a 3-T GE Signa scanner (GE Healthcare, Waukesha, WI) equipped with the MNS (multinuclear spectroscopy) hardware package. The RF coil used in these experiments was a dual-tuned ^{1}H–^{13}C coil with a quadrature ^{13}C channel and a linear ^{1}H channel constructed based on an earlier design [7] and used in prior hyperpolarized ^{13}C pyruvate studies [3–5]. The inner coil diameter was 8 cm and the length of the coil was 9 cm to accommodate rats of varying size.

T_2-weighted anatomical images were obtained in all three planes using a fast spin-echo sequence. Axial and sagittal images were each acquired in ~10 min with a 10-cm FOV, 192×192 matrix, 2-mm-thick slices and NEX=6. Coronal images were acquired with a 12-cm FOV, 192×192 matrix, 1.5-mm-thick slices and NEX=6 with a scan time of 10 min. The total imaging time required to obtain images in all three planes was, thus, approximately 35 min.

One polarized/dissolved sample was used to estimate the T_1 relaxation time of the hyperpolarized [1-^{13}C]lactate in solution. The polarized [1-^{13}C]lactate solution was transferred into a syringe and placed inside the dual-tuned coil used in this study. A small tip angle, nonlocalized MRS pulse sequence (identical to what is used in the ^{13}C in vivo dynamic MRS study described in the next paragraph) was used to measure the signal decay due to T_1 relaxation. A monoexponential fit was used to estimate T_1, and the reported T_1 value was corrected for RF tipping [8].

Five ^{13}C dynamic MRS studies were performed in five different rats using a double spin-echo pulse sequence with a nonselective 5° flip angle RF excitation pulse and a pair of 180° hyperbolic secant refocusing pulses (also without localization) [4]. A TE of 35 ms, a repetition time (TR) of 3 s and a readout filter of 5000 Hz/2048 points were used for these studies. The acquisition started at the beginning of a 12 s manual injection of the ^{13}C lactate into the rat tail vein.

3D ^{13}C MRSI data were acquired in 10 s in two normal rats using a double spin-echo pulse sequence with a slice-selective small tip angle excitation pulse and a flyback echo-planar readout trajectory [4]. An 8×6×1 phase-encoding matrix with flyback echo-planar trajectory on the z-axis (8×6×16 effective matrix) was used with 10×10×10 mm spatial resolution (1.0 cc voxel resolution) that resulted in an 80×60×160 mm FOV to cover the rat torso and abdomen. The flyback echo-planar trajectory was designed for a 581-Hz spectral bandwidth to include ^{13}C lactate, ^{13}C alanine and ^{13}C pyruvate without spectral aliasing. A total of 59 readout/rewind lobes were included during each readout for a spectral resolution of 9.83 Hz. With a readout filter of 25,000 Hz/2538 points, 16 k-space points were acquired during each TR and the SNR efficiency of this waveform was 61% [9]. The TE for the MRSI acquisition was 140 ms (readout was centered on the center of the second spin echo), and the TR was 215 ms.

2.4. Data processing

Dynamic data were apodized in the time domain with a 10-Hz Gaussian filter prior to a one-dimensional Fourier transform. 3D ^{13}C MRSI data acquired using the flyback echo-planar trajectory were processed using the same

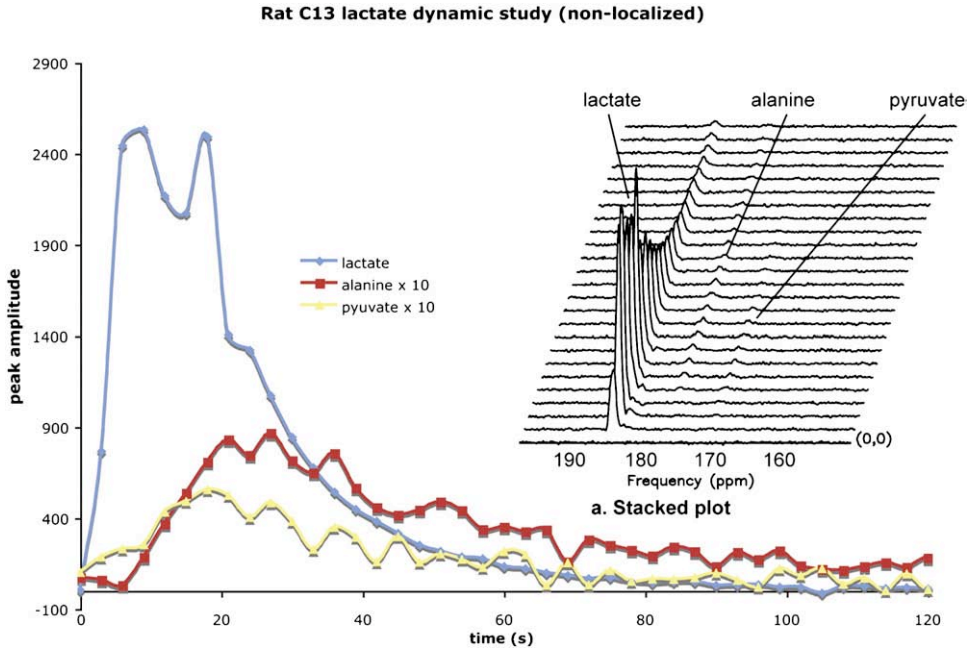

Fig. 1. Dynamic MRS data from a normal rat after injection of hyperpolarized ^{13}C lactate. Both a stacked plot of individual spectra from each time point (first 25 time points, top right) and a time course graph (bottom left) of the ^{13}C lactate, ^{13}C alanine and ^{13}C pyruvate peak amplitude are shown.

procedures described previously [4,9]. The *k*-space points corresponding to the constant portion of the flyback trajectory were selected out, and the data were reordered into a 4D data set with the time decay in the first dimension. The reordered data set was then processed in the same manner as a conventional 4D MRSI data set with the exception that the *k*-space points in the flyback dimension were each acquired at a slightly different time point [9]. This was corrected during the 4D Fourier reconstruction using the fact that an origin shift in *k*-space is equivalent to a phase shift in the transformed domain. The time domain signal was apodized by a 16-Hz Gaussian filter and zero-filled from 59 to 128 points. No apodization in the spatial domains was applied.

3. Results

The T_1 of hyperpolarized [1-^{13}C]lactate estimated from the MRS study performed on the syringe of hyperpolarized lactate solution was 45 s. In this experiment, polarization in solution was measured to be ~7%, which corresponds to a 28,500-fold increase in polarization compared to the thermal equilibrium polarization of 2.47 ppm at 3 T and 300 K.

From the dynamic MRS studies performed starting at the same time the prepolarized ^{13}C lactate was injected, the time course of hyperpolarized ^{13}C lactate uptake in the animal and the time course of the formation of its metabolic products were determined. Representative data in Fig. 1 show a stacked plot of dynamic ^{13}C data (first 25 time points, each 3 s apart) and a graph of the signal amplitude versus time from one of the in vivo dynamic studies. Resonances for ^{13}C lactate, ^{13}C alanine and ^{13}C pyruvate were observed in the spectra (Fig. 1). In the time course graph in Fig. 1, the peak amplitudes of ^{13}C alanine and ^{13}C pyruvate were scaled up by 10-fold for easier viewing. Also observed in this graph was that the ^{13}C pyruvate resonance appeared in the dynamic MRS study prior to the appearance of the ^{13}C alanine resonance, but the ^{13}C alanine signal amplitude becomes higher than that of the ^{13}C pyruvate at approximately 15 s after the start of the injection/experiment in this particular case. No discernable ^{13}C bicarbonate resonance was observed in this dynamic study. Table 1 summarized the lactate arrival time as well as the lactate, pyruvate and alanine time to peak (maximum amplitude in the time course) relative to the start of the injection time. The alanine time to peak was, on average, 14.4 s after the lactate time to peak and 7.4 s after the pyruvate time to peak.

In two out of the five dynamic studies, ^{13}C bicarbonate resonance was observed, with the representative data shown

Table 1
Summary of ^{13}C lactate dynamic MRS data

Rat number	Animal weight (kg)	Dose[a] (mg/kg)	Lactate arrival time (s)	Lactate time to peak (s)	Pyruvate time to peak (s)	Alanine time to peak (s)
1	0.31	34.9	0	9	15	21
2	0.36	30.0	3	9	18	27
3	0.36	24.5	0	12	18	27
4	0.32	31.3	0	12	18	24
5	0.33	29.1	3	12	20	27
Mean	0.34	30.0	1.2	10.8	17.8	25.2

[a] Injected solution was 44 mM lactate, yielding an estimated 5–6 mM blood concentration.

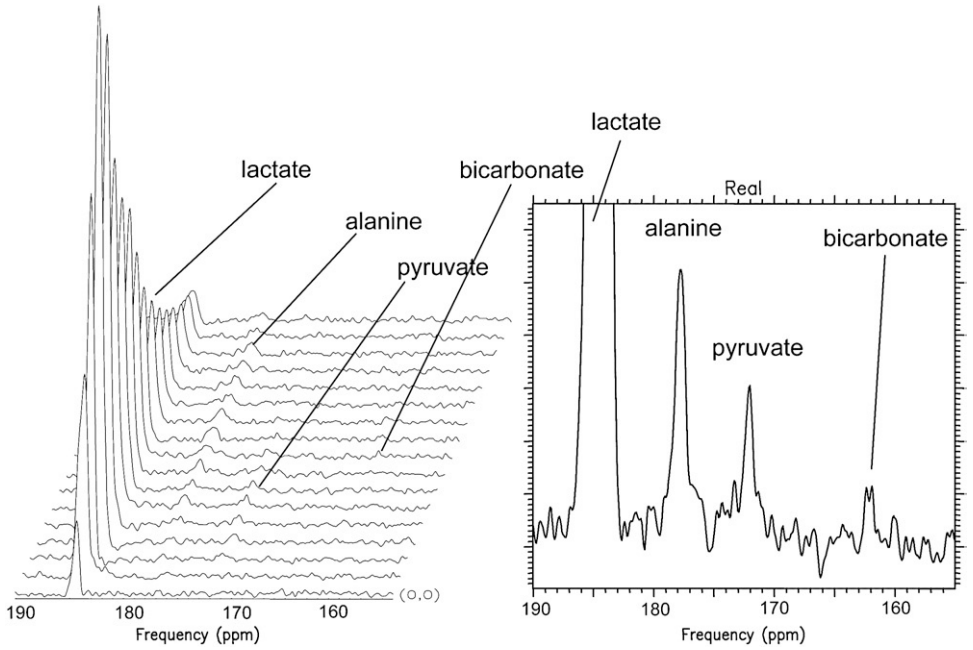

Fig. 2. Stacked plot of in vivo dynamic MRS spectra (first 16 time points, A) as well as a summed spectrum from the stacked shown (B). In addition to the ^{13}C lactate, ^{13}C alanine and ^{13}C pyruvate resonances, the ^{13}C bicarbonate resonance was also observed.

in Fig. 2. Due to the low SNR of the ^{13}C bicarbonate signal, the ^{13}C bicarbonate peak was more clearly observed in the summed spectrum (Fig. 2B) as compared with the individual spectra in the stacked plot (Fig. 2A).

From the 3D ^{13}C MRSI studies, high amplitudes of ^{13}C lactate were observed (Fig. 3); ^{13}C alanine and pyruvate were also observed in voxels centered on rat kidney, muscle and vasculature. Consistent with the data from the ^{13}C dynamic MRS studies, a higher ^{13}C alanine signal amplitude, as compared to that of ^{13}C pyruvate, was observed in the MRSI voxels where both resonances were present.

4. Discussion

Hyperpolarized ^{13}C MRS/MRSI provides an exciting new opportunity to study dynamic metabolic processes in vivo since small organic molecules including many metabolic intermediates have the potential to be compatible with the DNP process [3]. Oxidation, transamination and oxidative decarboxylation of prepolarized [1-^{13}C]pyruvate to produce ^{13}C lactate, ^{13}C alanine and ^{13}C bicarbonate have been reported in vivo [3]. Since high, nonphysiological concentrations of [1-^{13}C]pyruvate were used in these studies,

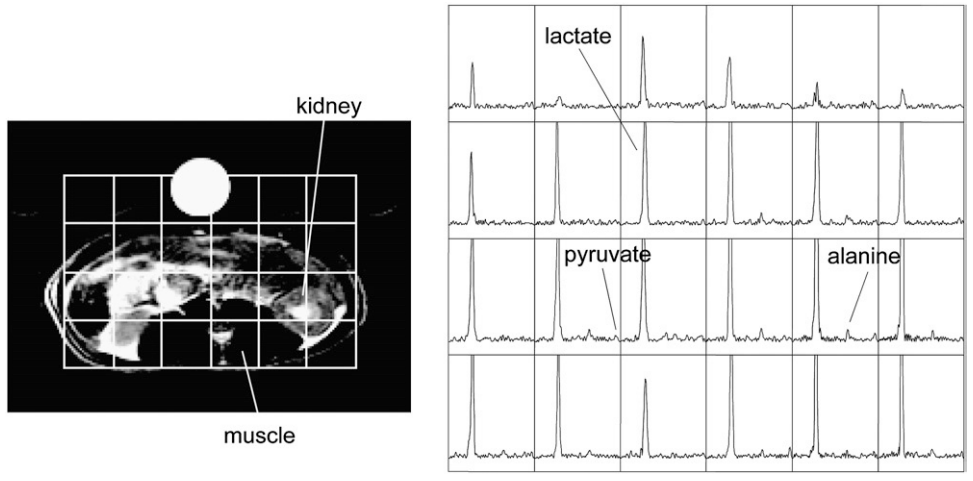

Fig. 3. Axial T_2-weighted anatomical image (left) from a rat through one of the kidneys and the corresponding ^{13}C MRSI data (right) are shown. High level of ^{13}C lactate signal was observed throughout the slice, and ^{13}C alanine and ^{13}C pyruvate resonances were observed in voxels containing kidney and voxels containing muscle and vasculature.

this substrate allowed investigation of the differences in the enzymatic conversion of pyruvate to lactate for different tissue types. However, the lactate-to-pyruvate process could not be studied with ^{13}C pyruvate as the substrate. The use of prepolarized ^{13}C lactate as the substrate provides a new opportunity to probe the LDH activity that converts lactate to pyruvate and to determine if secondary conversions from lactate through pyruvate to alanine and bicarbonate would be observable in vivo by hyperpolarized ^{13}C MRSI.

In this study, after injection of the hyperpolarized [1-^{13}C] lactate into rats, ^{13}C pyruvate was indeed observed (Figs. 1–3). In addition, ^{13}C alanine resonance was also observed in these experiments. The spectral pattern observed in these studies was very different from that observed in the studies in which [1-^{13}C]pyruvate was injected. The alanine signal intensity was higher than the signal intensity for pyruvate in both the dynamic MRS studies and the MRSI studies. It can be surmised that the ^{13}C pyruvate produced by the ^{13}C lactate substrate was rapidly consumed to produce lactate, alanine and bicarbonate, resulting in the relatively low level of ^{13}C pyruvate. The slight temporal offset between the ^{13}C pyruvate curve and the ^{13}C alanine curve in Fig. 1 (the pyruvate signal was initially higher than the alanine signal) and the delayed time to peak of the lactate seen in the dynamic MRS studies (Fig. 1; Table 1) further suggest that ^{13}C pyruvate was produced first after the injection of the ^{13}C lactate and that the ^{13}C pyruvate was then metabolized to ^{13}C alanine, ^{13}C bicarbonate and perhaps back to ^{13}C lactate. It is worth noting that while the prepolarized magnetization may have been decreased through T_1 losses in various enzymatic and membrane transport processes, ^{13}C bicarbonate was still observable in some cases through three enzyme-catalyzed reactions (LDH, pyruvate dehydrogenase and carbonic anhydrase). The fact that multiple downstream products from ^{13}C lactate were observed in this study demonstrates the added potential of using prepolarized ^{13}C substrates for in vivo metabolic studies in which multiple enzyme steps are required.

In the MRSI experiments performed in this study, high-intensity ^{13}C lactate signals were observed in most voxels within the body of the rat. ^{13}C pyruvate and ^{13}C alanine were observed mostly in the kidney and the muscle/central vasculature regions in the rat body. It appeared that the uptake of lactate did not differ significantly between different tissues in a normal healthy rat. The low amplitudes of the ^{13}C pyruvate and ^{13}C alanine signals observed in these studies relative to the ^{13}C lactate signal indicate that the conversion of the ^{13}C lactate to pyruvate is fairly low in normal tissues. More ^{13}C pyruvate and alanine were detected in voxels containing rat kidney, but with the low signal level of these metabolic products and the large voxel sizes used, it was not possible to see clear differences in metabolic activities between different tissue types in this study. Furthermore, the flyback echo-planar readout trajectory did not have adequate spectral bandwidth to include the ^{13}C bicarbonate resonance without spectral aliasing, and based on the relative SNR of the ^{13}C bicarbonate compared to the other metabolites, the ^{13}C bicarbonate signal was most likely below the detection limit in these 3D MRSI experiments. Perhaps a 2D MRSI experiment [5] that does not employ the flyback readout trajectory (to give larger spectral bandwidth) and a larger voxel size (to give more SNR) are required to allow observation of ^{13}C bicarbonate.

In all the in vivo experiments reported in this study, the boluses given corresponded to an approximate 5–6 mM concentration of ^{13}C lactate in the blood of the animals. This concentration is four to five times the endogenous blood lactate concentration in a resting animal (1–2 mM) but within physiological range for an exercising mammal. Thus, the results of this study demonstrate the feasibility for studying hyperpolarized lactate metabolism at concentrations within the physiological range for blood lactate levels.

A prior study using hyperpolarized pyruvate reported that higher levels of ^{13}C lactate were observed in regions of prostate cancer in a mouse model of prostate cancer after injection of prepolarized ^{13}C pyruvate [4]. However, whether the high level of lactate observed in the tumor reflected solely the higher level of LDH activity within the tumor, or the higher uptake of the ^{13}C lactate (produced in the blood and other organs) as well, was not clear. Although beyond the scope of this initial feasibility study, the use of prepolarized ^{13}C lactate as a substrate for in vivo MRSI studies may allow improved elucidation of the mechanism behind the high lactate observed in the tumor region in preclinical cancer models.

5. Conclusions

The feasibility of using [1-^{13}C]lactate as a substrate for prepolarized MRS and MRSI in vivo was demonstrated in this study. The metabolic products ^{13}C pyruvate, ^{13}C alanine and ^{13}C bicarbonate were observed in normal rats following injection of hyperpolarized ^{13}C lactate. The use of [1-^{13}C]lactate as a substrate provides the opportunity to study the conversion of lactate to pyruvate in vivo and to detect the secondary conversions to alanine and bicarbonate through pyruvate.

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