Hyperpolarized 1-[13C]-Ethyl-Pyruvate Metabolic Imaging in Anesthetized Rat Brain

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Introduction: Hyperpolarized 1-[^{13}C]-pyruvate has proven to be an excellent metabolic imaging technology, especially in oncology and cardiology (1,2). Dynamic and tissue level changes in 1-[^{13}C]-pyruvate and its metabolic products, 1-[^{13}C]-lactate, 1-[^{13}C]-alanine and [^{13}C] bicarbonate, have been shown to correlate with metabolic states of interest including disease progression (3) and response to therapy (4). However, for potential neurological applications, pyruvate transport into brain can be a limiting factor, given the 1-2 minute window of useful hyperpolarized lifetime of 1-[^{13}C]-pyruvate *in vivo*. This is especially true in the case of anesthetized normal rat brain where pyruvate transport has been reported to be 2-3 times slower than in conscious animals (5). A potential solution is the use of ethyl-pyruvate, a lipophilic analogue of pyruvate that may have faster uptake through the blood-brain-barrier. Like pyruvate, ethyl pyruvate is an anti-inflammatory that has therapeutic potential (6). It has been shown to attenuate kainic acid-induced neuronal cell death in the mouse hippocampus (7) and reduce the impact of stroke (8). In this study, we explore the feasibility of polarizing 1-[^{13}C]-ethyl pyruvate and compare initial spectroscopic images of uptake in normal anesthetized rat brain relative to the uptake and metabolism of hyperpolarized 1-[^{13}C]-pyruvate.

Material and Methods: ***Animal***: The experiments were performed on 6 healthy (250g-380g) Wistar rats anesthetized with 1-3% isoflurane in oxygen (~1.5 L/min). Respiration, rectal temperature, heart rate, and oxygen saturation were monitored throughout the experiment. Rectal temperature was kept at 37ºC by heating the animal with a temperature-controlled warm water blanket. Hyperpolarized solution was injected via tail vein catheter. Each rat was injected two times at a 2-hour interval with either PA or EP solutions. Preparation and physiological monitoring of the animals in the ^{13}C experiment followed the protocol approved by the local Institutional Animal Care and Use Committees. Maximum tolerated dose and injection rate of ethyl-pyruvate were determined by escalation. The primary limiting physiological response observed was a lowering of heart rate (transient dip following injection) at ethyl-pyruvate doses over 50mM and at rates over 24µmole.s^{-1}.kg^{-1}.

Hyperpolarized 1-[^{13}C] pyruvate solution: The hyperpolarized solution of 1-[^{13}C]-pyruvate was prepared as described previously (9) using a HyperSense™ polarizer (Oxford Instruments Molecular Biotools, Oxford, UK). Liquid state polarization was between 18 and 20%. The time delay between dissolution to injection into the animal ranged from 16 to 20 s. 200µmoles of pyruvate solution was injected at a rate of 75µmole.s^{-1}.kg^{-1}.

Hyperpolarized 1-[^{13}C] ethyl-pyruvate solution: 1-[^{13}C]-ethyl pyruvate (CIL, Cambridge MA) was freshly prepared as a 6.0M ethyl-pyruvate solution (20% v/v 100% ethanol) containing 15mM Trityl (Finland) and 1mM Prohance (Bracco International). 50µL of the formulation (300µmoles) was polarized using optimized conditions that were nearly the same as determined for pyruvate. Build up rate and polarization level were also very similar to the standard pyruvate formulation. Dissolution with 4mL (plus 0.1mL to compensate for the HyperSense™ dead volume) of 25mM NaOH and 100mg/L Disodium EDTA yielded a 50mM ethyl-pyruvate 25mM pyruvate mix, 2mL of which was injected at a nominal rate of 24µmole.s^{-1}.kg^{-1}.

MR Hardware and Methods: All experiments were performed on a 3T Signa™ MR Scanner (GE Healthcare, Waukesha, WI), using a custom-built dual-tuned (^{1}H/^{13}C) quadrature rat coil.

^{13}C Spectroscopic Imaging: Spectroscopic imaging of the brain used a modified FIDCSI sequence as previously described (9), except that in-plane resolution was reduced to 2.5mm to better separate brain signal from surrounding tissue. Images were collected 20s after injection.

Data Analysis: SAGE™ (GE Healthcare, Waukesha WI) was used for data analysis. The spectral domain was apodized with a 16Hz Gaussian filter and zerofilled once. Spectral grid displays were constructed without any spatial zero-fill. Metabolite Images were Fourier interpolated from 16 x16 to 32 x 32 in plane. A Masked image of an internal reference (8M ^{13}C Urea) was added to each metabolite image for metabolite-to-metabolite intensity comparisons. Color metabolite maps were overlayed on grey-scale proton images.

Results: The left panel of Figure 1 shows data from a 1-[^{13}C]-pyruvate image. Pyruvate signal is readily observed in the brain, but at levels lower than intravascular space and similar to that in the surrounding muscle. Lactate is detected in brain at levels similar to surrounding tissue. Alanine is only detected outside of brain. The right panel shows data from the corresponding 1-[^{13}C]-ethyl-pyruvate study. The most intense signals are focused within the brain. Ethyl-pyruvate hydrate is the most pronounced, followed by ethyl-pyruvate and lactate. All appear to be well distributed. Pyruvate in the injected EP solution is distributed similarly to that observed in the pyruvate-only image. The unidentified peaks U1 and U2, appear to be from the same contaminant (gHMBC data ref 10), and collocate on the in vivo images.

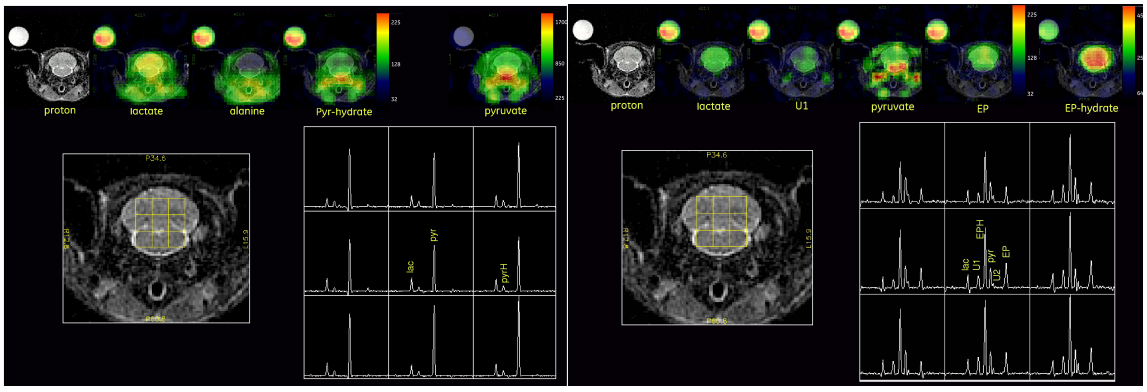

Figure 1.left: ^{13}C pyruvate spectroscopic image. Right: ^{13}C ethyl-pyruvate spectroscopic image. Grids displayed at sampled resolution, the color overlay images are Fourier interpolated once in each spatial direction

Discussion and Conclusion: We have successfully polarized 1-[^{13}C]-ethyl-pyruvate and demonstrated rapid and preferential uptake into brain, as well as the resulting metabolic signal from lactate. Chemical species other than ethyl-pyruvate and its hydrate, as well as safety of the rapidly injected solution continue to be issues. Despite these limitations, ethyl-pyruvate may prove to be good tool in neurological studies of animal models. The unique anti-inflammatory action proposed for ethyl-pyruvate (6) may also provide additional specificity in diseased tissues.

References: 1.Golman K et al., Cancer Res 2006;66:10855. 2.Golman K, et al., MRM 2008;59:1005. 3.Albers M et al., Cancer Res 2008 (in press) 4.Day SE et al., Nat Med 2007;13:1382. 5.Miller LP et al., J Neurochem 2006;46:1412. 6.Fink MP et al., Crit Care Med 2003;31:S51.. 7.Cho IH et al., J. Neurosci Res 2006;15:1505. 8.Yi YM et al., Stroke 2005;36:2238. 9.Kohler SJ et al., MRM 2007;58:65. 10.Hurd and John JMR 1991;91:648.