Purely endogenous hyperpolarized [1-13C]Pyruvate solutions for metabolic study in glioblastoma rat models

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Target audience: Scientists who have an interest in studying tissue metabolism *in vivo* using hyperpolarized precursors.

Introduction: Hyperpolarized [1-^{13}C]pyruvate prepared by dissolution dynamic nuclear polarization[1] has been shown as a promising new contrast for tumor diagnostic and treatment response[2, 3].The preparation of hyperpolarized pyruvate solutions requires the use of paramagnetic centers as polarizing agents which are commonly added in the form of persistent free radicals. The aim of the present study was to demonstrate that *in vivo* pyruvate metabolism can be measured in real time in brain tumors following the injection of purely endogenous hyperpolarized [1-^{13}C]pyruvate preparations[4].

Animal model: Orthotropic glioblastoma tumors were developed following stereotactic injection of cultured (p14) human glioma initiating cells (10^6 cells) into the striatum in the right hemisphere of imunodeficient 7 weeks old nude female rats[5] (200 gr ; n = 3).

Method: 35 uL of pure [1-^{13}C]pyruvic acid (PA) was placed in a synthetic quartz dewar filled with liquid nitrogen in form frozen beads. The frozen sample was then irradiated using a UV source (365-nm) for 1 hr to create photo-induced radicals[4]. The UV-irradiated PA was transferred into a sample cup together with beads of 10M NaOH (49uL). The sample was polarized at 7T custom-designed DNP polarizer[6] (196.8 GHz/1.00 ±0.05 K) for 4 hr before dissolution with 5 mL deuterated phosphate buffer. 1.3 mL of the solution was automatically infused into the rat vein as previously described[7]. MR measurements were carried out on a 9.4 T/ 31 cm actively shielded animal scanner (Varian/Magnex). The animals were anesthetized using 1.5% isoflurane and their physiology was monitored during the entire length of the experiments. Field inhomogeneity was corrected using the FASTMAP protocol. ^{13}C MRS measurements were acquired following a series of single adiabatic 30° BIR4 pulses applied every 3 seconds using a home-built ^{1}H quadrature ^{13}C single loop coil that was place on top of the rat head.

Results: Anatomical T_2W images acquired in rat model of glioblastoma tumor show that the boundaries of the tumor are indistinguishable due to the highly diffusible nature of the tumor. Typical time evolution of the LDH-mediated conversion of pyruvate to lactate following the infusion of solution of the purely endogenous hyperpolarized pyruvate preparation in orthotropic rat brain tumor is presented in Fig 1. Note that the time evolution of pyruvate transamination to alanine is also observable using this preparation. Bicarbonate-to-lactate ratio of $8.5\text{x}10^{-2}\pm2.4\text{x}10^{-2}$ was calculated from the sum spectrum of each animal.

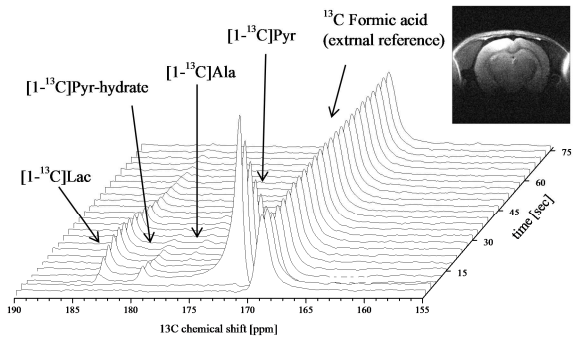

Figure 1: Hyperpolarized pyruvate metabolism studied in rat model of human GBM shown in the T2W image. Hyperpolarization pyruvate produced without persistent radical.

Discussion: The results show that it is possible to study tumor metabolism *in vivo* without the need for persistent radicals. Pyruvate-to-lactate conversion was detected with sufficiently large signal-to-noise ratio to allow studying its kinetic in the tumor. The bicarbonate-to-lactate ratio can be used as a marker for comparing PDH and LDH activities. It was found to be about half of the value reported in healthy rat brain[8]. The use of completely endogenous sample composition through all the stages of the dissolution DNP experiment can facilitate the application of hyperpolarized pyruvate in clinical application since the filtration step is eliminated.

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