In vivo comparison of total and hyperpolarized lactate levels assessed by localized ^{1}H MRS and hyperpolarized ^{13}C MRSI in glioblastoma models at 14.1Tesla

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INTRODUCTION

Dynamic nuclear polarization (DNP) combined with magnetic resonance spectroscopy (MRS) is a highly promising method for assessment of several metabolic reactions in real time. In particular, the conversion of HP [1-^{13}C] pyruvate into HP [1-^{13}C] lactate is of great interest for the investigation of tumor metabolism [1]. However, although the usefulness of the hyperpolarization technique has been clearly demonstrated, the mechanisms governing the *in vivo* modulations of HP [1-^{13}C] lactate levels produced from HP [1-^{13}C] pyruvate can be variable. The level of HP lactate is likely to depend on pyruvate delivery, monocarboxylate transporter activities, NADH levels, lactate dehydrogenase activity and the size of the lactate pool. Because glioblastomas often show evelated levels of lactate as detected by ^{1}H MRS, we questioned whether the levels of HP lactate would depend primarily on the level of total lactate, or whether the information provided by the hyperpolarized experiments differs from that provided by ^{1}H MRS. The goal of this study was therefore to use HP ^{13}C MRSI and localized ^{1}H MRS in order to evaluate the information provided by these two techniques when comparing two orthotopic glioblastoma models with different intracellular lactate levels at 14.1Tesla.

MATERIAL & METHODS

Cell production and culture Two cell lines derived from U87 GBM cells were compared: U87 cells transduced with the wild-type isoform of isocitrate dehydrogenase 1 (U87IDHwt) and U87 cells transduced with a mutant form of IDH (U87IDHmut). Both cell lines were cultured in high glucose-supplemented DMEM at 37°C in 5% CO_2. Intracellular lactate content was measured using high-resolution ^{1}H MRS of cell extracts (1-1.5x10^{8} cells, dual-phase extraction method). In a separate study (data not shown) we demonstrated using ^{1}H MRS of cell extracts that the intracellular lactate level was significantly elevated in U87IDHmut cells as compared to U87IDHwt: $[Lac]_{U87IDHmut}$=22.2±7.1 fmol/cell versus $[Lac]_{U87IDHwt}$=6.9±3.9 fmol/cell, $p<0.001$.

Tumor-bearing animals Athymic mice were anesthetized using ketamine/xylazine (100/20 mg.kg^{-1} respectively) and a suspension of U87IDHwt (n=4 animals) or U87IDHmut (n=5 animals) cells (~3x10^{5}) was injected in the right caudate putamen using the free hand technique.

Experimental set-up Experiments were performed on a 600 MHz wide bore vertical NMR system (Ø$_{I}$=55 mm, 100 G.cm^{-1} gradients, Agilent Technologies, Palo Alto, CA). MR imaging and ^{1}H MRS were performed using a Varian millipede ^{1}H coil (Ø$_{I}$=40mm, 5cm length). A ^{13}C volume coil with the same geometry as the ^{1}H coil was used for hyperpolarized studies. Mice were anesthetized using isoflurane (3% in O_2, 1.5 L.min^{-1}) and a 27G catheter was secured in the tail vein of the animal.

Localized ^{1}H MRS Anatomical imaging was performed to assess the location of the tumor (2D Spin Echo (SE), axial and coronal, TE/TR=20/1200ms, FOV=40x40mm, matrix 256x256, 20 slices, thickness=1mm, acquisition time 5min7s, NT=1). Shimming was performed on a 2.5x2.5x2.5mm^{3} voxel positioned inside the tumor (Figure 1A). The size of the voxel was chosen to match the voxel size of the ^{13}C MRSI grid. An optimized PRESS sequence was used to acquire short (TE=20ms, NT=128) and long echo time (TE=136ms, NT=640) spectra from the tumor voxel (TR=4000ms, SW=10KHz, 4096 points, VAPOR water suppression, 10mm OVS).

HP ^{13}C MRSI [1-^{13}C]-pyruvic acid containing 15 mM of radical OX063 was hyperpolarized using the HyperSense DNP polarizer (Oxford Instruments) as described previously [1]. After 1 hr, polarized pyruvic acid was rapidly dissolved in isotonic buffer (40mM Tris, 80mM NaOH, 0.1 mg/L Na_2EDTA) to obtain a 80mM solution. Within less than 10s, 300µl of this solution was injected through the iv catheter over 12s. ^{13}C 3D-MRSI was acquired 25s after injection: TE/TR=55.21/80ms, variable flip angle, matrix=16x16x16, SW 1600Hz, FOV= 40x40mm, 2.5x2.5x2.5mm^{3} resolution, 6.1s acquisition time). ^{13}C MRSI grids were positioned such as ^{1}H and HP ^{13}C data were obtained from the same 2.5x2.5x2.5mm^{3} voxel.

Post-processing ^{13}C 3D-MRSI data were processed using in-house software to calculate the integrals of lactate and pyruvate and the standard deviation of the noise in the tumor voxel in order to derive lactate-to-pyruvate and lactate-to-noise ratios. Localized ^{1}H MRS spectra were processed using the AMARES package in jMRUI [2].

RESULTS & DISCUSSION

In vivo lactate levels were calculated from the localized ^{1}H MR spectra at long echo time, in which the lactate peak is inverted and does not overlap with the lipid signal (peak *b*, Fig. 1B). Lactate levels normalized to total ^{1}H signal at TE=136ms were significantly higher in U87IDHmut tumors by 220 % ($p=0.02$, Figure 1C), in line with the previously determined cell extract results (Note that the total ^{1}H signal was not significantly different between the two tumor types). In contrast with the ^{1}H data, HP lactate-to-noise and lactate-to-pyruvate ratios from the tumor voxel were not significantly different between the two cell lines, even though a trend of increased lactate-to-noise ratio can be seen in U87IDHmut tumors (Fig. 2C &D). Additionnal metabolic differences were also detected between the two tumor models. Taurine levels were significantly elevated in U87IDHmut tumors ($p=0.04$, *d*, Fig. 1B) and 2-hydroxyglutarate was detectable in short TE spectra of U87IDHmut tumors (*h*, Fig. 1B).

This study demonstrates the differences between the lactate levels as detected by ^{1}H MRS and the lactate-to-noise and lactate-to-pyruvate ratios as assessed by HP ^{13}C MRSI. Even though lactate levels measured by localized ^{1}H MRS were significantly elevated in U87IDHmut, no difference in the level of lactate produced from HP pyruvate in the same voxel could be detected between the two tumor types. This discrepancy confirms that information provided by localized ^{1}H MRS is intrinsically different from the information provided by HP ^{13}C MRSI. The ^{1}H visible lactate reflects the sum of intra and extracellular lactate pools. In contrast, the HP lactate observed using HP ^{13}C MRSI reflects the amount of HP lactate generated from exogenous HP pyruvate during the time course of the experiment. As discussed above, HP lactate can thus be affected by several factors, not just total lactate levels. In our models, the level of lactate was clearly not a dominant factor in HP lactate production. To conclude, this study shows that HP ^{13}C MRSI and localized ^{1}H MRS provide distinct complementary information on the *in vivo* lactate levels in GBM. Furthermore, this study is also the first report of the combination of HP ^{13}C MRSI and ^{1}H MRS at high field to study the effect of the mutant IDH gene in gliomas *in vivo*, and should prove useful for the evaluation of metabolic reprogramming associated with this mutation.

REFERENCES 1. Chaumeil, Neuroimage 59(1):193-201 (2012) **2.** Vanhamme, J Mag Res 129(1):35-43 (1997) **ACKNOWLEDGMENTS** This work was supported by NIH UCSF Brain Tumor SPORE P50 CA097257, a grant from the Academic Senate (UCSF), a fellowship from the American Brain Tumor Association, a seed grant form the Radiology Department at UCSF, UC Discovery in conjunction with GE Healthcare, & center grant P41EB013598.

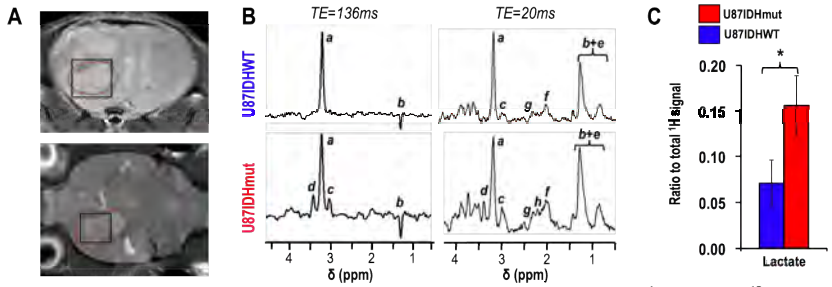

Figure 1 – ***(A)*** *Localization of the tumor voxel (black square) used for both ^{1}H and HP ^{13}C studies (SE images, axial top, coronal bottom)* ***(B)*** *Long (left) and short (right) echo time PRESS spectra from U87IDHwt (top) and U87IDHmut (bottom) tumors: a-choline, b-lactate, c-creatine, d-taurine, e-lipids, f-NAA, g-glutamine, h-2-HG* ***(C)*** *^{1}H Lactate levels for U87IDHwt (blue) and U87IDHmut (red).*

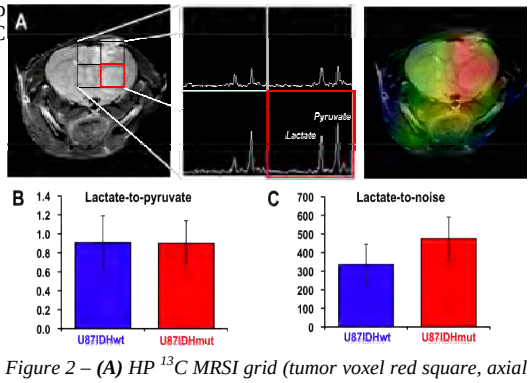

Figure 2 – ***(A)*** *HP ^{13}C MRSI grid (tumor voxel red square, axial SE image), corresponding spectra and lactate heatmap* ***(B)*** *Lactate-to-pyruvate and* ***(C)*** *lactate-to-noise ratios for U87IDHwt and U87IDHmut tumors.*