In vivo detection of PI3K pathway inhibition by hyperpolarized ^{13}C MRSI at 14 Tesla

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INTRODUCTION

Glioblastoma (GBM) is the most common and lethal primary malignant brain tumor in humans [1]. Despite surgical resection, chemotherapy and radiotherapy treatments, the median survival for GBM patients is ~ 1 year [2]. One of the new promising treatment approaches targets the phosphatidylinositol-3-kinase (PI3K) signaling pathway, which plays a crucial role in cell growth, proliferation and survival and is frequently activated in human cancers [3]. In particular, the anticancer drug Everolimus (RAD001), an inhibitor of the PI3K downstream effector mTOR, is currently in phase II clinical trials. However, the assessment of response to PI3K/mTOR inhibitors using traditional imaging methods remains a challenge, as drug action is often associated with tumor stasis rather than shrinkage. Using hyperpolarized (HP) ^{13}C magnetic resonance spectroscopy (MRS), the effect of PI3K/mTOR inhibition was previously investigated by monitoring HP [1-^{13}C]-lactate levels produced from HP [1-^{13}C]-pyruvate in cells [4, 5]. These studies showed a drop in HP lactate associated with a drop in lactate dehydrogenase (LDH) expression and activity resulting from a drop in the hypoxia inducible factor (HIF-1), which is controlled by PI3K/mTOR signaling. Based on these findings, we designed a study aimed at detecting PI3K/mTOR inhibition by Everolimus in tumors *in vivo* using HP ^{13}C MRS at 14 Tesla.

MATERIAL & METHODS

Tumor-bearing animals 4 weeks-old athymic mice (Nu/Nu, Simonsen, Gilroy, CA) were included in the study. For tumor implantation, animals were anesthetized using ketamine/xylazine (100/20 mg.kg^{-1} respectively) and a suspension of GS2 cells (~1x10^7) was injected in the left flank. When the tumor reached a diameter of ~6mm, treated animals received a daily intraperitoneal injection of Everolimus (10mg.kg^{-1}.day^{-1} in DMSO, v=20µL, Molcan Corporation, Canada) while control animals received the same volume of DMSO. During treatment, tumor size was monitored by caliper measurement. All experimental procedures were approved by the UCSF Institutional Animal Care and Use Committee.

MR system & Experimental set-up Experiments were performed on a 600 MHz wide bore vertical NMR system (Ø$_I$=55 mm, 100 G.cm^{-1} gradients, Varian Inc, Palo Alto, CA). MR imaging was performed using a Varian millipede ^{1}H coil (Ø$_I$=40mm, 5cm length). A custom-built ^{13}C surface coil (Ø$_I$=20mm) 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. The tumor region was placed in the center of the ^{13}C coil, and the animal was positioned in the magnet using a custom built cradle. A glass tube containing ^{13}C-enriched urea (c=10M, Ø$_I$=4mm) placed at the center of the surface coil was used for position and chemical shift reference. Temperature and respiration were monitored throughout the experiment.

MR acquisitions Anatomical imaging was first performed to assess the positioning of the tumor and of the urea sample (2D Spin Echo (SE), coronal, TE/TR=20/2000ms, FOV=32x32mm, matrix 256x256, slice thickness=0.5mm, gap=0.5mm, at=8min32s, NT=2). [1-^{13}C]-pyruvic acid (Isotech; Champaign, IL) containing 15 mM of the trityl radical OX063 (Oxford Instruments; Abingdon, UK) was hyperpolarized using the HyperSense DNP polarizer (Oxford Instruments) as described previously [6, 7]. After 1 hr, polarized pyruvic acid was rapidly dissolved in isotonic buffer (40mM Tris, 100mM NaOH, 0.1 mg/L Na_2EDTA) to obtain a 100mM solution. Within less than 10s, 300µl of this solution was injected through the iv catheter over 12s. ^{13}C 2D-MRSI was acquired 37s after injection, the time point when, based on non-localized ^{13}C dynamic data, the hyperpolarized ^{13}C lactate reached a maximum as previously described [8]. The ^{13}C 2D-MRSI parameters were as follows: TE/TR=0.195/125ms, frequency dimension=512; phase dimension=8x8, SW 5000Hz, FOV= 32x32mm, at=8s). A rectangular pulse (pw=100us) equivalent to 20° FA at 5mm from the coil was used for excitation.

Post-processing Tumor volume was calculated from caliper measurements and confirmed from SE images assuming an ellipsoid shape (volume=4/3.π.a.b.c). ^{13}C 2D-MRSI data were processed using jMRUI software [9]. Basic preprocessing was performed (zerofilling 2048 points, lb=20Hz, 0 and 1st order phase corrections). For each voxel, the lactate-to-pyruvate ratio was calculated as the ratio of lactate and pyruvate intensities quantified using the AMARES package for MRSI. From the correspondence between anatomical images and MRSI data, the voxels including more than 75% of a tumor region were considered as tumor voxels and the lactate-to-pyruvate ratios from these voxels were averaged.

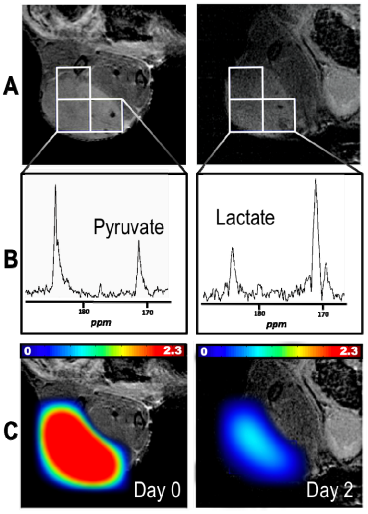

Fig 1 - Effect of Everolimus treatment on GS-2 tumor xenografts *(A) Coronal SE image overlaid with tumors voxels (B) Corresponding 2D-MRSI spectra and (C) lactate-to-pyruvate ratio maps.*

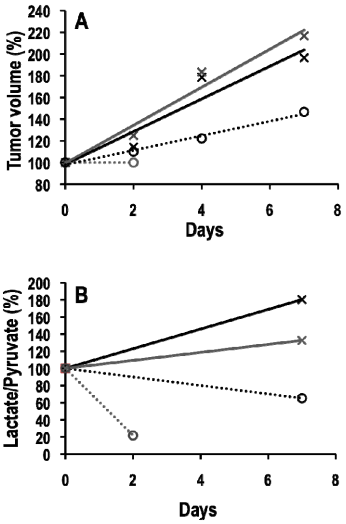

Fig 2 *– (A) Tumor volume and (B) lactate-to-pyruvate ratio as calculated from MRSI data as functions of days of treatment. [x/solid lines] controls, [o/dashed lines] treated.*

RESULTS & DISCUSSION

Figure 1 presents data obtained from one animal. The left column corresponds to data acquired before treatment; the right column to data acquired 2 days post Everolimus treatment. Coronal SE reference images (1A) acquired at 7mm from the surface coil allow assessment of the location and size of the tumor. The tumor voxels (n=3) were overlaid on the SE images. Average spectra (magnitude mode, lb=20Hz) from the tumor voxels are displayed in (1B), showing the decrease in lactate-to-pyruvate ratio after Everolimus treatment. Corresponding lactate-to-pyruvate ratio maps reconstructed from MRSI data are shown in (1C). Figure 2 summarizes results obtained from all animals (controls in solid lines, treated in dashed lines). As shown in (1A), tumor growth as determined from caliper measurement was inhibited in treated animals relative to controls (56±6% inhibition after 7 days of treatment). The lactate-to-pyruvate ratio increased by 56±34% after 7 days of carrier treatment in controls (n=2) and decreased by 78% and 35% in treated animals relative to controls after 2 and 7 days, respectively (2B).

The drop in lactate-to-pyruvate ratio following Everolimus reported in this study is in line with the findings in treated cells [4,5] and likely indicates a decrease in LDH activity in treated tumors. More extensive studies are needed to assess the dynamics of pyruvate to lactate conversion within each voxel and confirm the underlying mechanisms of tumor response to Everolimus treatment. Nonetheless this preliminary *in vivo* study demonstrates the likely value of hyperpolarized ^{13}C MRS for noninvasive monitoring of the effect of PI3K inhibitors and is, to our knowledge, the first report using this method for detection of response to PI3K pathway inhibition *in vivo*.

REFERENCES 1- Belda-Iniesta C et al. Clin Transl Oncol (2008) **2**- Stupp R et al., Lancet Oncol (2009) **3**-Workman P et al., Nat Biotechnol (2006) **4**- Venkatesh et al., AACR (2009) **5**- Ward et al., Poster #2421, ISMRM (2009), **6**- Kohler SJ et al., Magn Reson Med (2007) **7**- Albers MJ et al, Cancer Res (2008) **8**-Park I et al, Neuro Oncol (2009) **9**- Naressi A et al, Comput Biol Med. (2001)

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