In vivo 2D L-COSY detects decreased PC in gliomas after treatment with MN58b, a choline kinase inhibitor

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Introduction: Increases in choline metabolism are consistently observed in cancer and elevated total choline (tCho) is typically used as a biomarker of malignancy in both pre-clinical tumor models and in human tumors. Increased choline kinase (ChoK) activity and subsequently increases in phosphocholine (PC) are observed in proliferating cancer cells and *in situ* tumors using *in vivo* magnetic resonance spectroscopy (MRS). Upon treatment with anticancer drugs, the catabolic pathway of choline metabolism leads to increased glycerophosphocholine (GPC) with a concurrent decrease in PC. Consequently, a change in the PC/GPC ratio occurs in the early phase of treatment, which can be used as a pharmacologic marker of early response. Inhibition of ChoK using specific ChoK inhibitors, such as MN58b (1-2), is a promising new strategy for treatment of tumors. Measuring this treatment response requires non-invasively detecting GPC separately from PC, which can be difficult because these peaks typically overlap in conventionally-acquired one-dimensional (1D) MR spectra. Localized correlated spectroscopy (2D L-COSY) (3) can resolve such peaks by adding a second spectral dimension through indirectly monitoring t_1 evolution with incrementing delays. Thus, this study aimed to use *in vivo* 2D L-COSY to detect changes in PC and GPC in brain tumor models in response to MN58b treatment.

Materials & Methods: Tumor cell culture and implantation: Intracranial tumors were induced by stereotactic injection of F98 cells ($5x10^4$ cells in a 10µl suspension) implanted into syngeneic Fischer (F344) rats. The cell implantation was performed as described previously (4). Animals were allowed to recover and monitored periodically for two weeks then baseline experiments were performed.
Animal Preparation for MRI Scan: The isoflurane anesthetized rat was placed in the coil in an in-house developed restraining device. During the scan, anesthesia was maintained with 1.5% isoflurane in air. Body temperature was maintained at 37±1°C by blowing warm air through the magnet bore.
***In-vivo* 2D L-COSY Spectroscopy:** *In vivo* 2D L-COSY experiments were performed on six normal rat brains and five rats bearing an intracranial tumor. Out of these five tumors, three were subsequently treated with Mn58b (2 mg/kg i.p. daily for 5 days) and the L-COSY experiments were repeated to monitor the effects of treatment. All experiments were performed on a 9.4 T horizontal bore scanner (Varian, Palo Alto, CA) equipped with 25 G/cm gradients. A 35mm i.d. quadrature birdcage coil (M2M, Cleveland, OH) was used for signal transmit and receive. Multi-slice spin-echo and T_2-weighted images were acquired for planning the voxel. Acquisition parameters were as follows: TE/TR = 30/3000ms, averages = 24, bandwidth = 3500 Hz (F_1) and 3500Hz (F_2), 256 Δt_1 increments and $5mm^3$ voxel size. The free induction decays were filled to 1024 x 512 points before Fourier transformation. The total time for 2D L-COSY was around 5 hrs for each scan.
High Resolution NMR: Samples of normal tissue, untreated and treated tumor were scanned *in vitro* using a 500 MHz NMR scanner (Bruker, Billerica, MA).
Data Quantification: *In vivo* spectroscopy data were post-processed using a custom MATLAB-based (The Mathworks, Boston, MA) program and metabolite concentrations were quantified using peak volume integrals.

RESULTS & DISCUSSION: 2D L-COSY was able to uniquely resolve several metabolite peaks including resonances due to creatine (Cr), Choline (Cho), N-acetylaspartate (NAA) and lactate (Lac) which are typically observed in 1D spectra, but also cross-peaks due to alanine (Ala), aspartate (Asp), glutamate/glutamine (Glx), Lac/threonine (Lac/Thr), the aspartyl group of NAA, taurine (Tau), hypotaurine (HTau), phosphoethanolamine (PE), phosphocholine (PC) and glycerophosphocholine (GPC) along with lipids and the glycerol backbone (G) (5-6). Figure 1 shows these identified metabolites in a normal rat brain spectrum. The zoomed-in regions on the right show the L-COSY spectrum from the choline region of an untreated (right, top) and treated (right, bottom) F98 tumor. Table 1 shows quantified metabolite concentrations based on peak volume integrals, with emphasis placed on PC and GPC. The PC/GPC ratio was higher in tumors (1.04) compared to normal brain (0.88). The PC/GPC ratio was found to decrease to 0.86 in tumors following treatment. These findings agreed with the *in* vitro high resolution NMR findings from tissue extracts, as shown in Figure 2, where a higher PC/GPC ratio was noted in tumor before treatment compared to the PC/GPC ratio after treatment.

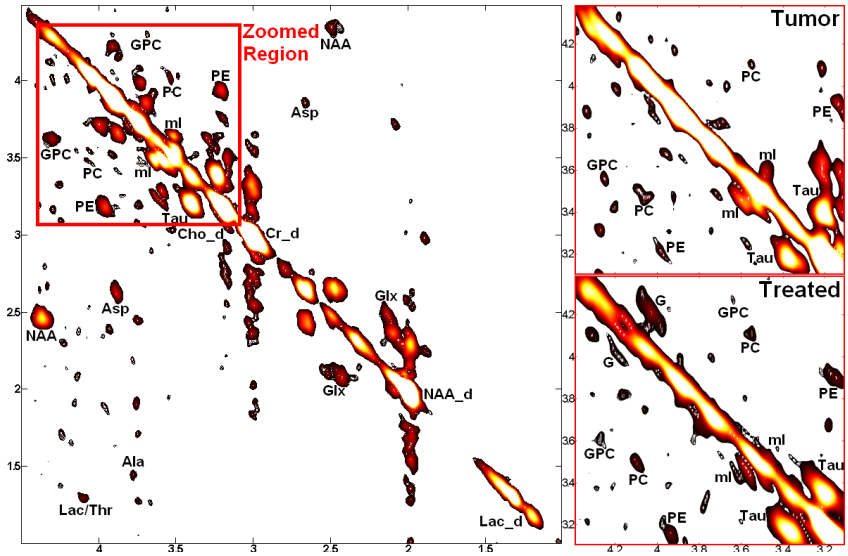

Figure 1: L-COSY spectrum from normal rat brain (left). Zoomed-in spectra show PC in both the untreated (right, top) and treated (right, bottom) glioma tumors. GPC is increased in treated tumor

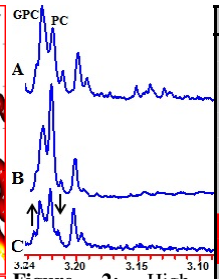

Figure 2: High resolution NMR spectra acquired *ex vivo* from normal (A), untreated tumor (B) and treated tumor (C). Decrease of PC and increase of GPC can be seen after treatment in (C).

Met	P	Normal	Tumor	Treated
Cr3.9	d	40 ± 4.3	22 ± 3.3	10 ± 3.4
Cho	d	33 ± 11	39 ± 12	29 ± 8.7
Cr3.0	d	49 ± 10	27 ± 2.2	17 ± 2.8
NAA	d	38 ± 14	26 ± 2.7	18 ± 5.1
Lac12	d	13 ± 2.6	17 ± 9.8	33 ± 15
Lip0.9	d	19 ± 2.7	17 ± 0.2	8.8 ± 0.2
NAA	C	15 ± 2.6	9.0 ± 0.9	9.0 ± 2.6
GPC	C	4.8 ± 2.4	4.5 ± 0.9	5.1 ± 2.6
PC	C	4.2 ± 0.9	4.6 ± 0.6	4.4 ± 0.8
PE	C	7.0 ± 2.3	5.7 ± 0.6	4.3 ± 0.1
Asp	C	6.8 ± 1.2	4.0 ± 1.7	3.6 ± 1.1
Lac	C	3.6 ± 0.7	9.5 ± 3.2	11 ± 2.2
Ala	C	3.3 ± 0.6	4.0 ± 1.3	6.0 ± 1.2
Tau	C	21 ± 5.8	21 ± 7.3	17 ± 5.8
HTau	C	2.2 ± 0.3	5.8 ± 3.0	5.3 ± 0.7
Glx	C	6.7 ± 3.0	5.5 ± 0.8	3.3 ± 0.5
Lip	C	2.8 ± 0.6	2.7 ± 1.0	3.5 ± 1.7

Table 1: Metabolite concentrations in normal, untreated tumor and treated cases. PC and GPC values are highlighted. Diagonal(d)/cross(C) peaks are also labele

Conclusion: Cross peaks arising from J-coupling interactions in GPC and PC were successfully detected and resolved using 2D L-COSY, and then quantified using volume integrals. Data from rat glioma models indicates a relative increase of GPC relative to PC in the treated F98, which is consistent with ex-vivo findings. Due to the small sample size, tests of statistical significance were not performed for these findings. Future studies will include a larger sample size and advanced quantification techniques, including prior-knowledge based peak fitting to improved characterization of the treatment response of tumor to MN58b.

References: 1. Al-Saffar NM et al. Cancer Res. 2006;66:427-34., 2. Lacal JC. IDrugs. 2001;4: 419-26., 3. Thomas MA et al., NMR Biomed 2003:245-251 4. Kim S. Et al. NMR Biomed 2008;21:208-216., 5. Roman S. Int. J. Cancer. 1997;73:570-579., 6. Velan S., Magn reson Med 2007;26:405-409