Glycine as a biomarker in brain tumors

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Introduction– Glycine is a major inhibitory neurotransmitter in the mammalian central nervous system [1]. High levels of glycine have been detected in the brain of patients with hyperglycinemia and in brain tumors in particular glioblastoma multiforme (GBM) [2-4]. Given its significance, an accurate measure of brain glycine is desirable; this is hindered by the fact that its resonance overlaps with the resonances of myo-inositol. Because of myo-inositol's short TE, glycine can be detected in long TE and when editing, TE-averaged point resolved spectroscopy (PRESS) or 2D J-PRESS are used [1]. Here we employed HRMAS ^{1}H MRS to quantify glycine (Gly) in brain tumor biopsies using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence.

Materials and Methods– Fifty-five samples of control biopsies from epileptic surgeries (9) and tumor biopsies (46) were analyzed. The tumor biopsies belonged to three categories: high-grade [20 cases: 12 glioblastoma multiforme (GBM); 8 anaplastic astrocytoma (AA)], low-grade or LG (17 cases: 7 meningioma (MN); 7 schwannoma; 3 pylocitic astrocytoma), and brain metastases or Met (7 cases: 3 from adenocarcinoma; 4 from breast cancer). Subjects ranged in age from 17 to 54 years. experiments were performed on a Bruker Bio-Spin Avance NMR spectrometer (600.13 MHz) using a 4mm triple resonance (^{1}H, ^{13}C, ^{2}H) HRMAS probe (Bruker). Specimens were pre-weighed and transferred to a ZrO2 rotor tube (4mm diameter, 50 µl), containing an external standard [trimethylsilyl propionic-2,2,3,3-d4 acid (TSP), Mw=172, d=0.00 ppm) that functioned as a reference both for both resonance chemical shift and quantification. The HRMAS ^{1}H MRS was performed at -8°C with 3 kHz MAS speed to minimize tissue degradation. One dimensional (1-D) water suppressed, fully relaxed spectra were acquired with an optimized rotor synchronized Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence [90-(τ-180-τ)$_n$-acquisition]. CPMG is preferred over simple Free Induction Decays (FIDs), as it acts as a T2 filter that reduces the interference of very broad features in the spectrum baseline, originating from tissue water and macromolecules. Additional parameters for the CPMG sequence include inter-pulse delay (τ= $2\pi/\omega_r$ = 400 µs); 256 transients; spectral width of 7.2 kHz; 8k data points; and TR = 3 sec. For quantification purposes we measured the T2 relaxation time, by varying the CPMG evolution time ($T_{CPMG} = 2n\tau$) [n from 7 to 800 (~ 5 to 530 ms)]. *Myo*-inositol (Myo) consists of six CH groups that generate a complex spectral pattern in the ^{1}H MR spectrum and can be modeled as an AM2N2P spin system. The M protons resonate at 3.52 ppm (M2), the N resonates at 3.61 ppm (N2), and the A and P protons resonate at 4.05 and 3.27 ppm, respectively.

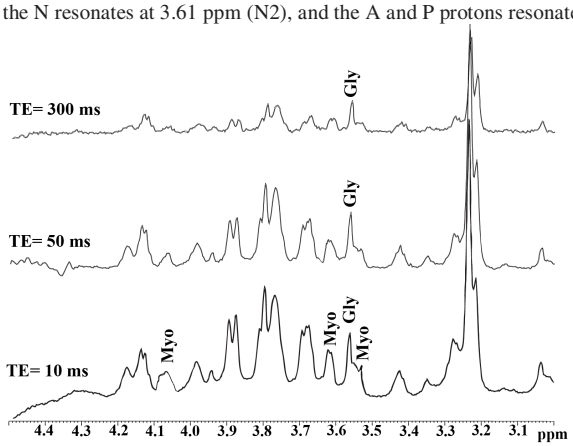

Figure 1. CPMG spectra with different echo-time (TE) of brain tumor metastasis. TE of 50 ms is optimal to quantify Gly.

Results– Figure 1 shows CPMG spectra of metastasis acquired in different echo times. The spectrum acquired at TE=10 ms does not exhibit J- modulation, then at TE=50 ms we detect a strong peak of Gly and the reduction of Myo doublet at 3.52 ppm without losing signal. In longer TEs (i.e. TE=300ms) we detect the Gly singlet but we have a general reduction of signal in the spectrum. We expected a T2 in the range of 10 to 360 ms, thus we acquired a series of spectra to cover a period between 5 and 530 ms. In this manner we aimed to reduce the signal of Myo in favor of Gly singlet using a spin echo sequence. This was possible because of the large J-coupling (9.9 Hz) of Myo proton signal at 3.52ppm and its fast J-modulation. The two protons of Gly constitute a singlet at 3.55 and, like all singlets, do not have phase modulation during the spin echo single excitation. When we used a single spin-echo coherence, the signal of Myo at 3.52ppm and 3.63 ppm underwent a rapid J-modulation and at TE= 100 ms the intensity of the Myo resonance was strongly reduced. At TE of 50 ms the signal intensity of Myo showed a 50% decrease due to J-modulation. Figure 2 shows CPMG spectra (TE= 50 ms) of different brain tumor biopsies. Normal brain tissue and low-grade brain tumors have a higher amount of Myo and the Gly signals are decreased, so we don't see the Gly singlet in their spectra. GBMs exhibit a strong Gly signal. In these tumors the decrease of Myo resonance at 3.52 ppm permitted quantification of the Gly. Our spectra are scaled with respect to TSP signal (not shown in figures). In figure 3 we report averaged scaled ratios of Gly peaks calculated in normal (N) and tumor brain tissues. We also report

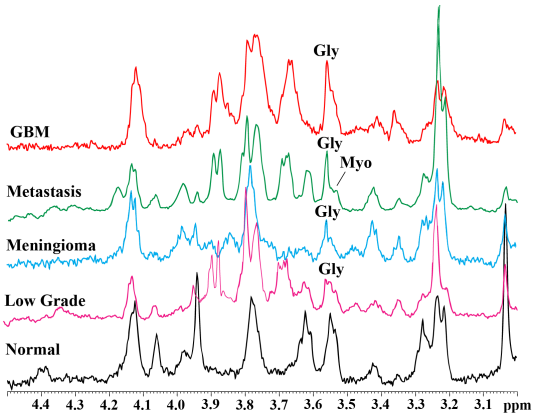

Figure 2. CPMG spectra acquired with TE of 50 ms.

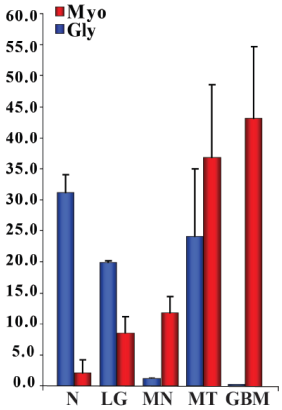

Figure 3. Average scaled ratios of Myo and Gly. The errorbars represent standard errors (SE).

Table I. *P-values* of normal *vs* brain tumor, and GMB *vs* other.

	N/LG	N/MN	N/MT	N/GBM
Myo	0.3730	0.0004	0.6011	-
Gly	0.0494	0.049427	0.0567	0.0310
	GBM/LG	**GBM/MN**	**GBM/MT**	
Gly	0.0494	0.0584	0.7516	

comparisons for Myo and Gly in normal (N) *vs* brain tumors (Low Grade (LG), Meningioma (MN and GBM), Metastasis (MT)) using a t-test (two-tailed, $p < 0.05$) (Table I).

Discussion– In the present study, we show that reliable detection of Gly in brain tumor biopsies is feasible using HRMAS ^{1}H MRS at TE of 50 ms with CPMG. With respect to the choice of TE, this is a TE at which the amplitude of the Myo resonance is sufficiently reduced to allow for Gly detection. The Gly resonance, being a singlet at 3.55 ppm that is overlapped by the more intense Myo resonances, cannot be directly edited, but requires approaches that minimize the signal contribution from Myo. Our results show that we can differentiate brain tumor types based on the amount of Gly they contain and are in agreement with prior observations [4]. Especially the distinction between high-grade tumors (i.e., GBM) and metastasis is of clinical significance because it is a distinction not made adequately at present [5]. Here, it is the reduced Myo and high glycine that distinguish GBM from MT, which exhibit both Myo and Gly resonances. We propose Gly as a useful biomarker in brain tumors.

References

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