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***In vivo* ^1H MR Spectroscopic Imaging of Glycine in Brain Tumors at 3T**

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Abstract

Purpose—MR spectroscopic imaging of glycine (Gly) in the human brain is challenging due to the interference of the abundant neighboring J-coupled resonances. We aim to accomplish reliable imaging of Gly in healthy brain and brain tumors using an optimized MR sequence scheme at 3T.

Methods—Two dimensional ^1H spectroscopic imaging (SI) was performed with a point-resolved spectroscopy (PRESS) scheme. An echo time of 160 ms was used for separation between Gly and myo-inositol (mIns) signals. Data were collected from eight healthy volunteers and fourteen subjects with gliomas. Spectra were analyzed, with LCModel, using numerically-calculated basis spectra. Metabolite concentrations were estimated with reference to creatine in white matter regions at 6.4 mM.

Results—From a linear regression analysis with respect to the fractional gray matter content, the Gly concentrations in pure gray and white matter in healthy brain were estimated to be 1.1 and 0.3 mM, respectively. Gly was significantly elevated in tumors. The tumor-to-contralateral Gly concentration ratio was more extensive with higher grades, showing ~10 fold elevation of Gly in glioblastomas.

Conclusion—The Gly level is significantly different between gray and white matter in healthy brain. Our data indicate that spectroscopic imaging of Gly may provide a biomarker of brain tumor malignancy.

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Keywords

Glycine; Human brain; Gliomas; ^1H MR spectroscopic imaging; 3T; PRESS (point-resolved spectroscopy)

INTRODUCTION

Glycine (Gly), a non-essential amino acid, is a post-synaptic inhibitory neurotransmitter and acts as co-agonist of glutamate on N-methyl D-aspartate receptors (1,2). It is a precursor for the synthesis of proteins and is a primary source of one-carbon units in cellular metabolism (3). Gly is derived from serine, as a product of the Gly, serine and threonine metabolism pathway, and is hypothesized to reside in two separate pools, i.e., metabolic and transmitter pools (3,4). Recent studies indicate abnormal Gly levels in a wide range of brain tumor subtypes (5–12) including gliomas, as well as a variety of cancer cell lines (13). Thus Gly may be a biomarker for primary as well as metastatic brain tumors. Given the cellular heterogeneity as well as regional variations in metabolic alterations (14–16) in brain tumors, imaging of Gly may have important clinical application in the management of patients with brain tumors.

Gly has two uncoupled co-resonating protons, giving rise to a singlet at 3.55 ppm (17). *In vivo* detection of Gly using ^1H MRS in the human brain is challenging primarily due to the presence of the overlapping multiplets of myo-inositol (mIns), whose concentration is much higher (5 – 7 fold) than the Gly concentration in normal physiological conditions (10,11,18,19). Thus, sufficiently strong suppression of the mIns multiplet in the proximity of the Gly resonance is essential for reliable detection of Gly. Several single-voxel localized MRS approaches were proposed to overcome the spectral complexity in Gly measurement, which include TE averaging (19) and triple refocusing (20) at 3T, and short-TE single-spin echo (18) and long-TE PRESS (point-resolved spectroscopy) methods (11) at 7T. Prior *in vivo* studies for Gly elevation in brain tumors used single-voxel localized MRS (8,10) and multi-voxel MRS imaging (7,12). While single-voxel MRS is technically simple and may be preferable for precise measurement of Gly, given the high heterogeneity of brain tumors (14–16) and the presence of regional variation of Gly levels in the normal human brain (11), applicability of Gly spectroscopic imaging is very high.

In the current paper, we report ^1H MR spectroscopic imaging of Gly in the human brain at 3T, which was developed from a Gly-optimized PRESS TE = 160 ms scheme (10). Following the validation in a phantom solution, the feasibility of the Gly spectroscopic imaging method was tested in healthy volunteers and subjects with brain tumors. Spectral fitting was used to resolve the Gly signal from the neighboring metabolite signals. Regional variations of Gly in healthy brain are discussed with linear regression analysis. Numerical simulations were performed to investigate the effect of linewidth and signal-to-noise (SNR) on reliable estimation of Gly. Preliminary tumor data are presented in comparison with the contralateral regions in subjects with brain tumors.

METHODS

Fourteen adult patients with gliomas (median age 43, range 24 – 69) were enrolled in this study. The set of tumor grade comprised 5 grade II, 4 grade III, and 5 grade IV. The tumor subtypes included 7 oligodendrogliomas, 2 astrocytomas, and 5 glioblastomas. The tumor grade and subtypes were determined by histology on biopsy, with World Health Organization criteria. In addition, 8 healthy volunteers (median age 27.5; range 26 – 29) were studied as controls. The study protocol was approved by the Institutional Review Board at the University of Texas Southwestern Medical Center and written informed consent was obtained prior to MR scans.

MR experiments were carried out on a 3.0 T whole-body scanner (Philips Medical Systems, Best, The Netherlands), equipped with an integrated body coil for radio-frequency (RF) transmission and an 8-channel phased-array coil for signal reception. ^1H MR spectroscopic imaging (SI) data were obtained using phase encoding gradients within a PRESS sequence for spatial localization. The volume of interest (VOI) was prescribed by the PRESS sequence with $\text{TE} = 160$ ms ($\text{TE}_1 = 60$ ms and $\text{TE}_2 = 100$ ms). The PRESS sequence included 9.8 ms 90° (along the anterior-posterior direction) and 13.2 ms 180° (along the left-right and head-foot directions) RF pulses, with bandwidths of 4.2 and 1.3 kHz, respectively, at an RF field intensity of 13.5 μT . Validation experiments were performed on a 10 cm diameter spherical phantom that contained Gly, mIns and creatine (Cr) at 1.1 mM, 10 mM and 8 mM, respectively. Phantom SI data were obtained with a 32×32 data matrix with a field of view (FOV) of 160×160 mm², VOI of 50×50 mm², using a TR of 3000 ms and four signal averages. For comparison with SI data, single-voxel spectroscopy (SVS) data were obtained from a voxel size of $10 \times 10 \times 10$ mm³, using a TR of 3000 ms and 256 signal averages.

For *in vivo* experiments in healthy volunteers, the MR protocol included survey imaging followed by a T_1 -weighted high-resolution sagittal Magnetization Prepared RAPid Gradient Echo (MPRAGE, $\text{TE}/\text{TI}/\text{TR} = 3.8/875/1360$ ms, 160 slices with 1 mm thickness, $256 \times 256 \times 160$ mm³ field of view) sequence. For brain tumors, the MR protocol included survey imaging followed by T_2 -weighted fluid attenuated inversion recovery ($T_2\text{w-FLAIR}$) imaging ($\text{TR}/\text{TE}/\text{TI} = 11000/125/2800$ ms, $\text{FOV} = 230 \times 230$ mm², slice thickness of 5 mm, 28 slices along each of transverse and sagittal directions) for tumor identification.

For SI data acquisitions, a PRESS-prescribed transverse slice was positioned to cover the region of interest with spatial resolution of 10×10 mm² and slice thickness of 15 mm. In each SI scan, a 20×16 data matrix was obtained from a 200×160 mm² FOV. A vendor-supplied 2D elliptical k-space sampling scheme was used (21), to reduce the acquisition time by $\sim 30\%$. Each k-space point with 1024 complex points was acquired with two averages at $\text{TR} = 1.2$ s and spectral width = 2000 Hz. The SI data were reconstructed with 2D Fourier transformation of the k-space data, after zero filling the non-acquired k-space points and zero-padding the data matrix to two fold along each phase encoding direction (*i.e.*, 40×32) to obtain interpolated spatial resolution of 5×5 mm². The PRESS RF pulses were tuned to 3 ppm which is halfway between the N-acetylaspartylglutamate 4.6 ppm and lactate 1.3 ppm resonances. Water suppression was performed using a four RF-pulse scheme

(22). In healthy brain a VOI of $80 \times 80 \text{ mm}^2$ was positioned to cover the central brain region above the corpus callosum and for tumor scans the VOI included the tumor mass as well as normal-appearing brain regions. Four outer volume suppression bands were placed in the periphery of the VOI to minimize potential contamination from extracranial lipids and water outside the VOI. First- and second-order shimming was carried out on VOI using FASTMAP (23). To minimize motion artifacts, foam pads were placed to restrict head motions inside the reception coil. The scan time of a single SI acquisition was approximately 10 minutes.

During post-processing, residual water signals were further minimized using a singular value decomposition algorithm in jMRUI (7,10,24,25). A 1-Hz exponential function was multiplied to FIDs prior to Fourier transformation, in order to suppress potential distortions in the later part of the FIDs. Correction for frequency differences across the FOV was performed using in-house programs written in MATLAB® (MathWorks Inc., Natick, MA, USA). The FIDs were zero filled to 4096 points and spectra were analyzed with LCModel (Version 6.3) (26,27) using numerically simulated basis sets, which were calculated according to a published method (25). The basis function included simulated spectra of sixteen brain metabolites; Gly, mIns, tCr (creatine + phosphocreatine), tNAA (N-acetylaspartate + N-acetylaspartylglutamate), tCho (phosphocholine + glycerophosphocholine + free choline), glutamate, glutamine, γ -aminobutyric acid, glutathione, lactate, alanine, taurine, scyllo-inositol, aspartate, phosphoethanolamine, and serine. Spectral fitting was undertaken between 0.5 – 4.1 ppm. For validation purpose, additional LCModel fitting was performed without Gly in the basis sets. For estimation of mIns, tCr, and tCho concentrations, the transverse relaxation effects were corrected using published T_2 values of 200, 150, and 230 ms, respectively (10,28). The T_2 of Gly was assumed to be equal to the mIns T_2 . Metabolite estimates in molar concentrations (mM) were then obtained with reference to tCr in white matter regions at 6.4 mM (29–32), ignoring potential difference in T_1 saturation effects between metabolites in healthy brain and tumors (33). The linewidth (FWHM) estimates were obtained from the LCModel output. Maps of metabolite estimates and Cramér-Rao lower bounds (CRLB) for the VOI were generated. The maps (e.g. matrix of size 16×16) were then interpolated 10 fold (e.g. 160×160) using the nearest neighbor method. The correction for chemical shift displacement effects was performed by shifting the metabolite matrix grid according to the ratio of the chemical shift difference of metabolite resonances from the PRESS RF carrier frequency (3.03 ppm) with respect to the PRESS RF pulse bandwidths. For this, a major resonance was chosen for each metabolite based on the highest signal selectivity. The major resonances used were 3.55, 3.62, 3.21, and 3.03 ppm for Gly, mIns, tCho, and tCr, respectively. For Gly, the chemical shift displacement of the VOI was 2% and 5% along the anterior-posterior (selected by the 90° pulse) and left-right directions (selected by the 180° pulse), respectively. After the shift, the matrix grid was reduced to the original matrix size by averaging over 10×10 pixels. In case of healthy brain data, the central 13×13 grid was selected for analysis of GM and WM linear regression and to generate average metabolite maps. The chemical shift displacement along through-slice direction, selected by the second 180° pulse, was ignored.

For healthy brain data only, gray and white matter (GM and WM) segmentation was performed on MPRAGE images using SPM 5. Fifteen slices of MPRAGE corresponding to an SI slab were selected from the segmented images and averaged to obtain the GM, WM and CSF contents within each of the SI voxels. Metabolite concentrations from healthy brain data were corrected for cerebrospinal fluid contamination (34). To obtain the metabolite concentrations in pure GM and WM, the tissue concentration of metabolite was fitted with a linear function of fractional GM content, $f_{GM} = GM/(GM+WM)$, individually for each of the subjects, and subsequently the intercepts at $f_{GM} = 0$ and 1 were averaged over the 8 subjects. Paired two tailed t-tests were conducted for comparing the metabolite concentrations and CRLBs between pure GM and WM in the healthy brain. For tumor data, paired and unpaired two-tailed t-tests were used to compare the metabolite levels between tumors and normal-appearing brain and between tumor grades. Statistical significance was declared for p-value 0.05. All statistical analyses were conducted using software SAS 9.3 (SAS Institute, Gary, NC).

RESULTS

Phantom Studies

The PRESS (TE_1, TE_2) = (60, 100) ms method was tested in a phantom solution with Gly and mIns for both SI and SVS acquisitions. The multiplet of mIns at ~3.55 ppm was substantially attenuated, affording good separation of the Gly singlet from the mIns background signal (Fig. 1c and 1d). For a prepared mIns-to-Gly concentration ratio of 10/1.1 and a singlet linewidth (FWHM) of 3 Hz, a computer simulation of 90°-acquisition indicated that the largest peak of mIns at ~3.55 ppm is 9.5 fold greater than the Gly signal. The peak amplitude of mIns in the phantom was decreased by 17 fold at $TE = 160$ ms when compared to 90°-acquisition and consequently the mIns-to-Gly peak amplitude ratio at 3.55 ppm was 0.57. This mIns signal suppression occurred similarly in SI and SVS data, and the Gly+mIns composite signal pattern was about the same for SI and SVS. The Gly and mIns signals were well reproduced by LCModel fitting, with uniform residuals between 2.7 and 4.2 ppm in both SI and SVS. Following the correction for transverse relaxation effects using measured phantom T_2 's of Gly (1.5 s) and mIns (0.75 s), the Gly and mIns concentrations were estimated as 1.1 and 10.2 mM, respectively. T_2 of mIns was obtained by fitting phantom data with the simulated signal of mIns at various echo times.

Healthy Brain Studies

The PRESS $TE = 160$ ms SI method was evaluated for imaging of Gly in healthy brain. Figure 2 shows representative data from a healthy subject. For an SI slab positioned above the corpus callosum (Figs. 2a and 2b, blue line), the spectra within a 65×65 mm² volume (Fig. 2b, brown line) showed well defined metabolite signals without substantial baseline distortions (Fig. 2c). Two spectra from GM and WM dominant regions were analyzed using basis sets with and without Gly (Figs. 2d and 2e). In the spectral fitting with a basis set with Gly the *in vivo* spectra were closely reproduced by the fit. In the spectra from the GM dominant region (Fig. 2d), the signals of Gly (3.55 ppm) and mIns (3.62 ppm) were readily discernible, similarly as in the phantom data (Fig. 1c). The Gly level was estimated to be 0.8 mM with CRLB of 9%, and the mIns was 9.2 mM with CRLB of 4%, with reference to tCr

in white matter at 6.4 mM. When Gly was excluded from the basis set, residual signals were clearly present at ~3.55 ppm, indicating that the signal at 3.55 ppm was primarily attributed to Gly. The absence of Gly in the basis set influenced mIns estimation, resulting in higher estimate (11.0 mM). In contrast, spectra from WM dominant region (Fig. 2e) gave lower estimate of Gly (0.3 mM) and mIns (6.2 mM), with larger CRLBs compared to the data from the GM dominant region. The residual difference between the fittings with and without Gly was relatively small in spectra from WM region. The linewidth of the spectra from GM and WM dominant regions were 5.7 and 5.6 Hz, respectively. The LCModel returned SNRs of the spectra were very similar between the fittings with and without Gly (*i.e.*, 50 vs. 49 for GM and 45 vs. 45 for WM).

Concentration and CRLB maps were averaged over the eight healthy subjects, using axial T₁w-MPRAGE images as anatomical reference. The averaged concentration map of Gly showed regional variations of Gly levels in the brain (Fig. 3a). The Gly concentration was constantly high in the GM dominant regions along the anterior-posterior midline, whereas the left and right parietal WM-rich regions showed markedly low concentrations of Gly. The Gly concentration was estimated to be 1.0 ± 0.4 mM (mean $\pm$ SD, $n = 8$) for the GM dominant regions (as indicated by a black line Fig.3a, regions with GM/(GM+WM) > 80%) and 0.4 ± 0.2 mM for the WM rich regions (red line in Fig.3a, regions with WM/(GM+WM) > 95%). The mean CRLBs of Gly and mIns were $16 \pm 17\%$ and $9 \pm 3\%$ for GM regions, and $24 \pm 16\%$ and $6 \pm 1\%$ for WM regions, respectively. The Gly CRLBs showed significant difference between GM and WM ($p < 0.001$), while the mIns CRLB difference between the regions was not significant ($p > 0.05$). The mean correlation coefficient between Gly and mIns, returned by LCModel, was -0.31 ± 0.10 and -0.19 ± 0.09 in GM and WM dominant regions, respectively. The negative value of the correlation coefficient was as expected given the equal polarity of the Gly and mIns signals at the echo time used. Figures 3c and 3d show the linear regression of the Gly and mIns concentrations versus fractional GM content for an individual subject. The Gly and mIns concentrations both increased with fractional GM content. From the y-intercepts at unity and zero fractional GM contents, the concentrations in pure GM and WM were estimated to be 1.1 ± 0.2 and 0.3 ± 0.1 mM for Gly, and 10.2 ± 1.1 and 5.7 ± 1.2 mM for mIns, respectively. The difference of Gly level between pure GM and WM was statistically significant ($p < 0.001$). Similar regional variation was observed in mIns and tCr, with significantly higher concentrations in GM than in WM ($p < 0.001$ for both).

Due to the small spectral distance between the Gly singlet (3.55 ppm) and the largest peak of mIns at (3.62 ppm), detection of the small Gly signal in healthy brain may depend on linewidth. The effect of linewidth on Gly detection was investigated with spectral fitting on simulated spectra with various linewidths (Fig. 4). Simulation included Gly, mIns, NAA, Cr, Cho, Glu and Gln spectra. With the linewidth of 4 – 7 Hz in our *in vivo* data, spectra were generated for singlet linewidths of 3.5 – 7.5 Hz. Based on our estimated Gly-to-mIns ratios in GM and WM dominant regions, the simulations were performed with Gly-to-mIns concentration ratios of 1:10 and 1:20 for GM and WM, respectively. The spectral pattern of the Gly+mIns composite signal was well preserved across the linewidth and the Gly signal was constantly discernible. LCModel fitting of the calculated spectra with *in vivo* noise level

reproduced the Gly and mIns concentrations used for generating the spectra. The Gly-to-mIns estimated ratio was 0.099 ± 0.002 and 0.047 ± 0.001 for simulated Gly-to-mIns concentration ratios of 1:10 and 1:20, respectively. Gly and mIns estimates were within 5% of the true values. The Gly CRLBs were $6 \pm 1\%$ and $18 \pm 2\%$ for Gly-to-mIns ratio of 1:10 and 1:20, respectively.

Brain Tumor Studies

The PRESS SI method was used to acquire data from fourteen subjects with gliomas. Figure 5 shows data from a subject with glioblastoma (grade IV), in which T₂w-FLAIR imaging identified two distinct lesions in the left mid-to-posterior brain. Spectra from the two lesions (labeled A and C in Fig. 5) showed a large singlet like signal at 3.55 ppm with a much smaller signal at 3.62 ppm, compared to the contralateral normal brain (labeled B and D). Spectral analyses of the data showed that Gly was higher in the posterior lesion (labeled C; 4.2 mM) than in the other lesion (labeled A; 2.8 mM). The ratio of the elevated Gly to the contralateral normal Gly level was 9.3 and 7.0 for the posterior and mid brain lesions, respectively. In contrast, tCho was extensively elevated (~5 mM) in the mid brain tumor (labeled A) compared to the other lesion (labeled C; ~3 mM). The variations in the Gly and tCho levels between the lesions were clearly contrasted in the concentration maps.

For the 14 tumor patients, the Gly level was evaluated by averaging the estimate over ~4 voxels for each of the tumors and each of the contralateral normal appearing brain. Necrotic regions were not present in the tumors and no significant lipid or macromolecule signals were observed. The mean concentration of Gly in 14 patients was 1.8 ± 1.4 mM (CRLB of $11 \pm 7\%$) and 0.4 ± 0.2 mM (CRLB of $18 \pm 10\%$) for tumor and contralateral regions, respectively. Figure 6 presents the group comparison of the metabolite estimates. For grade II, III and IV gliomas, the mean Gly level in tumors was estimated to be 1.2 ± 0.6 (N = 5 subjects), 0.7 ± 0.1 (N = 4), and 3.3 ± 1.5 (N = 5) mM respectively, which were all significantly higher than the normal levels in the contralateral brain (0.5 ± 0.2 , 0.3 ± 0.2 , and 0.5 ± 0.3 mM respectively). Here the contralateral Gly level in glioblastoma patients was calculated from 3 patients, excluding 2 cases in which the tumor was located in brainstem. The mean Gly level was lower in grade III than in grade II tumors (0.7 vs. 1.2), but the ratio of the elevated Gly in tumors with respect to the contralateral brain was higher in grade III than in grade II (3.0 vs. 2.3). This was largely because the contralateral Gly estimate was very low in the grade III patients in whom the tumors were located mostly in WM dominant regions. Glioblastoma (grade IV) showed significantly higher Gly level compared to grade II and III tumors ($p < 0.05$ and 0.01 respectively). Excluding the 2 glioblastomas in brainstem, the mean tumor-to-contralateral Gly ratio in glioblastoma was calculated to be 10.5, significantly higher than those in grade II and III ($p < 0.01$ and $p < 0.02$ respectively). Taken together, the Gly elevation was significantly higher in grade IV than in grade II and III, indicating that elevated Gly may be a marker of tumor malignancy. For tCho, the mean concentration in tumors was higher than the contralateral value for all three grades, but the difference between the tumor and contralateral regions was significant only in grade III. The mean tCho level or the tumor-to-contralateral tCho ratio was not significantly different between the grades. The Gly-to-tCho ratio in tumors was significantly larger in grade IV

compared to grade II and grade III ($p < 0.05$ and $p < 0.01$, respectively) and a significant difference was not observed between grade II and grade III tumors.

DISCUSSION AND CONCLUSION

The present study reports *in vivo* ^1H MR spectroscopic imaging of Gly in healthy brain and brain tumors at 3T. The use of an optimized PRESS TE (160 ms) gave an effective suppression of the mIns signal in the proximity of the Gly resonance and consequently allowed Gly measurement with minimal contamination from the mIns background signals. The data indicated the presence of a regional variation of Gly concentrations in the healthy brain. The Gly concentration was estimated to be higher by > 2 fold in GM than in WM from a linear regression analysis, in good agreement with a recent single-voxel MRS study at 7T (11). The mean Gly CRLB was much smaller in GM voxels than in WM voxels (16% vs. 24%), which may be largely due to the regional difference of Gly. The Gly level was significantly increased in tumors. Compared to the contralateral normal appearing brain, Gly elevation in tumors was more extensive with increasing tumor grade, suggesting Gly as a potential biomarker of tumor malignancy. The observation of elevated Gly in all tumors in the current study is contrasted with the result from a prior study (10), which reported that Gly was elevated only in a subset of glioblastomas as measured using a single-voxel localized PRESS TE = 160 ms method. Given that in this prior study abnormal Gly levels in tumors were evaluated with respect to the normal level (~ 0.6 mM) in the GM dominant region (medial occipital brain), it is most likely that elevated Gly in some tumors in WM dominant regions (*e.g.*, Fig. 4c in reference 10) was interpreted as normal. Of note, for the location of the tumor in the left-hemisphere WM dominant region, the normal Gly level was estimated to be approximately 0.3 mM in the present study.

The capability to discriminate Gly singlet from mIns signals could be affected by shimming. The *in vivo* linewidth was 4 – 7 Hz in healthy brain and 4 – 6 Hz in tumors in our data set. LCModel analysis of simulated spectra indicated that Gly estimate was not substantially influenced by increase in linewidth up to 7.5 Hz (see Fig. 4). Also, Gly detection may depend on SNR. In the present study we observed a mean NAA SNR (calculated as ratio of peak amplitude of NAA and standard deviation of noise from signal-free region) of 74 ± 15 in the voxels with $\text{GM}/(\text{GM}+\text{WM}) > 70\%$ and 84 ± 12 in the voxels with $\text{WM}/(\text{GM}+\text{WM}) > 80\%$ in healthy brain data. The mean NAA SNR was 78 ± 12 for an estimated Gly concentration of 0.3 mM or more. Based on the analysis of 169 spectra from each of the 8 healthy subjects, Gly concentration of 0.3 mM or higher was measurable with $\text{CRLB} < 20\%$ when NAA SNR is > 70 . LCModel analysis on *in vivo* healthy brain SVS data at TE = 160 ms indicated reliable Gly detection at NAA SNR of 70 or greater (data not shown). The voxel size of the SI was set at 1.5 mL at acquisition and reconstructed at 0.375 mL, to achieve a sufficient SNR for Gly detection within an acceptable time frame. The spatial resolution of 1.5 mL is lower than those in some prior SI studies (~ 0.8 mL) (7,35–37). The spatial resolution of SI may be possibly enhanced without substantial loss of spectral information using echo-planar spectroscopic imaging (38), parallel imaging (36,37), and compressed sensing (39,40) techniques.

The T_2 of Gly was assumed to be identical to T_2 of mIns (200 ms), and this assumption may introduce small error in estimation of Gly, depending on the difference in Gly and mIns T_2 s. In phantom studies Gly T_2 was measured to be long compared to T_2 of mIns (1.5 s vs. 0.75 s). However, the phantom T_2 data may not be directly applicable to the *in vivo* situation since T_2 may differ between aqueous solutions and brain tissue. For instance, the *in vivo* T_2 values of the CH_3 group protons of Cr and NAA are quite different in human brain (*i.e.*, 150 vs. 300 ms) while their T_2 values in phantom solutions are very similar. Also, the mIns T_2 may be longer than the Cr CH_3 T_2 in brain (28,41), but it is the opposite in an aqueous solution. Given the small molecular size of Gly, the Gly molecules may be very mobile *in vitro* and consequently the Gly CH_2 proton T_2 is measured to be long (*i.e.*, much longer than the Cr CH_2 T_2 and similar to the Cr and NAA CH_3 T_2 s). For proton NMR, although the intra-molecular dipole-dipole interaction is a dominant mechanism for T_2 relaxation in most cases, there may be several other factors that affect the TE dependence of PRESS signals (namely, apparent T_2), which may include molecular diffusion, magnetization transfer (42), and the environments in which the molecules reside. In addition, the T_1 saturation effects between GM and WM were assumed to be equal, based on our *in vivo* experiments (data not shown), in which the Gly and mIns composite signal pattern and estimate ratios from GM and WM dominant regions were very similar between TR = 1.2 and 2.5 s. The potential difference of metabolite T_1 between healthy brain and tumors was ignored, as reported in a prior study (Li *et al.* JMRI 2008).

Spectroscopic imaging of Gly may provide a clinically useful tool compared to single-voxel MRS. The brain Gly level appears to be quite different between GM and WM, as shown in a prior study (11) and in the current study. The Gly elevation may be regionally different between many brain lesions, as shown in Fig. 5. Our data indicated ~2 fold difference in Gly levels between the lesions and more than 4 fold elevations when compared to contralateral normal brain regions. To date, noninvasive *in vivo* detection of elevated Gly in malignant tumors has been achieved largely using single-voxel MRS (8,10). Gly spectroscopic imaging may provide a unique opportunity to study multiple lesions and to investigate the disease infiltration into surrounding normal tissue. In a prior Gly imaging study at 3T by Hattingen *et al.*, data were acquired at both short (~30 ms) and long echo times (~140 ms) and the spectral difference between the two TEs was analyzed to obtain the Gly portion of the composite signal (7). As demonstrated in the current study and in a prior study (12), imaging of Gly and mIns can be achieved using a single refined optimized TE, which simplifies the spectral analysis without need of additional data analysis. A major drawback of our SI method is extensive T_2 signal loss due to the use of long TE (160 ms), but selected signals are often better resolved at optimized long TE, benefiting from attenuated macromolecule signals (11,43). With its improved ability for Gly detection, the proposed Gly SI method may have extended applications for which alterations of Gly levels are relatively small.

The ability to image Gly in the human brain may have important applications in the diagnosis and management of malignant brain tumors. Tumors reprogram their metabolism to meet the needs of rapid cell growth and survival in harsh environments (44). Thus, changes in metabolite abundance relative to normal tissue may serve as a biomarker of malignancy. Evidence suggests that production of Gly is under oncogenic control,

emphasizing its importance in tumor biology (45). A recent mass spectrometry study in a wide range of cancer cell lines (NCI-60) (13) indicates that Gly may play a critical role in rapid cell proliferation. Elevation of Gly in tumors relative to contralateral is distinctly pronounced in GBM compared to lower grade tumors (~10 fold vs. ~3 fold). Thus longitudinal monitoring of Gly in serial measurements in patients may provide a noninvasive tool for early detection of malignant transformation. A recent study indicated correlation of Gly elevation with increase in tCho, cerebral blood flow, and enhancement of contrast in MR imaging (12). Hence the capability to monitor the changes in Gly levels noninvasively by means of spectroscopic imaging would help to identify diffuse infiltration into normal brain and/or metabolically active regions in the periphery of the tumor mass.

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FIG. 1.

SI and SVS data were acquired with PRESS TE = 160 ms from a phantom containing Gly (1.1 mM), mIns (10 mM) and Cr (10.0 mM). (a) The FOV (blue line; $160 \times 160 \text{ mm}^2$) and VOI (brown line; $50 \times 50 \text{ mm}^2$) are shown in a gradient echo image. Fine grids indicate the individual voxels of the SI. (b) Spectra from the VOI are presented between 2.7 and 4.2 ppm. (c,d) SI and SVS spectra and LCModel fitting results. Vertical dashed lines are drawn at 3.55 and 3.62 ppm. Data were acquired using a TR of 3000 ms. The SI data were acquired

with a single average for each k-space point, while the SVS spectrum was acquired with 256 signal averages. The spectra are broadened to singlet linewidth of ~ 3 Hz.

FIG. 2.

Representative ^1H SI data from a healthy volunteer. (a, b) The FOV (blue line) and VOI (brown line) of the SI are shown in T_1 w-MPRAGE images. The VOI was prescribed by PRESS localization ($TE = 160$ ms). Fine grids indicate the individual voxels of the SI. The mean tCr estimate over the voxels within a red line was set to 6.4 mM after CSF correction and used as reference for metabolite quantification. (c) Spectra from voxels within the VOI are shown between 4.1 to 1.0 ppm. (d, e) Spectra from a voxel in the gray matter (blue box) and from a voxel in the white matter (green box) dominant regions are shown together with

LCModel fits, baseline, and residuals that were obtained using basis sets with and without Gly. The Gly and mIns signals, returned by LCModel, are shown with the concentration estimates and CRLBs. Vertical lines are drawn at 3.55 ppm.

FIG. 3.

(a) Concentration and CRLB maps of Gly, mIns, and tCr for the VOI (green box in a T_1w -MPRAGE image), averaged over eight healthy volunteers. The maps were smoothed by eight fold using a bi-cubic polynomial function, after the corrections for the CSF contamination and chemical-shift displacement effects. Averaged Gly-mIns correlation coefficients (returned by LCModel) are mapped on linear scale between -0.5 and 0.5 . (b) Gray matter and white matter segmented brain images are shown with the SI VOI. (c, d) Linear regression of Gly and mIns tissue concentrations with respect to fractional GM

contents from a single subject. Dashed lines indicate 95% confidence intervals of the linear fit.

FIG. 4.

Numerically-calculated spectra and LCModel fitting results are shown for Gly-to-mIns = 1:10 (a) and 1:20 (b). Spectra were broadened to singlet linewidth of 3.5 – 7.5 Hz and random noise was added to the spectra such that the ratio of the NAA singlet amplitude to the noise standard deviation was 50 for all the linewidths. The metabolites include Gly (1 and 0.3 in (a) and (b) respectively), mIns (10 and 6 in (a) and (b) respectively), NAA (10), Cr (8), Cho (2), Glu (9), and Gln (2), where the numbers in brackets are the concentrations.

FIG. 5.

SI data from a subject with multi-focal glioblastoma. Spectra from tumors (labeled A and C) and the corresponding contralateral locations (labeled B and D) are shown together with spectral analysis results. The concentrations of Gly and mIns were estimated with reference to the mean tCr estimate within a black box in the T₂w-FLAIR image at 6.4 mM.

FIG. 6.

The mean Gly and tCho concentrations in the tumor and contralateral brain regions in 5 grade II, 4 grade III, and 5 grade IV gliomas are bar graphed with standard deviations. The contralateral estimates in grade IV were calculated from 3 patients, excluding 2 patients in whom the tumor was located in brainstem. Statistical significance is shown for $p < 0.05$, with an asterisk (*) for paired t-test and a dagger (†) for unpaired t-test.