Improvement of 2-hydroxyglutarate detectability by optimized triple-refocusing at 3T *in vivo*

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PURPOSE: Mutations in isocitrate dehydrogenase (IDH) 1 and 2 result in production of 2-hydroxyglutarate (2HG) [1-3]. IDH mutations are associated with longer patient survival when compared to IDH wild type tumors [4]. Thus precise measurement of 2HG by MRS may provide an effective tool for diagnosis and prognosis. The 2.25 ppm resonance of 2HG gives a large signal in most cases. The proximity of this 2HG resonance to the GABA 2.28 ppm makes it difficult to separate the signals between the metabolites. The purpose of the study was to develop a new MRS for reducing potential contamination of GABA in 2HG estimation.

METHODS: ^{1}H MRS data were obtained from 10 glioma patients, using single voxel localized PRESS (TE_1, TE_2) = (32, 65) ms and 2HG optimized triple-refocusing sequence (TE_1, TE_2, TE_3) = (30, 86, 21) ms with 26 ms long non-slice selective refocusing pulse which was tuned to 2.5 ppm (Fig. 1). Data were acquired with 8-channel head coil in a 3T whole-body scanner (Philips Medical Systems). Voxel size was 4 – 8 mL depending on tumor volume identified with T_{2w}-FLAIR. Data acquisition parameters included TR = 2 s, sweep width = 2.5 kHz, number of sampling points = 2048, and number of averages = 128 – 512. Water unsuppressed data was acquired for eddy current compensation and multi-channel combination. Spectral fitting was performed with LCModel software [5], using basis spectra calculated incorporating the volume localizing RF and gradient pulses of PRESS and triple-refocusing sequence with published chemical shift and J-coupling constants [6].Metabolite quantification was using water as a reference at 42 M.

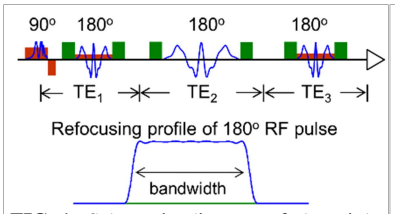

FIG 1. Schematic diagram of the triple-refocusing sequence, used for 2HG detection, is shown together with the refocusing profile of 180° RF pulse. The second 180° pulse was non-slice selective (durations 26 ms and bandwidth 650Hz).

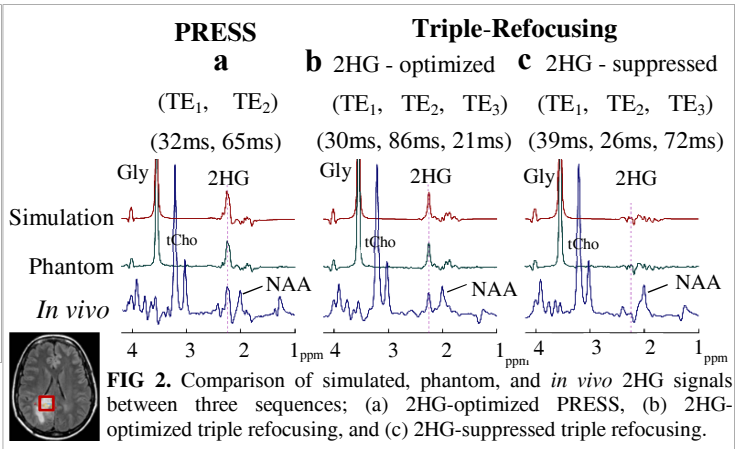

FIG 2. Comparison of simulated, phantom, and *in vivo* 2HG signals between three sequences; (a) 2HG-optimized PRESS, (b) 2HG-optimized triple refocusing, and (c) 2HG-suppressed triple refocusing. The total echo time is identical (137 ms) for the triple refocusing sequences. Simulated and phantom spectra were broadened to *in vivo* linewidth. Vertical dotted lines are drawn at the 2HG C4-proton resonance (2.25ppm). The tumor was IDH-mutated oligoastrocytoma, as confirmed from biopsy. 2HG of *in vivo* data was estimated as 17mM in both sequences.

RESULTS: Numerical simulations indicated that the 2HG signal is temporally maximum at triple-refocusing (TE_1, TE_2, TE_3) = (30, 86, 21) ms, and temporally minimum at (TE_1, TE_2, TE_3) = (39, 26, 72) ms. Calculated 2HG signals for these triple-refocusing schemes were in excellent agreement with phantom data (Figure 2). The 2HG signal intensity from the 2HG-optimized triple refocusing was slightly smaller than that from the previously reported PRESS TE=97ms method [7], but for identical singlet linewidth, the triple refocused 2HG signal was narrower than the PRESS 2HG signal, suggesting improved resolution of 2HG *in vivo* (Fig. 2a,b). The manipulated signal pattern and strength of 2HG by triple refocusing were reproduced *in vivo*. While a signal at 2.25 ppm was clearly discernible in the 2HG-optimized triple refocusing, the 2HG resonance was essentially null in the 2HG-suppressed triple refocusing data, confirming the detected signal at 2.25 ppm is truly attributable to 2HG (Fig. 2c). 2HG was detectable by the two methods, but the 2HG estimate was larger in triple refocusing than in PRESS; 1.1 mM vs. 0.6 mM in patient 1 (Fig. 3a,b) and 2.2 mM vs. 1.4 mM in patient 2 (Fig. 3c,d). In contrast, the estimate of GABA, which has a resonance at 2.28 ppm, was opposite; namely smaller in triple refocusing than in PRESS. The 2HG CRLBs from triple-refocusing (9% and 6%) were overall smaller than those from PRESS (13% and 6%). Ten glioma patients were scanned with both triple-refocusing and PRESS, of which 9 patients showed relatively low 2HG levels (< 3mM). For these 9 patients, the 2HG estimates were higher with lower CRLBs and lower GABA estimates in triple refocusing than in PRESS. When 2HG levels were high, the estimations between PRESS TE = 97 ms and triple-refocusing were about the same.

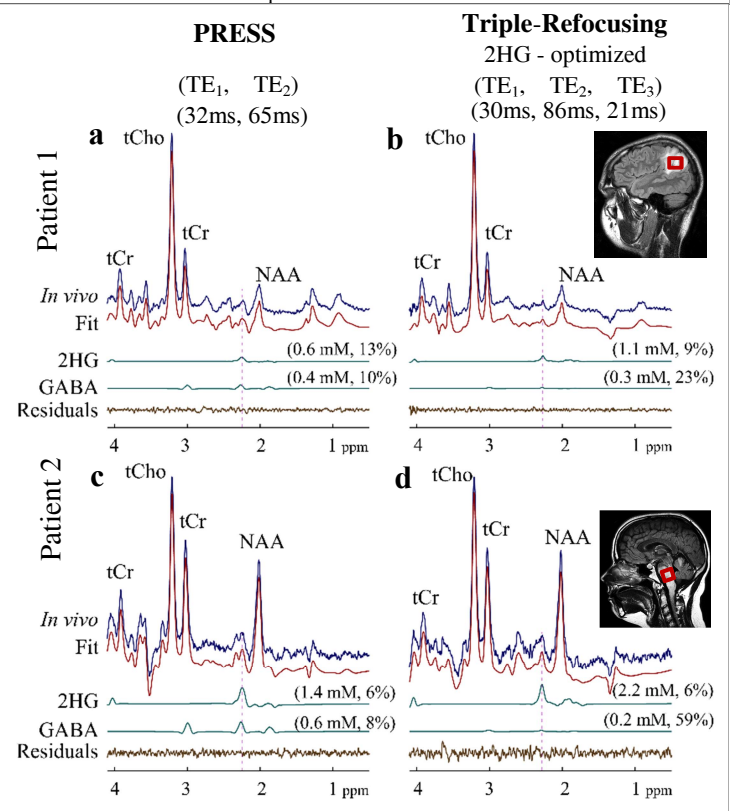

FIG 3. Comparison of PRESS TE=97ms and 2HG-optimized triple refocusing for 2HG detection in two patients *in vivo*. LCModel-returned signals of 2HG and GABA are shown together with the concentration estimate and CRLB. Vertical dotted lines are drawn at 2.25ppm. The tumor type and IDH mutation status are unknown (pre-biopsy scans).

DISCUSSION AND CONCLUSION: We report a new triple refocusing method for detection of 2HG in brain tumors, which was designed for maximum 2HG signal at 2.25 ppm and reduced GABA signal at 2.28 ppm in order to minimize potential GABA contamination to 2HG measurement. The estimation of low GABA with high 2HG by triple refocusing is likely the case in tumors since in tumors, GABAergic neurons may be depleted and consequently GABA may be decreased similarly to the neuronal marker NAA. A drawback of the triple refocusing is that due to the relatively long TE, the T_2 relaxation effect may decrease SNR. Further study is required to investigate the effects of improved resolution and T_2 relaxation on 2HG detectability.

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