Measurement of Reduced Glutathione (GSH) in Human Brain using MEGA Editing.

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Glutathione [GSH] was measured at 4 T using ^{1}H MRS with MEGA-PRESS editing in the occipital lobe of normal humans. The spectral pattern of the edited GSH resonance *in vivo* was consistently identical to an accordlingly line broadened phantom GSH spectrum. The difference-editing technique allowed for B_0 and phase correction, which resulted in improved spectral quality. Suppression of water using VAPOR as well as signals outside the volume of interest resulted in a flat and stable baseline for fitting the edited GSH multiplet. The concentration of GSH was 1.5±0.1 μmol/g (mean ± SD, n=6).

INTRODUCTION

GSH is an antioxidant which may be indicative of oxidative stress, which has been implicated in many neurodegenerative diseases as well as in schizophrenia (1).

Its low concentration (on the order of 2 mM), proximity/overlap with creatine and other signals, and the multiplet ^{1}H resonances all contribute to difficulty of GSH detection *in vivo*. Recently, detection of GSH was reported using double quantum coherence filtering (2). Difference editing methods (3,4) permit the determination of the overall phase/frequency shift of the spectrum from the singlet signals in the subspectra on a scan-per-scan basis.

Our goal was to detect and quantify the glutathione multiplet at 2.95 ppm at a higher B_0, where it is more separated from the nearby Cr signal.

METHODS

Six normal subjects were positioned supine in a 4 T magnet (Siemens) with a ^{1}H quadrature transceiver subjacent to the occipital lobe. After MRI and shimming a 3x3x3 cm^3 volume of interest, GSH signal was measured using a pulse sequence consisting of MEGA-PRESS (3) combined with VAPOR water suppression as described recently (4). In 4 subjects, editing was achieved using MEGA by switching a 20 ms Gaussian pulse applied at 4.56 ppm on/off on alternate scans. In 2 experiments the editing pulse was placed symmetrically about water resulting in a reduced editing efficiency. Editing efficiency was determined by comparing the glutathione signal relative to glycine in accordingly line broadened phantom spectra. GSH concentration was determined from the corrected area relative to total Cr (set to 8 μmol/g wet wt) in the subspectrum. The edited GSH peak at 2.95 ppm was fit as a multiplet with a fixed line width equal to that determined for Cr.

RESULTS AND DISCUSSION

In all 6 subjects a resonance consistent with that of GSH (arrow) was detected (Fig. 1A). The finite bandwidth of the editing pulse resulted in additional coherences at 2.5-2.75 ppm, which were assigned mainly to the non-equivalent aspartyl methylene multiplets of NAA. The coupling of the 2.5 ppm resonances to the αH at 4.4 ppm and the ensuing partial editing was verified by comparison with a phantom containing equimolar GSH and NAA (Fig. 1B). B_0 and phase correction performed on a scan-by-scan basis improved spectral quality (Fig. 1B) and reduced subtraction errors to an average of 0.9% at NAA (2.0ppm). Excellent water suppression, as well as outer-volume suppression contributed to the quality of the spectra, allowing unequivocal identification of the edited GSH multiplet in all cases. Fitting of the GSH resonance and taking into account editing efficiencies resulted in a concentration of GSH of 1.5 μmol/g with a standard deviation of 0.1 μmol/g (CV = 7 %). The estimated concentration is in good agreement with previous reports using multiple quantum filtering (1,2,6) as well as with LCModel quantification at 7 tesla (7).

We conclude that difference-editing of the GSH spin system and subsequent quantification is possible in the human brain, resulting in a highly reproducible quantification of this important antioxidant.

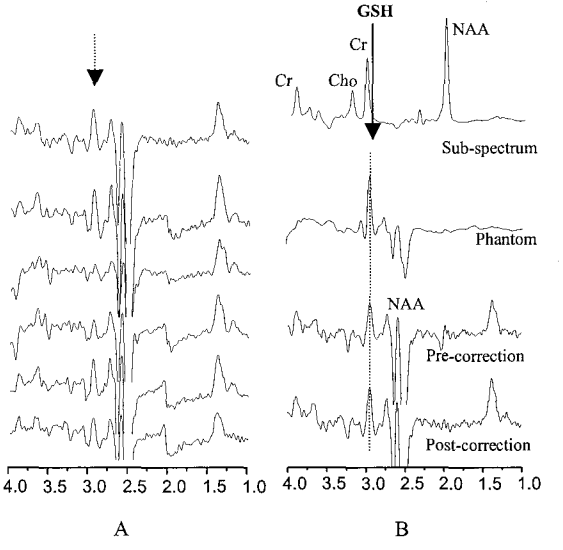

Fig. 1 ^{1}H NMR detection of GSH in the human brain.

(A) 6 edited *in vivo* spectra (TR=4.5 s, TE = 68 ms, NEX = 256 or 512). (B) *In vivo* ^{1}H NMR sub-spectrum (top), edited phantom (middle), and phase corrected and uncorrected (bottom) *in vivo* spectra (TR=4.5 s, TE = 68 ms, NEX = 512). The arrows indicate the chemical shift (2.95 ppm) of the edited GSH multiplet.

References & Acknowledgments

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