7 T ^{1}H MRS detects a human brain glutathione (GSH) response to N-acetylcysteine, an antioxidant precursor, in patients with Gaucher and Parkinson's disease

Melissa Terpstra[1], Mary Holmay[1], Lisa Coles[1], Usha Mishra[1], Matthew Ahlskog[1], James Cloyd[1], Paul Tuite[1], and Gulin Oz[1]

[1]University of Minnesota, Minneapolis, MN, United States

Introduction: One important utility of MRS is to noninvasively monitor how the human brain neurochemical profile responds to experimental treatments, and thus to guide development of optimal interventional protocols. N-acetylcysteine (NAC) is such a compound that is under consideration for the treatment of several psychiatric and neurodegenerative conditions. Among these are Alzheimer's[1], Gaucher (GD), and Parkinson's disease[2] (PD). Although the therapeutic mechanism is poorly understood, NAC can serve as a precursor for the formation of the antioxidant glutathione (GSH). As such, MRS of sufficient quality to detect GSH can be used to both understand NAC's mechanism of action and monitor efficacy of delivery to the pertinent brain region. Using ultra-high field MRS we tested the hypothesis that intravenous NAC would increase brain GSH levels.

Methods: In this study, 8 human subjects (4 males, average age 50 years) were scanned at 7 T before and during IV administration of NAC. Three of the subjects had GD, 3 had PD, and 2 were healthy controls. Subjects were not allowed to use antioxidants for 3 weeks prior to the study. The protocol was to measure brain GSH at baseline, remove the subject from the scanner to start the NAC infusion, and then return the subject into the scanner to follow the GSH time course from 50 to 120 minutes after the start of infusion (t=0). 150 mg/kg of NAC was administered intravenously over 1 hour. The blood GSH/GSSG redox ratio was measured using mass spectrometry throughout the protocol. MR spectra were measured at 7T (90-cm bore Siemens MAGNETOM) with an elliptical quadrature half-volume transceiver[3] using a modified semi-LASER sequence[4] (TE = 26 ms, TR = 5 s, NEX = 64). 22 x 22 x 22 mm³ occipital cortex volumes of interest (VOI) were selected using MPRAGE images. First- and second-order shims were adjusted using FASTMAP[5]. Unsuppressed water spectra acquired from the same VOI were used to remove residual eddy current effects and as an internal quantification reference. Single-shot data were saved during acquisition; individual FIDs were frequency and phase corrected prior to summation. Scout images were utilized to update the voxel position if the participant's head translated more than 3 mm. Metabolites were quantified using LCModel[6] and a typical basis set of neurochemicals[4]. The ratio of post NAC brain [GSH] to baseline was computed for analysis and plotting. Average brain GSH levels measured over 90 - 120 minutes were compared to baseline [GSH] using a paired, two-tailed t-test.

Results: The high quality illustrated in Fig. 1 was achieved for all spectra such that GSH was always fit reliably (CRLB ≤ 16%, 9 ± 2 mean ± SD, r > -0.5)[6]. Brain GSH increased by at least 10% ($33 \pm 17\%$ mean ± SD) in response to NAC in every participant, resulting in a strong group effect ($p < 0.001$) (Fig. 2). The effect remained robust ($p < 0.002$) when GSH was normalized to brain N-acetylaspartate or creatine. The average baseline GSH concentration was 0.8 µmol/g. The peak in brain GSH concentration (average 1.1 µmol/g) occurred on average 43 ± 7 (SD) minutes after the blood redox ratio (GSH/GSSG) peaked. The change detected in brain [GSH] could not be due to the contribution of blood [GSH] to the MRS voxel (~3% of VOI) because the 137% maximal increase in blood [GSH] would only lead to a MRS voxel [GSH] increase of 4%. No difference in brain GSH increase among subject groups was detected ($p > 0.4$).
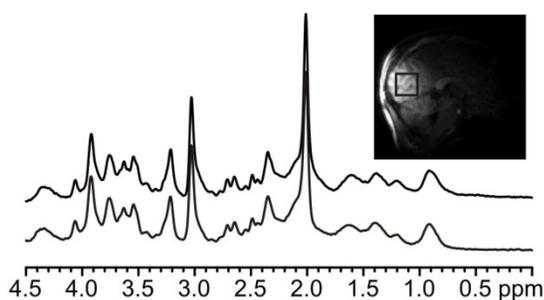

Fig 1. Spectra from a participant with GD at baseline (top) and 107 minutes (bottom) after starting NAC. Overlay: Image with illustration of volume of interest.
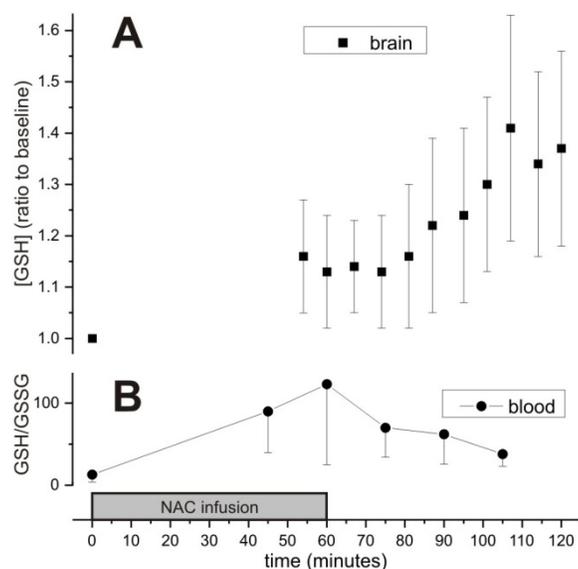

Fig 2. (A) Brain GSH concentration (ratio to baseline) throughout NAC infusion (mean ± SD, n=8). **(B)** Blood GSH/ GSSH ratio averaged over all participants (mean - SD, n=8). Inset bar: illustration of NAC infusion protocol.

Conclusions: This is the first demonstration in the human brain that the levels of the most potent intracellular antioxidant GSH can be modulated by administering an exogenous precursor. The data show that high field ^{1}H MRS is sensitive to monitor efficacy of NAC delivery and to investigate its mechanism of action. As expected, there was a delay in the changes in brain GSH as compared to the change in the blood redox status. The consistent increase in the concentration of this important antioxidant in the human brain suggests that NAC may be utilized to alleviate oxidative stress. This protocol is poised for determination of optimal delivery and dosage regimens, information that is needed in order to design controlled trials evaluating NACs safety and efficacy in these conditions and thus to study the important intermediate phase between dosing and amelioration of symptoms.

References & acknowledgments: [1] Pocernich & Butterfield *Biochim Biophys Acta* 2012 **1822**:625 [2]Martínez-Banaclocha *Med Hypotheses* 2012 **79**:8 [3]Adriany & Gruetter *J Magn Reson* 1997 **125**:178 [4]Oz & Tkac *Magn Reson Med* 2011 **65**:901 [5]Gruetter & Tkac *Magn Reson Med* 2000 **43**:319 [6]Provencher *NMR Biomed* 2001 **14**:260 &
NIH U54NS065768 , R01 AG039396, P41 RR008079, P41 EB015894, S10 RR026783, P30 NS076408, WM KECK Foundation.