Increased GABA Levels in Manganese Neurotoxicity: Biochemical Effect or Mn-induced Change of GABA T1 Relaxation Time?

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Purpose: Gamma-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the central nervous system, is of high interest in understanding manganese(Mn)-induced neurotoxicity due to GABA's important function in basal ganglia circuits[1]. Previous magnetic resonance spectroscopy (MRS) studies found elevated GABA levels in the thalamic region in Mn-exposed subjects[2,3]. However, parametric properties of divalent Mn cations (Mn^{2+}) could have an influence on the relaxation of the metabolites, confounding GABA quantification results. Because the basal ganglia region is a primary target for Mn deposition, this paramagnetic influence from Mn is of special importance to GABA measurements in this area, as significantly decreased water T1 relaxation times have been found in MRI studies of Mn exposed subjects[4]. Therefore our phantom study investigates the effects of Mn concentrations on the relaxation time of GABA using MEGA-PRESS[5].

Methods: Four 180 mL phantoms containing 8mM GABA, 10mM Creatine (Cr) and 12mM NAA were made, using a fixed amount of $NiCl_2$ to adjust the T1 relaxation time to in vivo levels. Additional concentrations of $MnCl_2$ in the four phantoms were chosen to be 0, 0.22, 0.44 and 0.88 µg/g respectively, with 0.22µg/g being approximately the average Mn concentration in a healthy human brain[6]. MRS acquisitions were performed on a 3T GE Signa MRI scanner equipped with an 8 channel head coil. GABA-edited MRS using MEGA-PRESS (TE=68 ms) was applied to measure T1 of GABA, NAA and Cr at five different repetition times (TR=1.2s, 2s, 3s, 5s, 10s) with 32 averages per TR. The voxel size of the measurement was 25×25×25 mm^3. T1 of water in each phantom was measured using spectra from water-reference scans. NAA and Cr were quantified from the spectra without the 1.9 ppm editing pulse. Spectra quantification was conducted using integration in SAGE (Spectroscopic Analysis, GE). Fig. 1 shows the example of GABA signals acquired in 5 TRs for one phantom. T1s were fitted using the model

$$S(TR,C_{Mn}) = S0(C_{Mn})\cdot(1-\exp(-TR/T1(C_{Mn})))+e_1 \quad [7],$$

where $T1=T1_0+\beta C_{Mn}+e_2$, $S0(C_{Mn})= S0_0+\alpha C_{Mn}+e_3$, $T1_0$ is the T1 of the metabolites without presence of Mn, $S0_0$ is the signal intensity without effect of Mn, C_{Mn} is the concentration of Mn, $S(TR,C_{Mn})$ is the signal intensity for each level of Mn concentration and TR, and e_1, e_2 and e_3 are error terms. Statistical analysis was performed using a hierarchical Bayesian approach with WinBUGS 1.4.

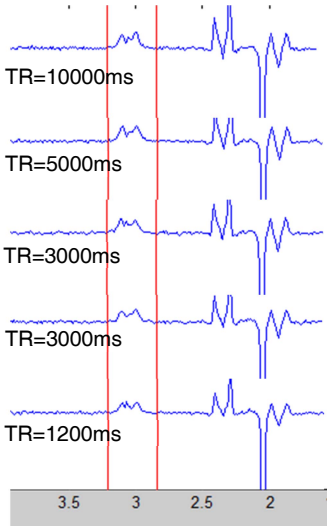

Fig. 1. Typical GABA-edited spectra from 5 TRs.

Results: The fitted T1 relaxation times with fitting errors for 4 phantoms are plotted in Fig. 2. Fitting errors for all metabolites were smaller than 12%. The estimated value and standard error of the slope β and $T1_0$ are listed in table 1, shown with a 95% Bayesian credible interval for β, CL, which is used to describe the effect of Mn on T1 from Mn. Based on the Bayesian credible limits both being negative, it was concluded that increasing Mn concentration predicts a decrease in the T1 relaxation time of water, which is consistent with other MRI studies. T1 of NAA also decreased with increasing Mn concentration. However the T1 relaxation times of GABA and Cr were not found to change with the increasing Mn concentration.

Table 1. Estimated parameters for T1 fitting

	GABA	Cr	NAA	Water
slope β (s/(µg/g))	-0.029	-0.140	-0.301	-0.202
SD (β)	0.178	0.286	0.065	0.008
2.5% CL	-0.385	-0.620	**-0.430**	**-0.269**
97.5%CL	0.328	0.610	**-0.170**	**-0.135**
$T1_0$ (s)	0.977	1.658	0.934	-1.603
SD ($T1_0$)	0.086	0.111	0.032	0.016

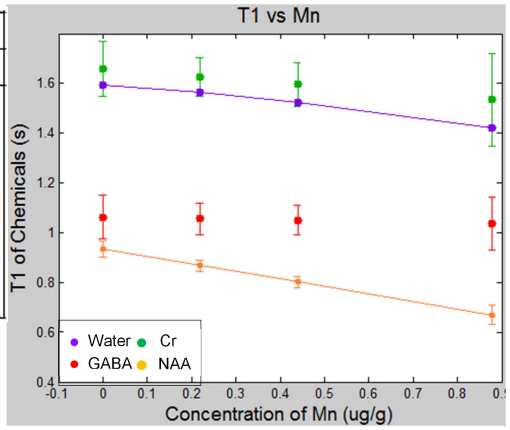

Fig. 2. Fitted T1(s) of water, Cr, GABA, and NAA vs. Mn concentration in each phantom

Conclusion: T1 relaxation times of GABA and Cr were not significantly affected by Mn concentration, indicating that changes in GABA (and GABA/Cr) levels measured by MEGA-PRESS in Mn-exposed brains are true biochemical effects of the Mn deposition in the brain representing GABA concentration changes rather than T1 changes confounding the MRI signal of GABA. However, GABA/NAA should not be used as indicator for GABA levels due to the decrease in NAA T1 relaxation time with brain Mn accumulation.

Reference: [1] Graybiel AM, Curr OpinNeurobiol, 2005; 15: 638-644; [2] Dydak U et al. Env. Health Persp. 2011;119:219-24;[3] Long Z et al. PLoS One. 2014; 4:9(2):e88220; [4] Krishna S et al. Arc of Toxicol, 2011; 88: 47-64; [5] Mescher M et al. NMR Biomed. 1998; 11: 266-272; [6] Markesbery WR et al. Neurotox; 1984; 5(1):49-57 [7] Puts et al. J Magn Reson Imaging 2013; 37:999-1003 [8]. Madsen et al. Proc. Intl. Soc. Mag. Reson. Med. 14 (2006). This study was funded by NIH/NIEHS R01ES020529.