Dose-Dependent Influence of Short-Term Binge Ethanol Intoxication on Cerebral Neurochemical Changes in Rats Detected by Ex Vivo 1H NMR Spectroscopy

Do-Wan Lee[1], Jung-Whan Min[2], Jung-Hoon Lee[1,3], Sang-Young Kim[1], Jin-Young Jung[1], Kyu-Ho Song[1], and Bo-Young Choe[1]

[1]*Department of Biomedical Engineering, and Research Institute of Biomedical Engineering, The Catholic University of Korea, College of Medicine, Seoul, Seoul, Korea,* [2]*Department of Radiological Science, The Shingu University College of Korea, Seongnam, Seongnam, Korea,* [3]*Department of Radiology, Kyunghee Medical Center, Seoul, Korea*

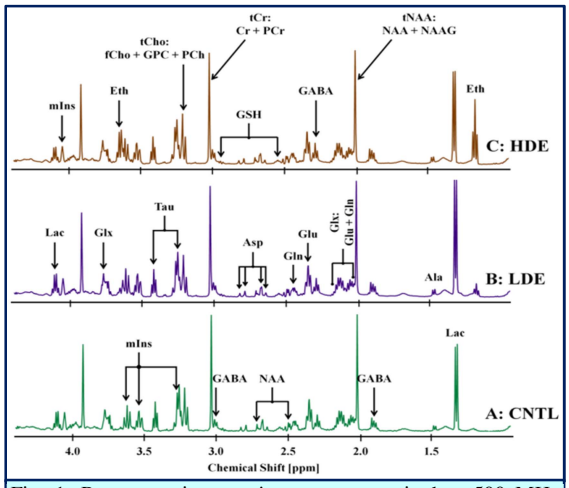

Fig. 1. Representative *ex vivo* spectra acquired at 500 MHz from the CNTL (A, green), the LDE- (B, purple), and the HDE-exposed rats (C, brown) in the frontal cortex. The chemical shift range was from 4.20 to 1.00 ppm.

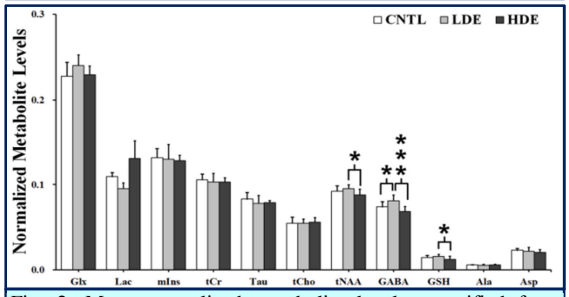

Fig. 2. Mean normalized metabolite levels quantified from CNTL, LDE, and HDE exposed rats in frontal cortex. The vertical lines on each of the bars indicate the (+) standard deviation of the mean values. Significance levels (one-way ANOVA): *, $p < 0.05$; ***, $p < 0.005$.

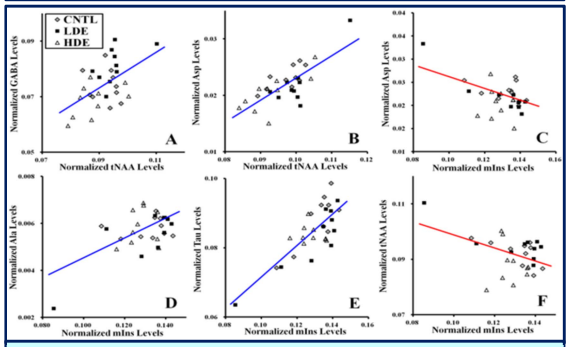

Fig. 3. Scatter plots of the metabolite-metabolite level correlations quantified from the individual rats that were distinguished by symbols among the CNTL (rhombus, gray), the LDE- (square, black), and the HDE- (triangle, white) exposed rats.

Target audience: Neurologist, medical doctors, and clinicians interested in MRS of the brain.

Purpose: To the best of our knowledge, investigations of the short-term binge ethanol intoxication effects on cerebral metabolite changes are scarce, and the literature is lacking. The influence of the short-term dose effects of binge ethanol intoxication on cerebral metabolite changes has not been experimentally investigated with ^{1}H *in vivo* MRS and *ex vivo* NMRS. Thus, the first goal of this study was to determine the influence of the dose-dependent effects of binge ethanol intoxication on cerebral metabolite changes among controls and low- and high-dose-ethanol-exposed rats with *ex vivo* high-resolution spectra. The second goal of this study was to determine the correlations between the metabolite-metabolite levels from all of the individual data from the frontal cortex of the binge ethanol-intoxicated rats.

Methods: 8-week-old male Wistar rats (n = 30) were divided into 3 groups (control [CNTL, distilled water]: n = 10; low-dose ethanol [LDE, 1.5 g/kg] group: n = 10; and high-dose ethanol [HDE, 2.5 g/kg] group: n = 10). The 20 rats in the LDE and HDE groups received an initial dose of 5.0 g/kg through oral gavage, and then received additional doses of 1.5 g/kg and 2.5 g/kg, respectively, every 8 h for 4 days. Thirty frontal cortical tissues were carefully harvested with the brain slicer matrix. *Ex vivo* ^{1}H high-resolution magic angle spinning (HR-MAS) NMR spectroscopy was performed on Agilent Technologies Korea VNMRS-500 (500.13 MHz). All HR-MAS spectra were acquired with a Carr-Purcell-Meiboom-Gill pulse sequence [complex data number, 16,384; spectral width, 8 kHz; acquisition time, 2.05 s; relaxation delay time, 5.0 s; presaturation time, 2.0 s; interpulse delay, 0.4 ms; number of acquisitions, 128; and total scan time, 15 min, 24 s]. The acquired raw data were analyzed and quantified with MestReNova. The 1-D FID data were zero-filled to 65,536 complex points, apodized with a 2.0 Hz Gaussian filter, and then Fourier transformed. The resulting spectra were manually phased, frequency referenced to TSP at 0.00 ppm, and baseline corrected. The postprocessed spectra were fitted with a global spectral deconvolution algorithm for an improved multiplet analysis. The *ex vivo* data were processed by the total signal intensity normalization method as described previously. [1] The metabolites were quantified with fitted spectra, and each deconvolution peak was as follows: Alanine (Ala), aspartate (Asp), free-choline (fCho), creatine (Cr), phosphocreatine (PCr), gamma-aminobutyric acid (GABA), glutamine (Gln), glutamate (Glu), glycerophosphocholine (GPC), glutathione (GSH), myo-inositol (mIns), lactate (Lac), N-acetylaspartate (NAA), N-acetyl-aspartyl-glutamate (NAAG), phosphocholine (PCh), ethanol (Eth), taurine (Tau), glutamine complex (Glx: Glu + Gln), total NAA (tNAA: NAA + NAAG), and total Cr (tCr: Cr + PCr).

Results: Fig. 1 (A to C) shows representative *ex vivo* 500-MHz spectra from the frontal cortex region of animals in the CNTL and the low- and the high-dose binge ethanol group. Fig. 2 illustrates the normalized cerebral metabolite levels that were quantified from the 30 acquired *ex vivo* spectra of the frontal cortex. Four days of binge ethanol intoxication resulted in altered metabolite levels for tNAA [$F(2,27) = 3.67$, $p = 0.039$], GABA [$F(2,27) = 10.43$, $p < 0.001$], and GSH [$F(2,27) = 3.49$, $p = 0.045$] among the 3 groups (CNTL vs. LDE vs. HDE). The GSH levels (*, $p < 0.05$) were significantly lower in the HDE-exposed rats than in the LDE-exposed rats. The GABA levels ($p < 0.05$) were significantly higher in the LDE-exposed rats than in the CNTL rats. However, the GABA levels (***, $p < 0.001$) in the HDE-exposed rats were significantly lower than that in the LDE-exposed rats. The tNAA levels (*, $p < 0.05$) were significantly lower in the HDE-exposed rats than in the LDE-exposed rats. To visualize the normalized metabolite levels quantified from the individual rat data and to assess the relationship among them, the pairs of normalized metabolite levels that changed the most were selected for linear scatter plots (Fig. 3 A–F). The clusters of individual data from 30 rats were significantly correlated (negatively or positively) in 6 scatter plots. Fig. 3 shows characteristic patterns of the neurochemical level changes among the 3 groups.

Discussion and Conclusion: In summary, the present study conducted *ex vivo* NMR spectroscopy in a rat model to quantitatively assess the dose-dependent influences of binge ethanol intoxication on cerebral neurochemical changes in the rat frontal cortex. From our results and those of previous studies, significantly lower tNAA levels might reflect that HDE binge ethanol intoxication results in neuronal degeneration and dysfunction in the frontal cortex of HDE-exposed rats than in that of CNTL and LDE-exposed rats. [2] Moreover, the significantly altered GABA levels between the HDE- and the LDE-exposed rats may reflect alterations in GABA synthesis and GABAA receptor densities. [3] Significantly lower GSH levels possibly indicate that HDE intoxication (ethanol doses over 2.5 g/kg) may lead to oxidative stress, possibly due to lipid peroxidation stimulation through the formation of free radicals and/or abnormalities of the antioxidant defense system in the frontal cortex of the high-dose-exposed rats. [4] Thus, our *ex vivo* ^{1}H HR-MAS NMRS results, which exhibited significant alterations for normalized tNAA, GABA, and GSH levels among the CNTL, LDE-, and HDE-exposed rats, might suggest that these markers can be utilized as key markers of the dose-dependent influence of short-term binge ethanol intoxication in the frontal cortex.

ACKNOWLEDGEMENTS: This study was supported by a grant (2010-0008096) from the Basic Science Research Programs through the National Research Foundation (NRF) and the program of Basic Atomic Energy Research Institute (BAERI) (2009-0078390) and a grant (2012-007883) from the Mid-career Researcher Program funded by the Ministry of Education, Science & Technology (MEST) of Korea.

References: **[1]** Lentz MR, Lee V, Westmoreland SV, et al. Factor analysis reveals differences in brain metabolism in macaques with SIV/AIDS and those with SIV-induced encephalitis. NMR Biomed. 2008;21(8):878-887. **[2]** Crews FT, Nixon K. Mechanisms of Neurodegeneration and Regeneration in Alcoholism. Alcohol Alcohol. 2009;44(2):115-127. **[3]** Ward RJ, Lallemand F, De Witte P. Biochemical and Neurotransmitter Changes Implicated in Alcohol-Induced Brain Damage in Chronic or "Binge Drinking" Alcohol Abuse. Alcohol Alcohol. 2009;44(2):128-135. **[4]** Uysal M, Kutalp G, Ozdemirler G, et al. Ethanol-induced changes in lipid peroxidation and glutathione content in rat brain. Drug Alcohol Depend. 1989;23:227-230.