Quantitative Assessment of Neurochemical Profiles in Rat Hippocampus after Short-Term Binge Ethanol Intoxication, Determined Using Ex vivo 1H High-Resolution NMR Spectroscopy

Do-Wan Lee[1], Jung-Hoon Lee[1,2], Jung-Whan Min[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 Radiology, Kyunghee Medical Center, Seoul, Korea,* [3]*Department of Radiological Science, The Shingu University College of Korea, Seongnam, Seongnam, Korea*

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

Purpose: A number of studies have suggested that acute binge alcohol abuse can lead to a variety of brain disorders, such as loss of brain volume, neurological dysfunction, functional abnormalities, and neurochemical alterations. [1] Previous studies have reported that the hippocampal region is especially vulnerable to the adverse effects of acute binge alcohol abuse. [2] Therefore, the purpose of present study was to provide *ex vivo* evidence of changes in the neurochemical profiles of rat hippocampus after 4-day binge ethanol intoxication, using high-resolution ^{1}H-NMR Spectroscopy.

Methods: 8-week-old male Wistar rats (n = 20) were divided into 2 groups (control [0.0 g/kg of ethanol; distilled water]: n = 10, binge ethanol-exposed rats [1.5 g/kg of ethanol; 25% w/v ethanol solution]: n = 10), were used in this study. The 10 rats in the binge ethanol group received an initial ethanol dose of 5.0 g/kg (30% w/v ethanol solution) via oral gavage, then received additional doses of 1.5 g/kg (25% w/v ethanol solution) every 8 h (at 10:00, 18:00, and 02:00) for 4 days. Oral gavage ethanol was administered according to body weight, using Majchrowicz binge alcohol protocol. [3] Body weights of the rats in both control and binge alcohol groups were recorded daily for 5 days (including the pre-administration [Pre-admin.] body weights). After 4 days of oral gavage, all animals were sacrificed and brain tissues were carefully harvested from the hippocampal region. *Ex vivo* ^{1}H HR-MAS NMR spectroscopy was performed using an Agilent VNMRS-500 (500.13-MHz). *Ex vivo* HR-MAS spectra were acquired from all 20 tissue samples with Carr-Purcell-Meiboom-Gill sequence [complex data number = 16384, spectral width = 8 kHz, acquisition time = 2.05 sec, relaxation delay time = 5.0 sec, presaturation time = 2.0 sec, inter-pulse delay (τ) = 0.4 msec, number of acquisitions = 128, and a total scan time = 15 min 24 sec]. Raw data obtained for the 20 samples were analyzed using LCModel with a simulated basis-set. The LCModel basis set for 500 MHz included spectra of 17 brain metabolites: 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). For the measured levels of each metabolite, we calculated metabolite ratios with respect to the tCr levels, and investigated metabolite changes in the hippocampal region.

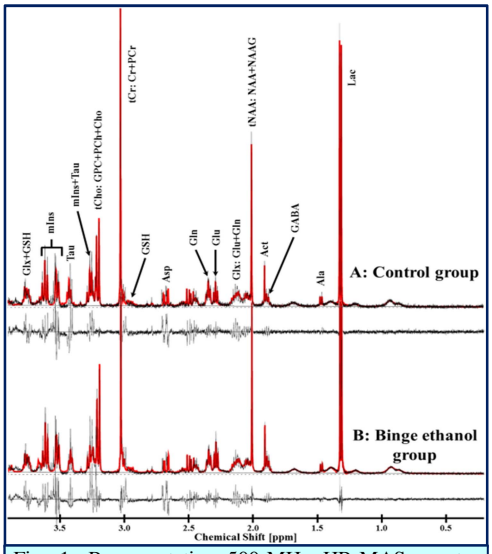

Fig. 1. Representative 500-MHz HR-MAS spectra from the hippocampal region of animals in the control (A) and binge ethanol (B) groups. The fitted LCModel spectra are represented in bold red. The residues are positioned under the fitted spectra. The chemical shift range is from 0.20 to 3.85 ppm.

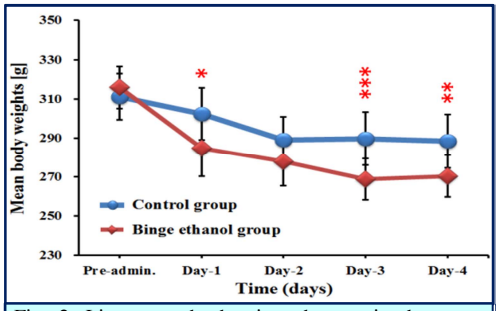

Fig. 2. Linear graph showing changes in the mean body weights (g) of rats in the binge ethanol group (red) and the control group (blue), over 5 days (including the preadministration [Pre-admin.] body weights). The vertical lines at data points indicate (±) standard deviations of the mean values. Significance level: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.005$.

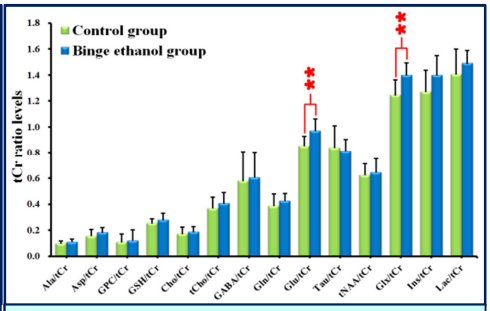

Fig. 3. Bar graph showing the significance levels of the metabolite to total creatine ratios in the hippocampal region of rats in the binge ethanol and control groups, which were quantified using LCModel software. Vertical lines on each of the bars indicate the standard deviation of the mean concentration values. Significance level: **, $p < 0.01$.

Results: Fig. 1 (A and B) shows representative *ex vivo* 500-MHz spectra from the hippocampal regions of animals in the binge ethanol and control groups. The *ex vivo* spectra provide a large amount of neurochemical information available from the tCr metabolite ratio levels and high-resolution spectrum. The body weights of rats in the binge ethanol group were markedly reduced compared with those of rats in the control group, with significant differences on day-1 (*: p = 0.011), day-3 (***: p = 0.001), and day-4 (**: p = 0.005) of the exposure period (Fig. 2). Fig. 3 shows the tCr ratio levels that were quantified from the 20 hippocampal tissue samples. The Glu/tCr (**: p = 0.007) and Glx/tCr (**: p = 0.006) ratios were significantly higher in the binge ethanol-exposed group than in the controls.

Discussion and Conclusion: The present study was conducted in rats using the Majchrowicz binge alcohol model for binge ethanol intoxication in order to observe the cerebral metabolite changes in the hippocampal region. We found significantly higher Glu/tCr and Glx/tCr ratios after binge ethanol intoxication, which may point to alterations in the glutamate-glutamine cycle, [4] and reflect an alteration in glutamatergic turnover in the neuron-glia shuttle. [5] On the basis of our findings and the results of previous studies, we suggest that significantly high Glu/tCr and Glx/tCr ratios may reflect the elevation of glutamate and glutamate+glutamine concentrations. Our main findings suggest that the glutamate signals and the glutamate-glutamine cycle in the hippocampal region are particularly sensitive to binge ethanol consumption. Future studies using a combination of human patients and animal MRS investigations, as well as other neuroimaging approaches, are required to strengthen our findings and to validate the translational component in the binge alcohol intoxicated condition.

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. This work was conducted with the *ex vivo* 500 MHz high-resolution NMRS system from Agilent Technologies Korea Ltd., Seoul, Republic of Korea.

References: **[1]** Crews FT, Braun CJ. Binge Ethanol Treatment Causes Greater Brain Damage in Alcohol-Preferring P Rats Than in Alcohol-Nonpreferring NP Rats. Alcohol. Clin. Exp. Res. 2003;27:1075-1082. **[2]** Crews FT, Nixon K. Mechanisms of Neurodegeneration and Regeneration in Alcoholism. Alcohol Alcohol. 2009;44:115-127. **[3]** Majchrowicz E. Induction of Physical Dependence upon Ethanol and the Associated Behavioral Changes in Rats. Psychopharmacologia 1975;43:245-254. **[4]** Hermanna D, Weber-Fahr W, Sartorius A, et al. Translational Magnetic Resonance Spectroscopy Reveals Excessive Central Glutamate Levels During Alcohol Withdrawal in Humans and Rats. Biol. Psychiat. 2012;71:1015-1021. **[5]** O'Leary-Moore SK, Galloway MP, A.P. McMechan AP, et al. Region-dependent alterations in glutamate and GABA measured by high-resolution magnetic resonance spectroscopy following acute binge inhalation of toluene in juvenile rats. Neurotoxicol. Teratol. 2007;29:466-475.