Influence of Short-Term Intermittent Ethanol Exposure and Abstinence on Cerebral Neurometabolite Concentrations Determined by Ex vivo 11.7-T Proton Nuclear Magnetic Resonance Spectroscopy

Do-Wan Lee[1,2], Jung-Whan Min[3], Jung-Hoon Lee[1,4], 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]*Asan Institute for Life Sciences, Asan Medical Center, Seoul, Seoul, Korea,* [3]*Department of Radiological Science, The Shingu University College of Korea, Seongnam, Korea,* [4]*Department of Radiology, Kyunghee Medical Center, Seoul, Korea*

Target audience: Neurologists, psychiatrists, and clinicians interested in MRS analysis of brain disorders.

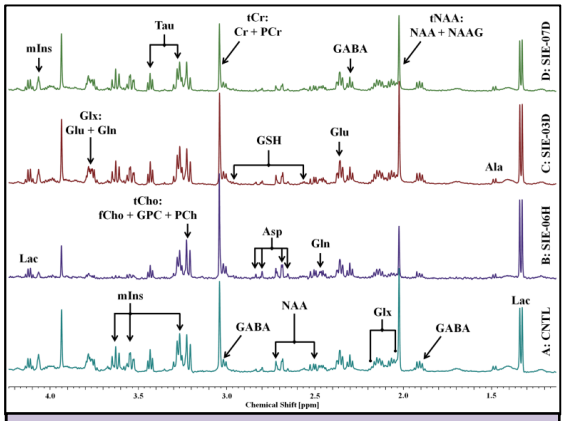

Figure 1. Representative *ex vivo* ^{1}H NMR spectra acquired in the frontal cortex at 11.7 T from the CNTL (A; control rats), SIE-06H (B; six-hour recovery from binge alcohol), SIE-03D (C; three-day recovery), and SIE-07D (D; seven-day recovery) groups. Complex data number, 16,384; spectral width, 8012.8 Hz; acquisition time, 2.05 s; relaxation delay time, 5.0 s; presaturation time, 2.0 s; interpulse delay (s), 0.4 ms; and number of acquisitions, 128. The chemical shift range was 4.20 to 1.00 ppm. Peak annotations are defined in the Methods section and are mostly neurotransmitters and small metabolites.

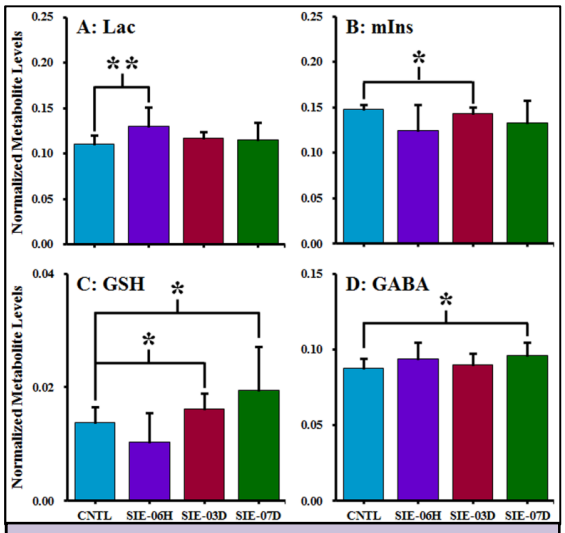

Figure 2. Mean normalized metabolite levels in the frontal cortex for the CNTL, SIE-06H, SIE-03D, and SIE-07D groups. The metabolite levels were normalized by dividing by the total signal intensities of the *ex vivo* 1H NMR spectra. The vertical lines on each of the bars indicate the (+) standard deviation of the mean values. Significance levels (independent t-test): *, p<0.05; **, p<0.01. Lac, lactate; mIns, myo-inositol; GSH, glutathione; GABA, gamma-aminobutyric acid.

Purpose: Alcohol is the most commonly abused intoxicating substance among young and middle-aged adults, and is a major cause of disability and mortality[1]. In general, the condition of patients with alcohol-dependence is affected by duration of alcohol dependence, drinking pattern, frequency of abstinence, as well as environmental variables like types of alcohol consumed[2]. Thus, a quantitative assessment of the neurochemical effects of binge ethanol intoxication on specific regions of the brain is necessary for a better understanding of the neurological effects of ethanol abuse. However, the influence of abstinence periods must be taken into account. We therefore studied the effects of short-term intermittent ethanol (SIE) exposure and abstinence on cerebral neurochemical changes with *ex vivo* ^{1}H nuclear magnetic resonance spectroscopy (NMRS), it has not been experimentally investigated. This study aimed to determine the influence of the time-dependent effects of SIE exposure on cerebral neurochemical differences and responses among control (CNTL) rats and rat groups at 6 h (SIE-06H), 3 days (SIE-03D), and 7 days (SIE-07D) after the last gavage procedure, using *ex vivo* high-resolution spectra.

Methods: 8-week-old male Wistar rats (n = 40; mean body weight, 304.4 ± 5.7 g; range, 291.0–314.5 g) were divided into 4 groups: control rats (CNTL, distilled water administration, n = 10); six-hour-recovery rats (SIE-06H, ethanol dose of 2.0 g/kg, n = 9); three-day-recovery rats (SIE-03D, ethanol dose of 2.0 g/kg, n = 11); and seven-day-recovery rats (SIE-07D, ethanol dose of 2.0 g/kg, n = 10). The 30 rats in the SIE-06H, -03D, and -07D groups received an initial dose of 5.0 g/kg through oral gavage, and then received an additional dose of 2.0 g/kg every 8 h (at 03:00, 11:00, and 19:00 h) for 4 days. The 10 rats in the sham CNTL group received an equivalent volume of distilled water at comparable times (at 04:00, 12:00, and 20:00 h). Oral-gavage ethanol was administered according to body weight, using the Majchrowicz binge-alcohol protocol[3]. Six hours (CNTL, and SIE-06H), 3 days (SIE-03D), and 7 days (SIE-07D) after the last gavage, all animals were euthanized by carbon dioxide inhalation and immediately decapitated. Forty frontal cortex tissue samples were carefully harvested using a brain slicer matrix. *Ex vivo* ^{1}H high-resolution magic angle spinning (HR-MAS) NMRS was performed on an Agilent Technologies Korea VNMRS-500 instrument (11.7-T). 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; 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 1.8-Hz Gaussian filter, and then Fourier transformed. The resulting spectra were manually phased, frequency referenced to trimethylsilylpropionate at 0.00 ppm, and baseline corrected. The post-processed 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[4]. The metabolites were quantified with fitted spectra, producing the following deconvolution peaks: 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. 1A–D** shows representative 11.7-T NMR spectra obtained from the frontal cortex regions of the 40 samples from the four groups (A, CNTL, n = 10; B, SIE-06H, n = 9; C, SIE-03D, n = 11; and D, SIE-07D, n = 10). The *ex vivo* NMR spectra were assigned the following cerebral metabolite signals: Lac, mIns, tCr, Glx, Tau, tCho, GSH, GABA, Asp, NAA, Gln, Glu, tNAA, and Ala. Fig. 2A–D illustrates the normalized cerebral metabolite levels quantified from the 40 acquired *ex vivo* spectra from the frontal cortex region. Our results showed significantly higher Lac (p = 0.010) and significantly lower mIns (p = 0.022) signals in the SIE-06H rats compared to the CNTLs. The GSH (p = 0.036) and GABA (p = 0.036) signals were significantly higher in the SIE-07D rats than in CNTLs. In addition, the GSH (p = 0.042) signals were also significantly higher in the SIE-03D rats than in the CNTLs.

Discussion and Conclusion: In summary, the present study conducted *ex vivo* ^{1}H HR-MAS NMR spectroscopy in a rat model to determine the time-dependent influences of SIE exposure on cerebral neurochemical changes in the rat frontal cortex. From our results and those of previous studies, significantly increased Lac signals can be observed when the brain is deprived of oxygen, or when anaerobic respiration increases[5]. Thus, significantly higher the Lac signals in rat frontal cortex 6 h after the SIE exposure may reflect alteration of the anaerobic respiration system or abnormal hypoxia. Significantly lower mIns signals in rat frontal cortex 6 h after the SIE exposure might indicate acute hypo-osmolality and hyponatremia in astrocytes[6]. Moreover, significantly higher GSH signals possibly indicate that increasing abstinence time after SIE exposure (3 days or more) may lead to oxidative stress, possibly due to stimulation of lipid peroxidation through the formation of free radicals and/or abnormalities of the antioxidant defense activities in the frontal cortex of the SIE-exposed rats[7]. Significantly higher GABA signals in the frontal cortex 7 days after the last SIE exposure may reflect alterations in GABA synthesis and GABAA receptor densities[8]. Our *ex vivo* ^{1}H NMR spectroscopy results indicate several potential metabolic markers for the time-dependent influence of short-term intermittent ethanol exposure on the frontal cortex.

References: **1.** M. Teesson, W. Hall, T. Slade, et al., Addiction 2010;105:2085–2094., **2.** S. Geibprasert, M. Gallucci, T. Krings. Eur. Radiol. 2010;20:1492–1501., **3.** Majchrowicz E. Psychopharmacologia 1975;43(3):245–254., **4.** Lee DW, Nam YK, Kim TK, et al., Neuroscience 2014;262:107–117., **5.** Cohen-Gilbert JE, Jensen JE, Silveri MM. Dev. Psychopathol. 2014;26:405–423., **6.** Braunová Z, Kašparová S, Mlynárik V, et al., Cell. Mol. Neurobiol. 2000;20:703–715., **7.** Agar E, Bosnak M, Amanvermez R, et al., Neuroreport 1999;10:1799–1801., **8.** Smith SS, Gong QH Alcohol 2007;41:223–231.

Acknowledgments: The authors declare no conflicts of interest. This study was supported by the program of Basic Atomic Energy Research Institute (BAERI) (2009-0078390), and the Basic Science Research Program (2010-0008096), and a grant (2012-007883) from the Mid-career Researcher Program through the National Research Foundation (NRF) funded by the Ministry of Science, ICT & Future Planning (MSIP) of Korea. And, this work was supported by the Industrial R&D program of MOTIE/KEIT.