Ex Vivo Quantification of Regional Cerebral Metabolites in Chronic Alcohol Consumption-Induced Rats

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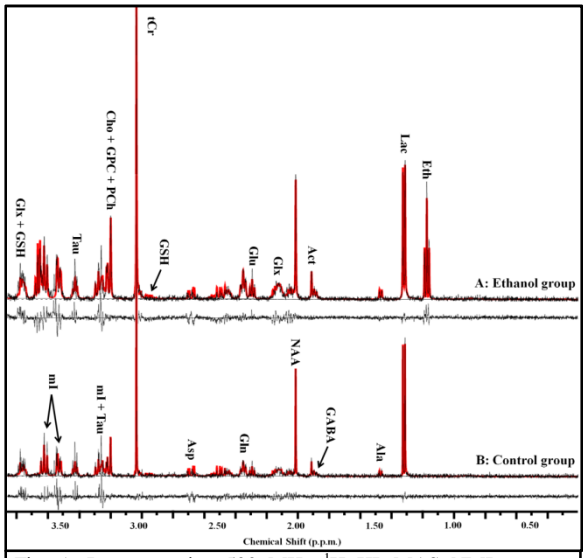

Fig. 1. Representative 500 MHz ^{1}H HR-MAS NMR spectra (total TR/TE = 5500/2 ms, CPMG sequence) of the ethanol treated-group and the control group, in region of the cerebellum.

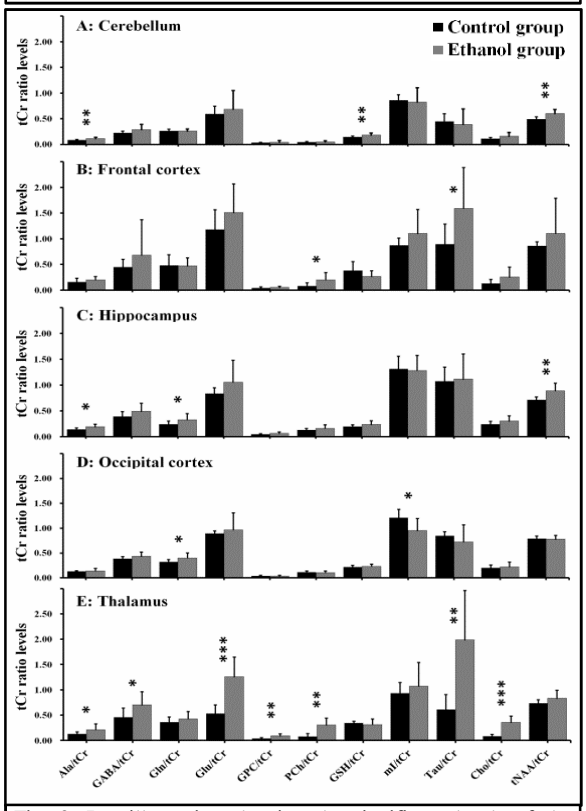

Fig. 2. Bar illustration showing the significant levels of the metabolite to total creatine ratio in the five investigated brain regions of the control group and the ethanol treated-group (Black bar: tCr metabolite levels of the control group; gray bar: tCr metabolite levels of the ethanol treated-group; range of the vertical axis: 0.0 to 2.5). Vertical lines on the bars indicate the (+) standard deviation of the mean values, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

Introduction:

To date, numerous studies have investigated the brain metabolic alterations in human chronic alcohol dependent patients, using ^{1}H MR spectroscopy. Previous studies emphasized that the chronic alcoholism in the human condition is affected by many factors, including the period of dependence, the pattern of drinking, types of alcohol, and frequency of withdrawals [1,2]. In addition, the effects of the alcohol appear differently in the brain regions [3,4]. Thus, the aim of this study was to quantitatively investigate the chronic ethanol consumption-induced cerebral metabolic changes in various regions of the rat brain, using 500 MHz ex vivo ^{1}H high resolution magic angle spinning (HR-MAS) NMR spectroscopy.

Materials and Methods:

Twenty two, six weeks-old, male Sprague Dawley Rats were used in this study. All animals were divided into two groups (control group: N = 11, ethanol-treated group: N = 11), and fairly fed with the ethanol and control liquid diets for 10 weeks. End of the experiment, all animals were sacrificed and harvested brain tissues of the five regions, including the cerebellum (Cere), the frontal cortex (FC), the hippocampus (Hip), the occipital cortex (OC) and the thalamus (Thal). *Ex vivo* ^{1}H HR-MAS NMR spectroscopy was performed using an Agilent VNMRS-500 (500.13-MHz [11.7-T]). One-dimensional HR-MAS spectra were acquired from all 110 tissue samples with Carr-Purcell-Meiboom-Gill (CPMG) sequence [complex data number = 12,000, spectral width = 8012.8 Hz, acquisition time = 2.05 sec, relaxation delay time = 4.0 sec, pre-saturation time = 1.5 sec, inter-pulse delay (τ) = 0.4 msec, big-tau (eighty 180-degree refocusing pulses) = 0.064 msec, number of acquisitions = 128, and a total scan time = 17 min 20 sec]. Raw data obtained for the 110 samples were analyzed in a fully automated spectral process, using LCModel with a simulated basis-set file. For the measured levels of each metabolite, we calculated metabolite ratios with respect to the total creatine (creatine + phosphocreatine [tCr]) levels, and investigated metabolite changes in five brain region.

Results:

Fig. 1 shows the high resolution 500 MHz HR-MAS spectra, in the region of the cerebellum. This figure indicated that the ethanol peak was fitted only ethanol treated group at 1.18 ppm. Fig. 2 show that the various metabolic changes in our studied brain regions. In the ethanol liquid diet group, there were significant increases in the metabolites ratio levels, as compared to control (Cere: alanine, glutathione, and N-acetlyaspartate; FC: phosphocholine and taurine; Hip: alanine, glutamine, and N-acetylaspartate; OC: glutamine; Thal: alanine, γ-aminobutyric acid, glutamate, glycerophosphocholine, phosphocholine, taurine, and free choline). However, in the ethanol liquid diet group, the myo-inositol levels of the OC were significantly lower.

Discussion:

In this study, we used *ex vivo* ^{1}H HR-MAS spectroscopy in the chronic ethanol-treated rat brain. Our results showed that the various metabolites were significantly altered in the five brain regions of the chronic ethanol-treated group, compared to the control group. The present study demonstrates that chronic ethanol consumption can lead to various cerebral metabolites changes in the chronic ethanol-treated rat. Our key finding is that the Glu/tCr and Cho/tCr ratio levels showed the most significant differences in the thalamus of the rat brain. These increased metabolic ratio levels of the thalamus might be utilized as the key marker in the human chronic alcoholism. Therefore, our results could be useful to pursue clinical applications for quantitative diagnosis in human alcoholism.

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