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BRAIN RESEARCH

Research Report

Ex vivo detection for chronic ethanol consumption-induced neurochemical changes in rats

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ABSTRACT

The aim of this study was to quantitatively investigate the chronic ethanol-induced cerebral metabolic changes in various regions of the rat brain, using the proton high resolution magic angle spinning spectroscopy technique. The rats were divided into two groups (control group: N=11, ethanol-treated group: N=11) and fed with the liquid diets for 10 weeks. In each week, the mean intake volumes of liquid diet were measured. The brain tissues, including cerebellum (Cere), frontal cortex (FC), hippocampus (Hip), occipital cortex (OC) and thalamus (Thal), were harvested immediately after the end of experiments. The *ex vivo* proton spectra for the five brain regions were acquired with the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence at 500-MHz NMR spectrometer. All of the spectra were processed using the LCModel software, with simulated basis-set file, and the metabolite levels were referenced to total creatine. 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,

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Abbreviations: MRS, magnetic resonance spectroscopy; NMRS, nuclear magnetic resonance spectroscopy; HR-MAS, high resolution magic angle spinning; DDW, distilled and deionized water; SNR, signal to noise ratio; p.p.m., part per million; CNS, central nervous system; CPMG, Carr-Purcell-Meiboom-Gill; CRLB, Cramér–Rao lower bound; LCModel, Linear Combination Model; %SD, percentage standard deviation; Ala, alanine; Act, acetate; Asp, aspartate; Cho, choline; Cr, creatine; PCr, phosphocreatine; GABA, gamma-aminobutyric acid; Gln, glutamine, Glu, glutamate; Glc, glucose; GPC, glycerophosphocholine; GSH, glutathione; sI, scyllo-inositol; mI, myo-inositol; Lac, lactate; NAA, N-acetylaspartate; NAAG, N-acetylaspartylglutamate; PCh, phosphocholine; Eth, ethanol; Tau, taurine

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taurine, and free choline). However, in the ethanol liquid diet group, the myo-inositol levels of the OC were significantly lower. The present study demonstrates how chronic ethanol consumption affects cerebral metabolites in the chronic ethanol-treated rat. Therefore, this result could be useful to pursue clinical applications for quantitative diagnosis in human alcoholism.

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1. Introduction

To date, numerous studies have investigated the brain metabolic alterations in human chronic alcohol dependent patients, using ^{1}H MR spectroscopy (Bartsch et al., 2007; Lee et al., 2007). However, 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 (Zahr et al., 2010). Most studies of the human chronic alcoholism have shown different patterns of metabolic alterations (Bloomer et al., 2004; Lee et al., 2007). Thus, in the study of chronic alcohol consumption, more quantitative investigation is necessary, using an animal model.

Several studies attempted quantitative investigations of the brain metabolic changes in chronic ethanol exposure, using animal models. In many ways, investigation of the chronic ethanol exposure has already been studied using the animal model. In the rodent model, most ethanol treatment studies have used the liquid diet technique (Lieber et al., 1989; Rao et al., 1986), the intragastric intubation (Cronise et al., 2001; Tran et al., 2000), the intraperitoneal ethanol injection (Denays et al., 1993; Yamakami et al., 1995) and ethanol inhalation using a vapor chamber (Izco et al., 2007; Lieber et al., 1989). Of all these, the intragastric intubation and the intraperitoneal ethanol injection were potentially stressful and invasive in animal models (Gil-Mohapel et al., 2010). Thus, these techniques cannot be used in chronic treatment studies. Ethanol inhalation using the vapor chamber was also ruled out because it could not control the level of nutrition (Gil-Mohapel et al., 2010). On the other hand, the liquid diet technique provides complete nutritional control and low procedural stress. Therefore, it has been used in chronic ethanol treatment studies conducted in animal models (Simonyi et al., 2002; Wills et al., 2008). In particular, the advantages of the chronic ethanol liquid diet model are continuous delivery of both ethanol and essential nutrients, without triggering aversion in animals. Also, one important advantage of the liquid diet is the absence of side effects caused by poor nutrition.

In vivo ^{1}H MRS technique provides non-invasive methods for the quantification of specific brain biochemical markers and neurotransmitters reflecting molecular processes (Opstad et al., 2010; Zahr et al., 2009). However, the low resolution and signal to noise ratio of the *in vivo* spectrum restricts the amount of biochemical information that can be quantified (Opstad et al., 2010; Tsang et al., 2005). In contrast to *in vivo* ^{1}H MRS, *ex vivo* ^{1}H HR-MAS is a powerful technique for observation of the brain metabolic alterations and allows high resolution spectra to be obtained directly from the biopsy tissues (Opstad et al., 2009, 2010). In particular, in case of extracted-tissues, the ^{1}H HR-MAS spectra can provide more detailed and valuable information on the biochemical tissue composition than the *in vivo* ^{1}H MRS (Martı´nez-Bisbal et al., 2004).

The present study was conducted in a rat model using the liquid diet technique for chronic ethanol exposure, in order to observe the metabolic changes in regional brain tissues. The aim of this study was to investigate the cerebral metabolic changes of the chronic ethanol-induced state in different regions of the rat brain, using the ^{1}H HR-MAS NMR spectroscopy technique.

2. Results

2.1. *Total intake of ethanol and liquid diet*

In the ethanol treated rats, mean intake volume of liquid diet data (Fig. 1A) indicated that a steady level of chronic ethanol exposure was maintained throughout the experiment. Consumption ethanol liquid diet volumes continued to increase after a slight decrease in the fifth week and the eighth week. The mean intake volumes of liquid diet increased gradually and reached values of 98.23±11.65 ml at the end of experiment. Net alcohol intake data (Fig. 1B) indicated that ethanol consumption volume of each of the rat was calculated as net alcohol intake amount per body weight per week (g/kg/week). The net alcohol intake amount was increased rapidly during second to fourth week, and then showing decreased patterns starting from the fourth week. Data from this study indicate that mean intake of net alcohol in amounts greater than 12 g/kg but less than 14 g/kg (reached values of 13.35±1.30 g/kg) occurred for fourth week. Since then, the net alcohol intake amount was slightly decreased and reached lowest values of 10.86±1.32 g/kg (reduction 18.65% compared to the highest value at fourth week) occurred for ninth week.

2.2. ^{1}*H HR-MAS NMR spectra*

The one-dimensional (1D) spectra were acquired from 99 regional brain tissue samples, as follows: Cerebellum (control group: N=11; ethanol group: N=10), frontal cortex (control group: N=11; ethanol group: N=10), hippocampus (control group: N=11; ethanol group: N=10), occipital cortex (control group: N=9; ethanol group: N=8), thalamus (control group: N=10; ethanol group: N=9). Figs. 2 (A and B) shows the representative fitted spectra from the cerebellum of the ethanol treated-group and control group. In the ethanol treated-group, unlike to the control group, spectra included the ethanol peak as triplet in all of the studied regions. Fig. 2 shows the high spectral resolution. Table 1 also shows that the strongly represented signals of creatine, total *N*-acetylaspartate, and *myo*-Inositol

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