Characterization of Hepatic encephalopathy in vivo by using 31P-MRS: A preliminary animal study at 9.4 T

Juyeun Park1, YoonSeok Choi1, Yunjung Lee2, Jisu Woo1, In-Chan Song3, Ji-Hoon Kim1, and Hyojeong Kim1

1Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Korea, Republic of; 2Lee Gil Ya Cancer and Diabetes Institute, Incheon, Korea, Republic of; 3Department of Radiology, Seoul National University Hospital, Seoul, Korea, Republic of

Introduction
Hepatic encephalopathy (HE) is a serious neuro-psychiatric complication of both acute and chronic liver disease [1]. To elucidate the pathogenesis of HE in vivo magnetic resonance spectroscopy technique (MRS) has been used, and for 1H-MRS previous studies have consistently reported the reduced myo-inositol (ml) and increased glutamine (Gln) concentrations in HE-affected brains [2,3]. For 31P-MRS, however, previous findings remain controversial [2], due, at least in part, to the relatively low sensitivity of the nucleus and limited spectral dispersion at low field. Given the information on energy state and cell membrane turnover and proliferation provided by 31P-MRS and its potential significance in the pathogenesis of HE, this is an important issue. To this end, we have characterized HE in animal by using 31P-MRS with substantially improved spectral dispersion and SNR at 9.4 T. The results from chemical analyses are presented for the validation of the animal model, and the in-vivo MRS findings are discussed.

Materials and Methods
A total of nine male Sprague Dawley rats (24 weeks of age) were used. HE model was induced in 5 rats (HE-group) by an intraperitoneal injection of CCl4 mixed with vegetable oil (25 μl CC4 in a 150 μl volume (1:6)) 3 times per week [4] for 18 weeks. All MRS studies were performed on a 9.4 T Bruker BioSpin system. 31P-MRS brain spectra were acquired using a double-tuned 1H/31P surface coil (Bruker, Germany) (a pulse-acquire sequence, TR = 8 s, spectral width = 10 kHz, number of data points = 2048, 256 averages). In vivo 1H-MRS spectra were also acquired from the prefrontal cortex by using a rat head coil (Bruker, Germany), and the results were compared with those from the previous studies [2,3] (a STEAM sequence, TR/TE/TM = 5000/2.8/5 ms, spectral width = 5 kHz, number of data points = 2048, 720 averages, a typical voxel volume of 48 mm³). MR data post-processing was carried out by using Matlab (Mathworks Inc., USA) and TopSpin software (v 2.0; Bruker, Germany). Following MR studies, the brains were harvested. Brain ammonia levels were estimated [5] for all rats by using an ammonia assay kit (Sigma-aldrich, AA0100, USA). Immunohistochemistry (IHC) was performed for 2 rats from each of the two animal groups by using glial fibrillary acidic protein (GFAP) staining for brain tissues embedded in paraffin, and the changes in astrocytic morphology were examined that are known to arise from ammonia intoxication [6].

Results
There was a significant difference in the brain ammonia levels between the control and the HE groups (Fig. 1; 0.0066±0.0002 vs. 0.0074±0.0005 mg/ml; p=0.05). Fig. 2 clearly depicted the changes in astrocytic morphology (cell swelling) in the treated rat (Fig. 2b) in contrast to the preserved, star-shaped, normal astrocytic morphology (Fig. 2a). In Fig. 3, the mean 1H-MRS spectrum of the control group (black line) is compared with that of the HE group (red line), where the reduced ml and increased Gln levels are in good agreement with the literature [2,3]. Fig. 4 shows a representative, 31P-MRS brain spectrum where the resonances from phosphomonooester (PME), inorganic phosphate (Pi), phosphodiester (PDE), phosphocreatine (PCr) and ATP are all well-separated, and the high SNR of the spectrum is clearly demonstrated. There were trends towards increased ATP to Pi ratio (ATP/Pi) and PDE to PME ratio (PDE/PME) in the treated group with respect to the control group (Fig. 5). There was also a trend towards increased ATP/Pi as a function of brain ammonia concentrations (r=0.6234, p=0.073). Most importantly, there was a strong correlation between PDE/PME and brain ammonia concentrations despite the small number of animals (r=0.7235, p=0.028).

Discussion
According to the results of chemical analyses and 1H-MRS, the animal model employed herein is suitable for studying HE. The higher ATP/Pi in the treated group with respect to the control group and its increase as a function of brain ammonia concentrations may depict the abnormal energy metabolism in HE brain that may results from marked increase in ADP and Pi giving rise to a considerable increase in relative ATP biosynthesis flux [7]. The higher PDE/PME in the treated group relative to that of the control group, and the strong correlation between this 31P-MRS measure and brain ammonia concentrations indicate altered cell membrane metabolism in response to the brain insult [2]. In conclusion, 31P-MRS at high field allows for highly resolved spectra in vivo, in particular, effective separation of PME, Pi and PDE, and thus, combined with 1H-MRS, can provide more vivid picture of the altered brain metabolism in the progression of HE.

References

ACKNOWLEDGEMENTS
This work was supported by Korean Ministry of Educational Science and Technology (2011-0002622 and 2011-0005432).

Fig. 1 Concentrations of brain ammonia in prefrontal cortex of normal (n=4) and CC14 treated (n=5) rats.

Fig. 2 Glial fibrillary acidic protein (GFAP) immunostaining. (a) Normal, star-shaped astrocyte (black arrow). (b) Abnormal astrocyte with cell swelling (white arrow).

Fig. 3 In vivo 1H-MRS spectra of the prefrontal cortex at 9.4T for the control (black line) and the HE groups (red line). Gln(2.46ppm) was increased and ml(3.52ppm) was decreased. HE = hepatic encephalopathy; Gln = glutamine; ml = myo-Inositol.

Fig. 4 In vivo 31P-MRS spectrum of the whole brain from a rat in the HE group.

Fig. 5 Difference in ATP/Pi and PDE/PME between the control and HE groups. Pi = inorganic phosphate; PDE = phosphodiester; PME = phosphomonooester.