Metabolite Profiling of Mild Hypothermia by 1H-MRS

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Introduction

Hypothermia has showed efficacy in protecting the brain and the heart from various insults, such as stroke, trauma, ischemia, cardiac arrest, in both animal and human studies[1,2]. However, the protective mechanism is not fully understood. *Ex vivo* NMR studies and analysis of body fluids showed changes in metabolites, like lactate, myo-inositol, taurine and glutamate during hypothermia[3,4]. In particular, taurine is regarded as the endogenous cryogen, and has a specific taurinergic pathway for thermoregulation[5,6]. We have investigated the metabolite profile of the brain before and after hypothermia, and the metabolic changes in the cortex and thalamus were studied at 7T. This is to demonstrate that single-voxel ^{1}H-MRS could be a mean of assessments, and the optimization of time and temperature for hypothermia.

Methods

Animal Preparation: Male Sprague-Dawley (SD) rats (280-300 g; $N = 5$) were under investigation. Each animal was anesthetized with ~1.5 % isoflurane and maintained at about 36.5°C. Body temperature was monitored by a rectal temperature sensor, and respiration and physiological parameters were monitored. Body temperature was kept at 33 °C for mild hypothermia[7]. MRS was performed on the cortex and thalamus of the same animal under normothermia and hypothermia. *In Vivo* MRS: ^{1}H-MRS experiments were performed on a 7T Bruker MRI scanner with a 72 mm birdcage transmit-only RF coil with an actively decoupled receive-only quadrature surface coil. The same voxel was used under normothermia and hypothermia. A 2.8×2.8×0.8 mm^3 voxel and a 2.8×2.8×2.8 mm^3 voxel was place on a homogeneous region of the cortex and thalamus respectively. MAPSHIM was used as the shimming protocol, and the first- and second-order localized voxel shimming with the field map based technique was applied. A FWHM linewidth of water signal of <20 Hz was achieved. The water signal was suppressed by variable power RF pulses with optimized relaxation delays (VAPOUR). Outer volume suppression (OVS) combined with point-resolved spectroscopy (PRESS) sequence was used for signal acquisition, with TR = 2500, TE = 20 ms, spectral bandwidth = 3 kHz, 2048 data points, 512 averages, and total scan time of ~20 min. Data Analysis: MR spectra were processed using the jMRUI software (version 4.0). The raw data was zero-filled, apodized with a 15-Hz Gaussian filter, phase corrected and filtered out the residual water signal with Hackel-Lanczos Singular Value Decomposition (HLSVD) algorithm. Peaks were assigned with reference to N-acetylaspartate (NAA) at 2.02 ppm. Metabolite area under peak is quantified by quantum estimation (QUEST) method with subtraction approach for background modeling. The numerical time-domain modal functions of 10 metabolites, including NAA, alanine (Ala), aspartate (Asp), creatine (Cr), choline (Cho), glutamate (Glu), taruine (Tau), γ-aminobutyrate (GABA), lactate (Lac) and myo-inositol (mI) were used in QUEST. NAA:Cr, Cho:Cr, Glu:Cr, Lac:Cr, mI:Cr and Tau:Cr ratios were statistically analysis using two-tailed paired Student's t-tests on the data before and that after hypothermia. Results were considered significant with $p<0.05$.

Results

Fig. 1a shows the ^{1}H-MRS spectra of the cortex during normothermia and hypothermia, and the position of voxel is shown in Fig. 1b. The statistical evaluation of the metabolites found that both mI and Lac were increased in the cortical region with $p<0.05$ in hypothermic rats (Fig. 1c). Fig. 2a shows the ^{1}H-MRS spectra of the thalamus before and after hypothermia. The region of thalamus subjected to MRS is shown in Fig. 2b. In the statistical analysis (Fig. 2c), we observed an increase in Tau under hypothermia with $p<0.05$.

Discussion and Conclusion

The mechanisms of mild and moderate hypothermia on neuroprotection are different and associate with a number of events[8]. ^{1}H-MRS enable us to study the changes in the metabolites and give us clue to clarify related mechanisms. Lactate is a substrate for energy, which is raised in case of neuroprotection. An increase in lactate was found in cerebral cortex from a microdialysis study[9]. This correlates with our findings that Lac increased in the cortex during hypothermia, and suggests the activation of neuroprotection. Brain cells are sensitive to temperature and extracellular tonicity, myo-inositoal and taurine are major osmolytes in brain. In case of hypertonicity, mI increases to prevent damage to neural cells[4]. Thus, an increase in mI in the cortex can be ascribed to the regulation of osmolality and prevent cell from swelling. For example under acute liver failure, mI was found to increase under hypothermia[3]. Taurine has a major role in thermoregulation[5], which also increased in case of acute liver failure after hypothermia. Tau is an agonist for $GABA_A$ and $GABA_B$, and activates the extrasynaptic GABA in the thalamus[6]. This leads to a hypoerpolarization of thalamic relay neurons, thus protect neurons from toxicity of hypothermia[10]. Therefore, an increase in Tau in the thalamus could help to regulate temperature and protect neurons from injury. The increase in specific metabolites in the cortex and thalamus tells us the mechanical aspect of neuroprotection under hypothermia. This study shows that ^{1}H-MRS provides a way to understand the mechanism of hypothermia and is advantageous for monitor this intervention in real time and at the specific site of interest.

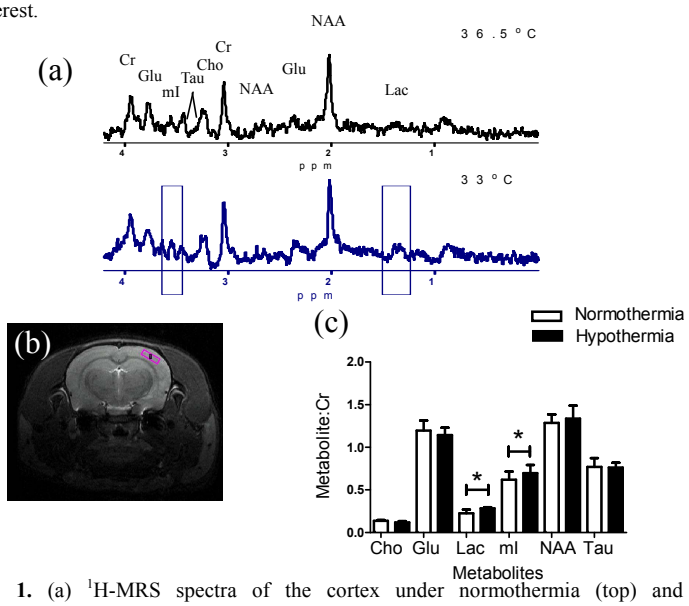

Fig 1. (a) ^{1}H-MRS spectra of the cortex under normothermia (top) and hypothermia (bottom); (b) Coronal view of the brain showing the voxel position; (c) List of metabolites with statistical change ($p<0.05$).

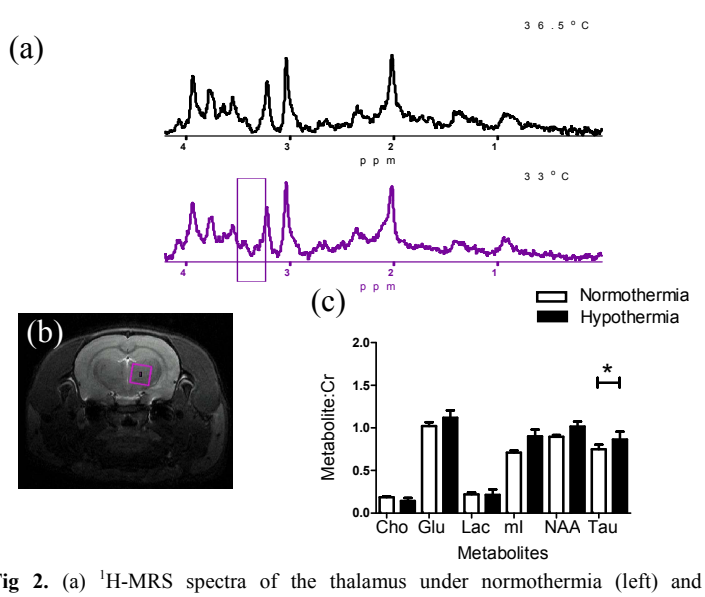

Fig 2. (a) ^{1}H-MRS spectra of the thalamus under normothermia (left) and hypothermia (right); (b) Coronal view of the brain showing the voxel position; (c) List of metabolites with statistical change ($p<0.05$).

References [**1**] Chatauret N et al. Gastroenterology 2003;125:815-824. [**2**] Marion D et al. J Neurotrauma 2009 ; 26 :455-467.[**3**] Zwingmann C et al. Brain Res 2004 ;999 :113-118. [**4**] Robertson NJ et al. Pediatr Res 2001 ;50 :692-700. [**5**] Frosini M et al. Adv Exp Med Biol. 2000 ;483 :273-282. [**6**] Frosini M et al. Br J Pharm 2003 ;139 :487-494. [**7**] Lo TP et al. J Comp Neurol 2009 ;514 :433-448. [**8**] Yager JY et al. Stroke 1996; 27:919-926. [**9**] Mendelowitsch A et al. J Neurol Neurosurg Psychiatry 1998;64:611-618. [**10**] Jia F et al. J Neurosci 2008;28:106-115.