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Magnetic resonance spectroscopy of the brain under mild hypothermia indicates changes in neuroprotection-related metabolites

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ABSTRACT

Brain hypothermia has demonstrated pronounced neuroprotective effect in patients with cardiac arrest, ischemia and acute liver failure. However, its underlying neuroprotective mechanisms remain to be elucidated in order to improve therapeutic outcomes. Single voxel proton magnetic resonance spectroscopy (^{1}H-MRS) was performed using a 7 Tesla MRI scanner on normal Sprague–Dawley rats (N = 8) in the same voxel under normothermia (36.5 °C) and 30 min mild hypothermia (33.5 °C). Levels of various brain proton metabolites were compared. The level of lactate (Lac) and myo-inositol (mI) increased in the cortex during hypothermia. In the thalamus, taurine (Tau), a cryogen in brain, increased and choline (Cho) decreased. These metabolic alterations indicated the onset of a number of neuroprotective processes that include attenuation of energy metabolism, excitotoxic pathways, brain osmolytes and thermoregulation, thus protecting neuronal cells from damage. These experimental findings demonstrated that ^{1}H-MRS can be applied to investigate the changes of specific metabolites and corresponding neuroprotection mechanisms *in vivo* noninvasively, and ultimately improve our basic understanding of hypothermia and ability to optimize its therapeutic efficacy.

Hypothermia confers a marked protection of the brain and heart from various insults, such as post-ischemic neurological injury and cardiac arrest, in both humans and animal models [3,18,24,39]. Mild hypothermia prevents hypoxic or ischemic damage, and has been applied for neuroprotection in stroke, ischemia [24,28], cardiac arrest [18] and hepatic encephalopathy (HE) [1]. It delays the onset of HE and prevents brain edema in acute liver failure [31]. It is a more effective intervention than osmotic diuretics for treating refractory intracranial hypertension in severe traumatic brain injury (TBI) and various acute neurological diseases [34]. However, underlying neuroprotective mechanisms of hypothermia are not yet fully understood.

Hypothermia has been found to affect a series of biological events, which range from the brain metabolism to immunoinflammatory processes [38]. Its neuroprotection is multifaceted and further complicated in specific diseases. Mechanisms of hypothermia include reduction of cerebral metabolism, cerebral blood flow and apoptosis, and prevention of excitotoxic damage by inhibiting the release of excitatory amino acids or inactivation of their receptors [30]. Other protective mechanisms include reduction in gliosis that leads to neuronal preservation [23], and dampening of the immune response and post-injury inflammation by preventing the immune cell infiltrations [26]. *Ex vivo* NMR studies and analysis of body fluids showed changes in levels of metabolites, like lactate, myo-inositol, taurine and glutamate during hypothermia [29,47]. In particular, taurine is regarded as the endogenous cryogen, and has a specific taurinergic pathway for thermoregulation [12,14].

The study of the associated neuroprotection mechanisms clarifies the role of hypothermia in disease treatments, especially in TBI [15]. Mild (34–35 °C) verse moderate hypothermia (32–34 °C), local verse systemic hypothermia and the duration of cooling could result in different therapeutic outcomes [18,22]. Brain and rectal temperature could vary in a range of 0.1–2 °C in patients [37]. Moreover, concentrations of metabolites (such as ammonia) or osmolytes could be different between the plasma and cerebral spinal fluid [31]. Hence, measuring the rectal temperature or plasma contents is not sufficiently specific for assessing the physiology of the brain in response to hypothermia in clinical practice. New parameters or methods are needed to better monitor and administer hypothermia. In particular, the ability to examine the metabolic changes and evaluate the outcomes of hypothermia *in vivo* is critical to the success of hypothermic therapy.

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Table 1
Metabolite to Cr ratios and corresponding Cramer-Rao lower bounds (CRLBs) under normothermia and hypothermia in the cortex and thalamus, respectively.

Metabolite:Cr	Cortex				p-Value*	Thalamus				p-Value*
	Normothermia		Hypothermia			Normothermia		Hypothermia		
	Mean ± SD	CRLB (%)	Mean ± SD	CRLB (%)		Mean ± SD	CRLB (%)	Mean ± SD	CRLB (%)	
Cho	0.13 ± 0.02	9.80 ± 2.55	0.13 ± 0.04	13.59 ± 8.31	ns	0.21 ± 0.02	4.31 ± 0.27	0.15 ± 0.01	4.95 ± 0.46	<0.01
Glu	1.12 ± 0.17	6.97 ± 8.54	1.13 ± 0.24	6.66 ± 3.68	ns	1.01 ± 0.13	3.45 ± 0.22	0.86 ± 0.33	3.48 ± 0.35	ns
Lac	0.21 ± 0.06	14.40 ± 2.93	0.30 ± 0.07	14.44 ± 3.57	<0.05	0.22 ± 0.05	11.82 ± 2.46	0.21 ± 0.09	15.71 ± 4.91	ns
mI	0.61 ± 0.18	5.70 ± 0.81	0.74 ± 0.18	6.55 ± 1.87	<0.01	0.66 ± 0.12	3.64 ± 0.53	0.72 ± 0.13	3.39 ± 0.56	ns
NAA	1.03 ± 0.47	4.58 ± 1.62	1.29 ± 0.49	6.68 ± 4.10	ns	0.87 ± 0.10	4.46 ± 1.12	0.94 ± 0.09	3.32 ± 0.46	ns
Tau	0.91 ± 0.26	5.05 ± 1.00	0.83 ± 0.14	8.03 ± 5.90	ns	0.63 ± 0.06	4.47 ± 0.50	0.73 ± 0.07	4.17 ± 0.30	<0.01

* Two-tailed paired Student's t-test.

In this study, proton magnetic resonance spectroscopy (^{1}H-MRS) was employed to examine the metabolic changes in the cortex and thalamus of normal Sprague–Dawley (SD) rats during mild hypothermia. This study aimed to determine the efficacy of using ^{1}H-MRS for assessing real-time metabolic profiles and neuroprotection mechanisms for better understanding of hypothermia.

All animal experiments were approved by the local institutional animal ethics committee. Normal adult male SD rats (280–300 g; 7–8 weeks; $N = 8$) were anesthetized by isofurane (with 3% induction and 1.5% maintenance) [42,46]. Respiration rate, heart rate, SpO_2 and rectal temperature were monitored throughout the scanning session. Body temperature was monitored by a rectal temperature sensor and maintained at 36.5 ± 0.5 °C with a heating pad during normothermia. Body temperature was decreased at a rate of 0.1 °C/min and then kept at 33.5 ± 0.5 °C for the induction of mild hypothermia [43]. ^{1}H-MRS was performed in the cortex and thalamus under both normothermia and mild hypothermia. MRS data acquisition under mild hypothermia was commenced after maintaining the body temperature at 33.5 ± 0.5 °C for 30 min.

Single voxel ^{1}H-MRS experiments were acquired on a 7 T MRI scanner with a maximum gradient of 360 mT/m (70/16 PharmaScan, Bruker Biospin GmbH, Germany). A 72-mm birdcage transmit-only RF coil with an actively decoupled receive-only quadrature surface coil was used. A 2.8 mm × 2.8 mm × 0.8 mm voxel and a 2.8 mm × 2.8 mm × 2.8 mm voxel were placed on a homogeneous region of the cortex and thalamus, respectively. Note that the same cortex or thalamus voxel was used for measurement under normothermia and mild hypothermia for each animal. The volume of interest was maximized to cover the frontal cortex and thalamus and avoid margins, and to optimize the signal-to-noise ratio. After first- and second-order localized voxel shimming with field map based shimming technique [27], a full-width half-maximum linewidth of water signal of ≤15 Hz was achieved. The water signal was suppressed by variable RF pulses with optimized relaxation delays (VAPOR). Outer volume suppression (OVS) combined with point-resolved spectroscopy (PRESS) sequence was used for signal acquisition using TR/TE = 2500/20 ms, spectral bandwidth = 3 kHz, 2048 data points and 512 averages, and total scan time of ~20 min [6]. RF pulses with Hermite envelop were used with bandwidth of 2000 Hz and 4000 Hz for 180° and 90° pulses, respectively. RF carrier frequency was set to the water resonance peak (4.7 ppm). Field map based shimming technique was used to shim B_0 homogeneity within the voxel.

MR spectra were processed using the jMRUI software (http://www.mrui.uab.es/mrui/) [6]. The raw data was apodized with a 15-Hz Gaussian filter and phase corrected. The residual water signal was filtered out using the Hankel-Lanczos singular value decomposition algorithm by preprocessing with 25 spectral components for modeling. Chemical shifts of peaks were assigned with reference to the CH_3-group of N-acetylaspartate (NAA) at 2.02 ppm. Metabolite area under peak was quantified by quantum estimation (QUEST) method with subtraction approach for background modeling. The metabolite parameters were decorrelated from the background with truncation of initial data points given that macromolecules and lipids signals decay rapidly in time-domain. The numerical time-domain model functions of ten 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 as prior knowledge in QUEST. These metabolite model signals were quantum mechanically simulated in NMR spectra calculation using operators (NMR-SCOPE) for the *in vivo* experimental protocol [16]. Errors in measurement of noise and inadequate modeling of the overlapping background signal were calculated by the Cramér-Rao lower bounds (CRLBs), which were used to assess the reliability of metabolite quantitation [9]. The quantification was considered as relevant only when the corresponding bound was below 25%. Cr is a marker for brain energy metabolism and was observed to be relatively constant *in vivo* [17]. To accurately extract the dominating changes of metabolites, a relative quantification method using creatine (Cr) peak as the internal spectral reference was applied [6]. NAA:Cr, Cho:Cr, Glu:Cr, Lac:Cr, mI:Cr and Tau:Cr ratios were statistically analyzed by using two-tailed paired Student's t-tests. The differences in metabolite:Cr between the normothermia and mild hypothermia was considered as significant with p-values less than 0.05. All data was expressed as mean ± SD.

Fig. 1 illustrates the typical voxel location (solid-line box) in the frontal cortex and the corresponding ^{1}H-MRS spectra during normothermia and hypothermia, including the spectra of QUEST quantitation. Similarly, Fig. 2 shows the results from the thalamus.

The mean values of metabolite to Cr ratios and CRLBs of peak quantitation were summarized in Table 1 for the cortex and thalamus, respectively. Both results of QUEST quantitation (Figs. 1 and 2) and CRLBs indicated reliable quantitations. In the cortical region, both mI and Lac increased significantly ($p < 0.05$) under hypothermia, which could also be observed qualitatively in the spectra in Fig. 1. Lac increased by 43% and mI increased by 21%. In the thalamus, Tau increased by 16% and Cho decreased by 29% with $p < 0.05$ during hypothermia. All metabolites had CRLB <20%, indicating their reliable quantitation [9].

Both cortex and thalamus are sensitive to temperature changes, where major neuroprotection metabolisms are found occur [19,25]. In this study, changes in levels of metabolites in the frontal cortex and thalamus were observed during mild hypothermia. These changes can be related to a number of neuroprotection processes. Lac is a substrate for energy which is responsible for anaerobic metabolism, interruption of oxidative phosphorlyation and anaerobic glycolysis [32]. An increase in Lac in cerebral cortex has been reported in a microdialysis study [25]. This correlates with our findings that Lac increased by 43% in the cortex during hypothermia, implicating the energy metabolism associated with neuroprotection that results from an increase in glycolysis [32] and a depression of tricarboylic acid cycle (TCA) in hypothermia [11,20]. The microdialysis study in ischemic patients also showed that glu-