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ORIGINAL PAPER

Neuroprotective effects of lithium treatment following hypoxic–ischemic brain injury in neonatal rats

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Abstract
Purpose Increasing evidence indicates that lithium is a neuroprotective agent against transient focal and global ischemic injury in the adult animal. In the developing brain, lithium has shown protective effects against neuroapoptosis induced by drugs. This study was designed to investigate the neuroprotective effects of lithium on hypoxic–ischemic brain injury in the neonatal rat.
Methods Seven-day-old Sprague–Dawley rats underwent hypoxic–ischemic injury (HII) induced by ligation of the common carotid artery followed by exposure to ~2.5 h of hypoxia (~7% oxygen). After HII, rat pups were randomly assigned into two groups: a control group (n=21), which received a daily subcutaneous injection of 0.9% normal saline for 14 days following HII; and a lithium group (n=32), treated with daily injection of lithium chloride. *N*-acetylaspartate/creatinine, choline/creatinine, lipid/creatinine ratios at 1.3 ppm ($Lip_{1.3}$/Cr) and 0.9 ppm ($Lip_{0.9}$/Cr) lipid peaks were evaluated by proton magnetic resonance spectroscopy on the day of HII and on days 7 and 14 after HII. Infarct ratios based on magnetic resonance images were also determined at the same time points.
Results Seven days after HII, the $Lip_{1.3}$/Cr and $Lip_{0.9}$/Cr ratios as well as the infarct ratio were significantly lower in the lithium group than in the control group. The $Lip_{1.3}$/Cr and $Lip_{0.9}$/Cr ratios were significantly correlated with infarct ratio.
Conclusion This study showed that post-HII treatment with lithium may have a neuroprotective effect in the immature brain. Further studies are needed to elucidate the mechanism of neuroprotective properties of lithium against HII-induced neonatal brain damage.

Keywords Hypoxic–ischemic injury · Lithium · Magnetic resonance spectroscopy · Neuroprotection

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Introduction

Hypoxic–ischemic injury (HII) during perinatal or neonatal stages impairs brain maturation, resulting in permanent neurologic sequelae. These serious complications include motor and learning disabilities, cerebral palsy, epilepsy, and even death [26]. In this context, prompt diagnosis and treatment is essential in rescuing the immature brain from HII. Proton magnetic resonance spectroscopy (^{1}H-MRS), which has been used for studying perinatal cerebral energy metabolism in vivo, may be useful as a real-time tool for localizing and monitoring the extent and evolution of brain HII [10, 15, 19, 32]. Moreover, it has been shown that the ^{1}H-MRS technique is capable of visualizing changes in cerebral metabolites throughout the execution of the apoptotic process [14, 25], proposed as therapeutic targets for neuroprotection following HII.

Lithium, a classic drug for the treatment of bipolar disorder, has been shown to possess neuroprotective properties, protecting against apoptosis-induced neuronal death, including aluminum-induced apoptosis [8], glutamate-induced excitotoxicity [16], and transient cerebral ischemia [1, 41, 42] in mature brain models of hypoxic or ischemic injury. It has been suggested that treatment with lithium provides protection against drug-induced neuronal apoptosis in an immature rodent model [36, 44]. Recently, it has also been proposed that lithium has neuroprotective properties by enhanced proliferation of neuronal cells and reduced apoptosis following neonatal HII [23, 24].

In the present study, we investigated the neuroprotective effect of lithium on HII in the neonatal rat brain using the Rice–Vannucci model. To analyze the neuroprotective effects of lithium, we used a morphologic approach based on magnetic resonance imaging (MRI) and ^{1}H-MRS that allows noninvasive evaluation of cerebral energy metabolism.

Materials and methods

Hypoxic–ischemic model of neonatal rats

This animal study was approved by the Animal Care and Use Committee of Ulsan University. Male, 7-day-old Sprague–Dawley rats (12–16 g) were used in the study. The rat brain at postnatal day 7 is known to be histologically similar to that of a 32- to 34-week gestation human fetus or newborn infant. The characteristic features include completion of cerebral cortical layering, involution of germinal matrix, and the presence of little myelinated white matter [40]. HII was induced using the previously described Rice–Vannucci model [39]. Briefly, rat pups were anesthetized with 1–2 vol.% isoflurane in oxygen through a nose cone. A cervical midline incision was made, and the right common carotid artery was ligated with 5-0 surgical silk. All rat pups were exposed to isoflurane within 5 min. After a 1-hour recovery period, rat pups were placed in an acrylic hypoxic chamber for 2.5 h. The oxygen concentration was maintained at ~7% and was monitored with an OX-90 oxygen monitor (Atom Medical, Tokyo, Japan). The temperature in the hypoxic chamber was maintained at 37°C using a heat lamp. Exhaled carbon dioxide was eliminated using a carbon dioxide absorbent placed within the breathing circuit. After HII, the surviving pups were returned to the dam.

Drug treatment

Following HII, the rat pups were randomly assigned into two groups: the control group (n=21) and the lithium group (n=32). The rats in the lithium group were treated daily with a subcutaneous injection of 1 mmol/kg of lithium chloride (Sigma-Aldrich, Seoul, Korea) dissolved in 0.9% normal saline (6 mg/ml). Lithium treatment was initiated immediately after HII and was maintained for 14 consecutive days. The rats in the control group received an injection of the same volume of 0.9% normal saline.

MRI and ^{1}H-MRS

The rats underwent MRI 7 and 14 days after HII. MRI scans were obtained using a 4.7-T Bruker Biospec imager (Bruker Biospin, Rheinstetten, Germany). The rats were anesthetized with 1–2 vol.% isoflurane during MRI scanning, and body temperature was maintained by warming the magnet bore. The imaging protocol included a T2-weighted image sequence (time to repetition=4,500 ms; time to echo=12 ms; slice thickness=1.5 mm) and a diffusion-weighted image sequence (time to repetition=2,000 ms; time to echo=12 ms).

Baseline ^{1}H-MRS was performed on the day of HII. Repeated ^{1}H-MRS measures were made 7 and 14 days after HII. ^{1}H-MRS spectra were acquired through a signal voxel ($3\times3\times3$ mm^3) in the right hippocampal region of the brain, which is an area commonly involved in HII [34, 39]. Spin acquisition mode was as follows: spectral width=5,000 Hz; 128 acquisitions; time to repetition=3,000 ms; time to echo=30 ms. ^{1}H spectra were analyzed using *X*WIN-NMR hardware and software (Bruker Biospin, Rheinstetten, Germany). The relative sizes of the metabolite peaks were compared semi-quantitatively. The volumes of *N*-acetylaspartate (NAA) and creatinine (Cr) peaks were measured at 2.02 and 3.03 ppm, respectively, and lipid (Lip) peaks were measured at 0.9 and 1.3 ppm. The choline-containing compounds (Cho) peak was estimated at a chemical shift of 3.2 ppm. Cho/Cr, NAA/Cr, $Lip_{0.9}$/Cr, and $Lip_{1.3}$/Cr peak ratios were calculated. Cr concentrations are less changeable than those of other metabolites between birth at full term and 1–2 years; therefore, we used the Cr peak as the internal reference for each rat to allow comparison of metabolite levels over time and among rats [22].

MRI analysis of infarct ratio

Measurements of infarct ratio (IR) were made on days 7 and 14 after HII based on the specific signal intensity on T2-weighted images. MRI analyses were performed using image analysis software (ParaVision 3.02, Bruker Biospin, Rheinstetten, Germany), and the boundaries of the infarct area were tracked manually using the "region of interest" tool [37]. The infarct area was calculated by an indirect method to avoid underestimation as a result of parenchymal

shrinkage. Briefly, the infarct area of the injured hemisphere was determined by subtracting the noninfarcted area of the injured hemisphere from the total area of the uninjured hemisphere. This area on each slice was then multiplied by the slice thickness, and the values were summed to yield the total volume. The IR for each pup was calculated as follows [38]: IR (%)=100×(total volume of infarct area/total volume of uninjured hemisphere). The IR is thought to be correlated with the infarct size [17]. This analysis was performed by a researcher blinded to the identity of the groups.

Morphologic scores and hematoxylin–eosin staining

Fourteen days after HII, all rat pups were anesthetized with isoflurane and perfused with 2.5 ml/g of normal saline containing 2 unit/ml heparin at 4°C, followed by the same volume of 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. After perfusion, the brain was removed and postfixed overnight in the same paraformaldehyde–phosphate buffer.

We assessed the morphology of the rat brain by scoring individual brains on a scale of 0 to 4 based on the gross morphologic appearance of the right hemisphere [31]. A score of 0 indicated no size difference between the two hemispheres. A score of 1 indicated the right side was smaller than the left side, but there was no visible infarct. A score of 2 was given when there was a difference in hemisphere size and a cystic change due to a slight infarct. A score of 3 was given when there was a marked discrepancy in size, with some preservation of the right hemisphere. Finally, a score of 4 indicated an extensive infarct with almost total destruction of the right hemisphere. The examiner was blinded to the groups.

After morphologic scoring, 2-mm-thick coronal brain sections were obtained 2 mm posterior to the optic chiasm and embedded in paraffin. Four-micron-thick sections were stained with hematoxylin and eosin (HE). An image of each slide was collected by digital photography for representative examples.

Statistics

The normality of the data distributions was assessed with the Kolmogorov–Smirnov test. Normally distributed data were expressed as means±SDs and where skewed as median values with interquatile ranges. Between-group differences in Cho/Cr, NAA/Cr, $Lip_{0.9}$/Cr, and $Lip_{1.3}$/Cr ratios were analyzed using unpaired *t* tests or Mann–Whitney rank–sum tests. A one-way repeated measures analysis of variance with the Holm–Sidak method for multiple pairwise comparisons was used to assess within-group differences in ^{1}H-MRS data. Differences in morphologic scores and IR between two groups were compared using Mann–Whitney rank–sum tests. Correlations between ^{1}H-MRS spectra and IRs were evaluated using nonlinear regression analysis. Correlations between ^{1}H-MRS spectra and morphologic scores were also analyzed by Spearman correlation. Values of $P<0.05$ were considered statistically significant. Sigmastat 3.10 software (Systat Software Inc., Richmond, CA, USA) was used for all statistical analyses.

Results

^{1}H-MRS

Fourteen days after HII, the Cho/Cr ratio was significantly decreased in all groups. The NAA/Cr ratio of the lithium group (0.95±0.22) was significantly increased ($P=0.009$) 14 days after HII compared with the baseline value (0.81±0.20). The $Lip_{1.3}$/Cr and $Lip_{0.9}$/Cr ratios of the control group were significantly increased 7 days after HII compared with baseline values ($P<0.001$ and $P=0.013$, respectively), whereas those of the lithium group were not increased. At 7 days after HII, the $Lip_{1.3}$/Cr ratio in the lithium group [1.43 (0.84–1.94)], [median (interquatile ranges)], was significantly lower ($P=0.004$) than that in the control group [2.20 (1.77–4.06)]. At 7 days after HII, the $Lip_{0.9}$/Cr ratio in the lithium group [1.01 (0.67–1.61)] was also significantly lower ($P=0.021$) than that in the control group [1.64 (1.13–2.36)]. $Lip_{0.9}$/Cr ratio of the control group remained elevated 14 days after HII. There were no significant differences in the Cho/Cr or NAA/Cr ratios between the two groups (Figs. 1 and 2).

IR and correlations

Seven days after HII, the IR in the lithium group [53.8 (43.0–61.9)%] was significantly lower ($P=0.004$) than that in the control group [64.0 (57.3–68.5)%]. The IR in the lithium group [55.5 (40.4–63.7)%] was also significantly lower ($P=0.014$) than that in the control group [67.0 (56.7–69.2)%] 14 days after HII (Fig. 3). The $Lip_{1.3}$/Cr and $Lip_{0.9}$/Cr ratios were significantly correlated with the IR 7 days after HII ($R^2=0.95$ and $R^2=0.97$, respectively; $P<0.0001$ for both). The correlation data could be fit to an exponential rise-to-max curve (Fig. 4).

Morphologic score and HE staining

The mean morphologic score of the lithium group [3.0 (3.1–4.0)] was significantly lower ($P=0.016$) than that of the control group [4.0 (3.0–4.0)]. The $Lip_{1.3}$/Cr and $Lip_{0.9}$/

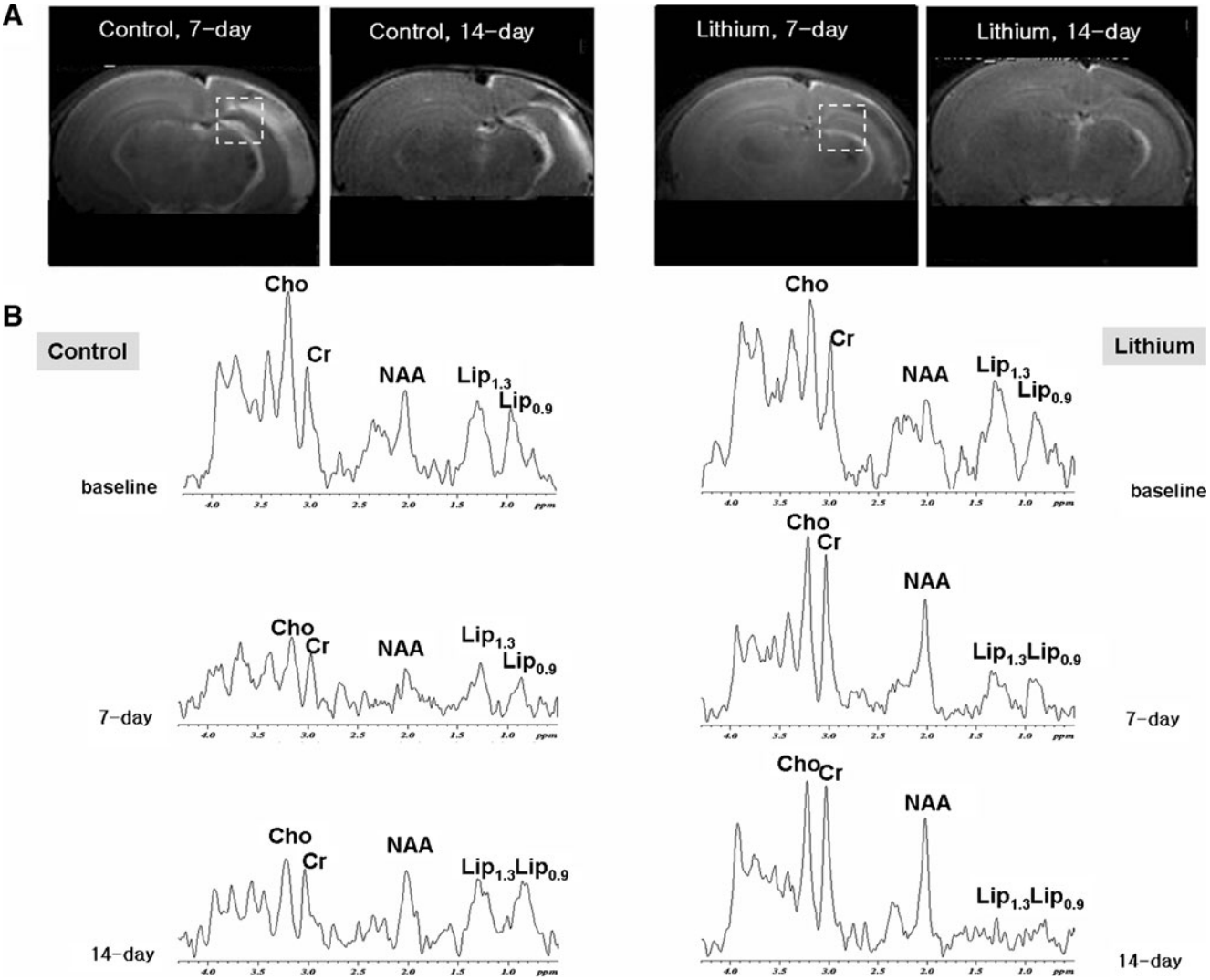

Fig. 1 Magnetic resonance spectroscopy (MRI) and proton magnetic resonance spectroscopy (^{1}H-MRS) spectra. **a** Representative MRI showing that infarct extent in a rat brain in the lithium group is slightly less than that in the control group 7 and 14 days after HII. **b** Representative ^{1}H-MRS spectra showing that lipid peaks at 1.3 ppm ($Lip_{1.3}$) and 0.9 ppm ($Lip_{0.9}$) relative to creatinine (Cr) are lower in the lithium group 7 days after HII than in the control group. *Dash boxes* indicate the volume of voxel including the hippocampal region. *Cho*, choline-containing compounds; *NAA*, *N*-acetylaspartate

Cr ratios were correlated with morphologic scores (R=0.259, P=0.066 and R=0.286, P=0.042, respectively). The HE staining of rat brains 14 days after HII revealed that sections in the lithium group were morphologically more preserved than those in the control group (Fig. 5).

Discussion

In the present study, we found that the $Lip_{1.3}$/Cr and $Lip_{0.9}$/Cr ratios of rats treated with lithium following HII were significantly lower than those in the control group 7 days after HII. The IR in the lithium group after HII was also significantly lower than that in the control group. These findings suggest that treatment with lithium after HII may have a neuroprotective effect against HII-induced neuronal injury in the neonatal rat brain.

To assess the neuroprotective effects of lithium treatment, we performed ^{1}H-MRS, which allowed us to measure the levels of cerebral metabolites and integrity in the brain [12, 20, 33]. ^{1}H-MRS is a versatile technique with noninvasive and real-time properties; thus, it provides useful information on brain metabolic processes in living tissue and can distinguish biochemical events associated with different neurologic conditions. Using this technique, we were able to obtain the metabolite ratios, Cho/Cr, NAA/Cr, $Lip_{0.9}$/Cr, and $Lip_{1.3}$/Cr.

The two peaks in spectra—$Lip_{1.3}$ and $Lip_{0.9}$—arising from macromolecular compounds underwent major intensity changes, which may have been caused by altered

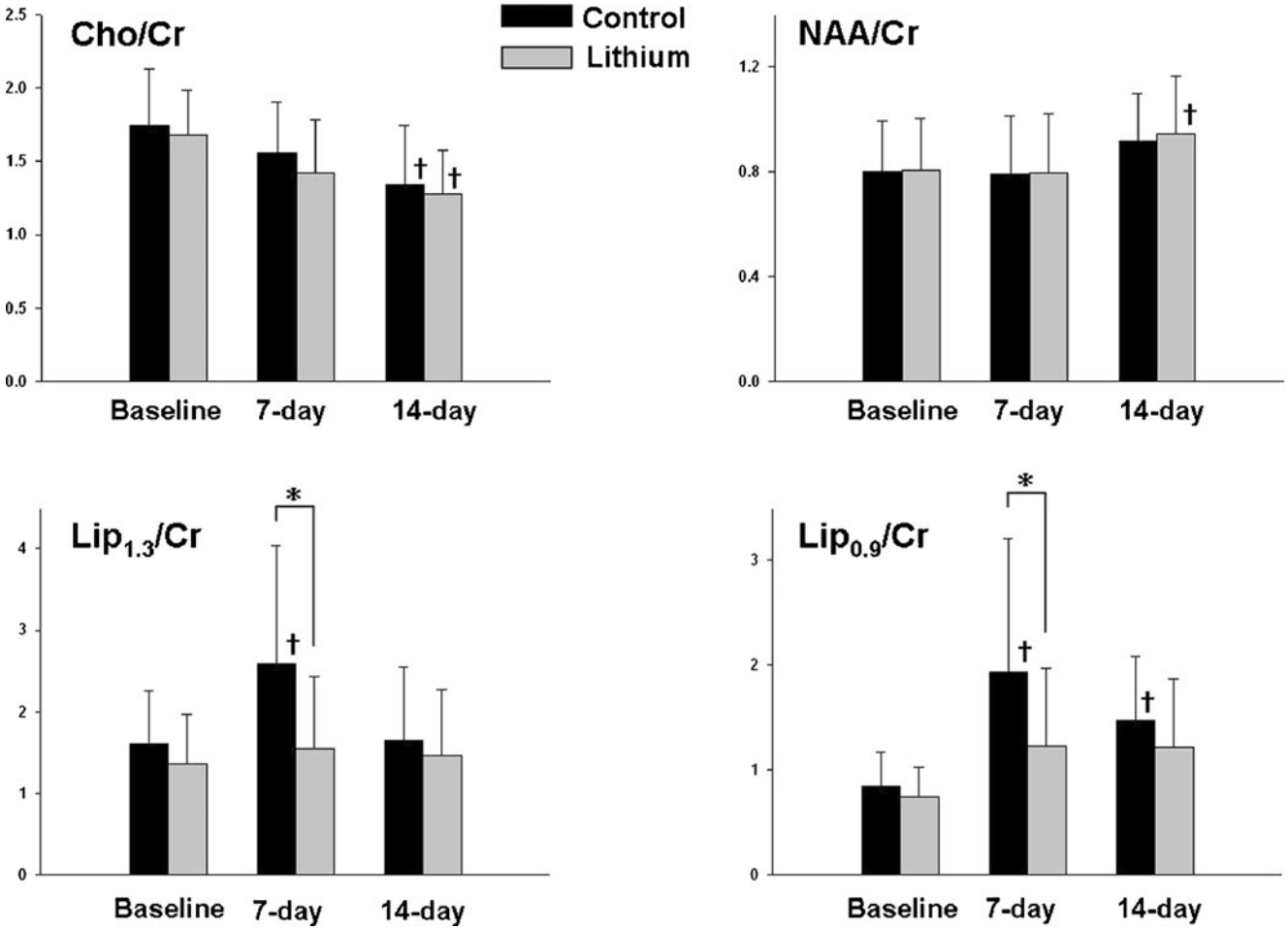

Fig. 2 Comparison of lithium and control groups using proton magnetic resonance spectroscopy. Lipid/Cr ratios at lipid peaks of 1.3 ppm (*Lip$_{1.3}$/Cr*) and 0.9 ppm (*Lip$_{0.9}$/Cr*) in the lithium group 7 days after hypoxic–ischemic injury are significantly lower than those in the control group. There are no significant differences in the *Cho/Cr* or *NAA/Cr* ratios between the two groups 7 and 14 days after HII. Data are presented as means±SDs (*P<0.05 between two groups; † P<0.05 compared with baseline in the same group). Cho, choline-containing compounds; NAA, *N*-acetylaspartate

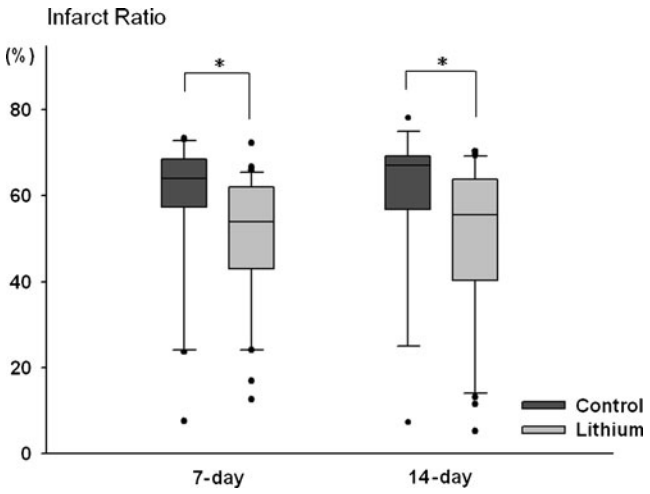

Fig. 3 Effect of lithium treatment on infarct ratio (IR). IRs on days 7 and 14 after hypoxic–ischemic injury are significantly lower in the lithium group than in the control group. The *box* indicates the interquatile ranges, and the *solid bar* indicates the median. The *error bars* represent the 5th and 95th percentiles. (*P<0.05 between two groups)

packing of the lipid bilayer, membrane blebbing, and/or decreased membrane microviscosity during cell breakdown [14]. The consequent decrease in membrane microviscosity and increase in cytoplasmic lipid droplets results in large methylene (Lip$_{1.3}$) and methyl (Lip$_{0.9}$) resonances. It has been shown that detection of Lip$_{0.9}$ and Lip$_{1.3}$ peaks by ^{1}H-MRS can be used to assess apoptosis and proliferation of tumor cells [2, 13, 27]. After ischemic brain injury, lipid signals on ^{1}H-MRS have also been reported to increase in the brain [7, 15, 35]. Our results showed significant correlations between Lip$_{0.9}$/Cr and Lip$_{1.3}$/Cr ratios and IR 7 days after HII, suggesting that lipid signals on ^{1}H-MRS may reflect the degree of overall neuronal injury following HII in the neonatal rat brain.

It is known that Cho is required as a substrate for the formation of cell membranes. In the present study, the Cho/Cr ratios of both lithium and control groups decreased 14 days after HII, indicating neuronal maturation in the developing brain [9]. The NAA/Cr ratio has been proposed

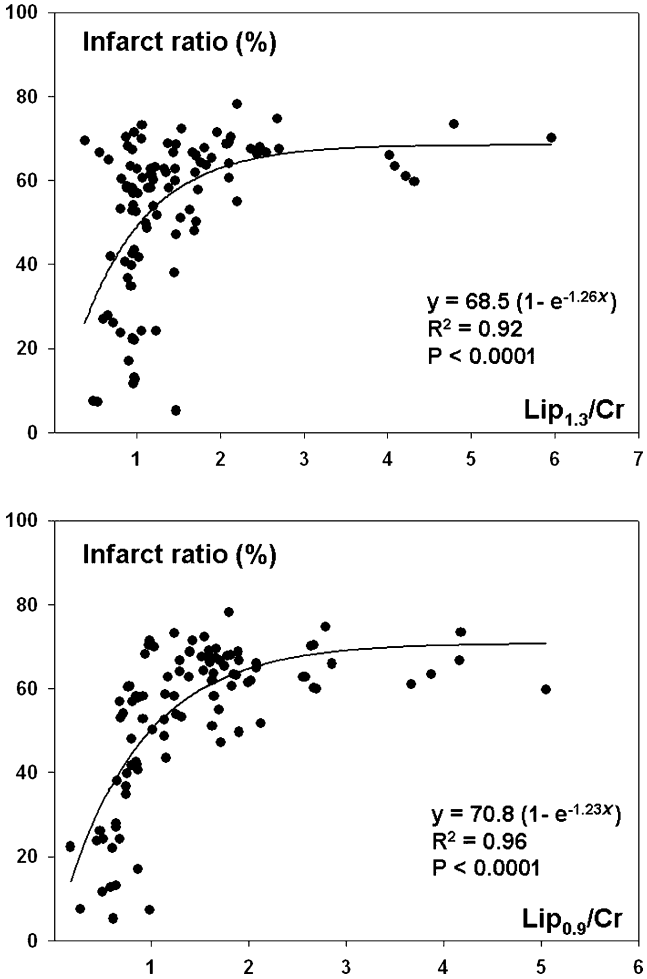

Fig. 4 Correlations between proton magnetic resonance spectroscopy spectra and infarct ratios (IR). The lipid/Cr ratios at lipid peaks of 1.3 ppm ($Lip_{1.3}/Cr$) and 0.9 ppm ($Lip_{0.9}/Cr$) are significantly correlated with the IR, and could be fit to an exponential rise-to-max curve

as an indicator of intact central nervous tissue in the neonatal brain injury; therefore, a decrease in NAA signals early after HII can be interpreted as destruction of normal brain tissue [18]. Consistent with the results of a previous study [12], we also found that the absolute baseline values of the NAA/Cr ratio decreased after HII. However, the NAA/Cr ratio in the lithium group significantly increased 14 days after HII compared with the baseline value. We postulate that the increase in NAA/Cr may be due to advanced gray matter development, including active myelination during maturation in the intact brain tissue [4]. Despite this increase in the lithium group, we found no significant differences in the NAA/Cr ratios between the two groups in this study. It is possible that this absence of a detectable difference in NAA/Cr ratios is a false-negative finding, caused by the small sized, single voxel ($3\times3\times3$ mm^3) used for ^{1}H-MRS measurement.

Lithium pretreatment has been reported to have neuroprotective effects, including reducing the cerebral infarct area and improving functional behavioral outcomes in an adult rat model of cerebral ischemia [1, 41, 43]. It has been reported that lithium has antiapoptotic properties, which can be explained by inhibition of *N*-acetyl-D-aspartate receptors [28], regulation of extracellular-regulated kinase (ERK) and Akt pathways [42], and alteration of gene expression, including upregulation of Bcl-2, heat shock protein 70 (HSP70) [1], and activator protein-1 (AP-1) [41] and downregulation of p53 and Bax [8]. In the developing brain, lithium has also been shown to protect against drug-induced neuroapoptosis [36, 44]. However, the neuroprotective effect of lithium treatment following HII in an immature brain remains unclear. In particular, therapeutic strategies based on pretreatment with neuroprotective agents are of limited value and would be difficult to test experimentally because chronic pretreatment in a 7-day neonatal rat model is impossible. In this regard, the significance of this study lies in its emphasis on the importance of post-HII lithium treatment in the immature brain.

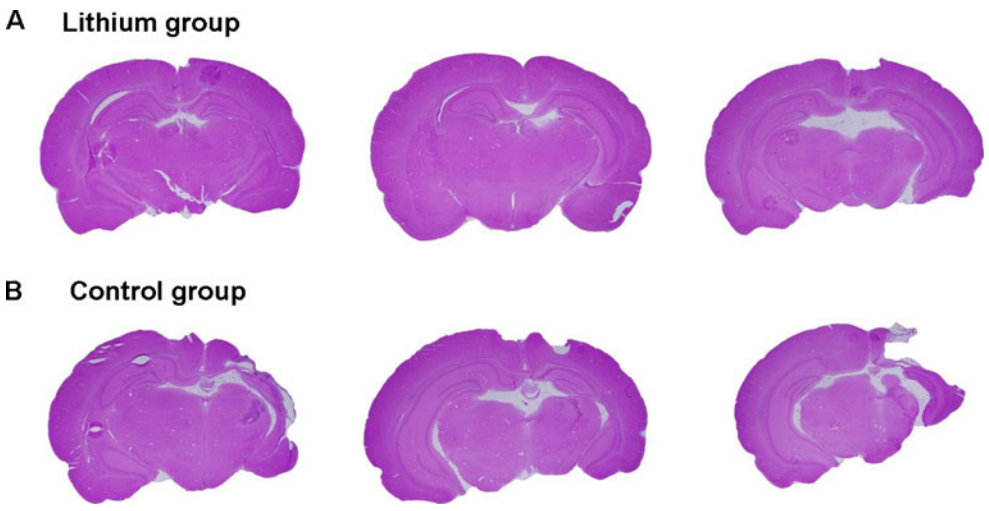

Fig. 5 Representative hematoxylin–eosin stained slides from six rat brains 14 days after hypoxic–ischemic injury. The *upper three coronal sections* from the lithium group (**a**) are morphologically more preserved than the *three lower sections* from the control group (**b**)

Although not directly addressed here, it is possible to speculate on possible mechanisms underlying the effectiveness of post-HII lithium treatment. Recent studies have demonstrated that the mitogen-activated protein kinase/ERK pathway has a role in the antiapoptotic effects of lithium against drug-induced neuroapoptosis in the developing brain [36, 44]. In the neonatal HII model, it has been reported that lithium may reduce apoptosis-inducing factor and inhibit postischemic autophagy [24]. They have also demonstrated that post-HII treatment with lithium reduces ischemia-induced brain damage in the neonatal rat by inhibiting inflammation and promoting neuronal stem/progenitor cell proliferation [23]. In addition to its effects on antiapoptotic processes, lithium may influence vascular formation. It has been shown that lithium contributes to the promotion of neurovascular remodeling after ischemic injury by promoting vascular endothelial growth factor expression [11]. Another study demonstrated that post-HII treatment with lithium enhanced cerebral blood oxygenation in an animal model of stroke [21].

Numerous studies have shown that lithium must be chronically administered to achieve its therapeutic effects [5, 28, 29]. Omata et al. [30] confirmed this for the neuroprotective effects, demonstrating that chronic, but not acute, treatment with lithium protects against the neurotoxic effects of hypoxia. On the basis of these reports, we administered lithium for 14 days in the present study. However, it is important to consider the narrow therapeutic index of lithium and the serious adverse effects that can be associated with its use, including lethargy, muscular weakness, confusion, seizure, renal insufficiency, and in some cases, death [6]. We did not directly measure serum lithium concentrations in rat pups, so we chose a low dose of 1 mmol/kg lithium [41, 42], which has been reported to achieve serum levels similar to the therapeutic lithium concentration required for the treatment of bipolar disorder in humans [41, 43]. Further study will be needed to determine an optimal dosing of lithium in neonatal rats.

Some limitations of this study should be noted. First, we could not confirm the predicted effects of lithium on apoptosis using histologic approaches, such as TdT-mediated dUTP-biotin nick end-labeling staining, because apoptotic cells induced by HII would not likely still be present at the end of the chronic 14-day treatment period used here [3]. Second, we did not evaluate functional behavioral effects. Therefore, further studies will be necessary to determine the mechanisms by which post-HII treatment with lithium exerts its antiapoptotic effects in the neonatal HII model and assess long-term outcomes. Finally, further studies are also needed to determine the acute neuroprotective effects of various dosages of lithium in the neonatal rat model of HII.

Using ^{1}H-MRS and morphological analysis, we demonstrated that lithium treatment following HII reduced markers of neuronal injury and the extent of gross infarct in neonatal rats. In conclusion, the present study suggests that treatment with lithium after HII might provide neuroprotective effects in a neonatal rat model, although beneficial effects on apoptosis were not clearly shown. It will be important to perform further studies to confirm the antiapoptotic properties of lithium and to characterize the long-term effects of lithium treatment after the HII-induced neonatal brain damage.

Disclosure statement The authors declare that they have no conflicts of interest.

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References

1. Bian Q, Shi T, Chuang DM, Qian Y (2007) Lithium reduces ischemia-induced hippocampal CA1 damage and behavioral deficits in gerbils. Brain Res 1184:270–276
2. Blankenberg FG, Storrs RW, Naumovski L, Goralski T, Spielman D (1996) Detection of apoptotic cell death by proton nuclear magnetic resonance spectroscopy. Blood 87:1951–1956
3. Brauer M (2003) In vivo monitoring of apoptosis. Prog Neuropsychopharmacol Biol Psychiatry 27:323–331
4. Cady EB, Penrice J, Amess PN, Lorek A, Wylezinska M, Aldridge RF, Franconi F, Wyatt JS, Reynolds EO (1996) Lactate, *N-acetylaspartate, choline and creatine concentrations, and spin–spin relaxation in thalamic and occipito–parietal regions of developing human brain. Magn Reson Med 36:878–886*
5. Calabresi P, Pisani A, Mercuri NB, Bernardi G (1993) Lithium treatment blocks long-term synaptic depression in the striatum. Neuron 10:955–962
6. Finley PR, Warner MD, Peabody CA (1995) Clinical relevance of drug interactions with lithium. Clin Pharmacokinet 29:172–191
7. Gasparovic C, Rosenberg GA, Wallace JA, Estrada EY, Roberts K, Pastuszyn A, Ahmed W, Graham GD (2001) Magnetic resonance lipid signals in rat brain after experimental stroke correlate with neutral lipid accumulation. Neurosci Lett 301:87–90
8. Ghribi O, Herman MM, Spaulding NK, Savory J (2002) Lithium inhibits aluminum-induced apoptosis in rabbit hippocampus, by preventing cytochrome c translocation, Bcl-2 decrease, Bax elevation and caspase-3 activation. J Neurochem 82:137–145
9. Girard N, Confort-Gouny S, Schneider J, Barberet M, Chapon F, Viola A, Pineau S, Combaz X, Cozzone P (2007) MR imaging of brain maturation. J Neuroradiol 34:290–310
10. Groenendaal F, Veenhoven RH, van der Grond J, Jansen GH, Witkamp TD, de Vries LS (1994) Cerebral lactate and *N*-acetylaspartate/choline ratios in asphyxiated full-term neonates demonstrated in vivo using proton magnetic resonance spectroscopy. Pediatr Res 35:148–151
11. Guo S, Arai K, Stins MF, Chuang DM, Lo EH (2009) Lithium upregulates vascular endothelial growth factor in brain endothelial cells and astrocytes. Stroke 40:652–655

12. Gwak M, Park P, Kim K, Lim K, Jeong S, Baek C, Lee J (2008) The effects of dantrolene on hypoxic–ischemic injury in the neonatal rat brain. Anesth Analg 106:227–233
13. Hakumaki JM, Poptani H, Sandmair AM, Yla-Herttuala S, Kauppinen RA (1999) 1H MRS detects polyunsaturated fatty acid accumulation during gene therapy of glioma: implications for the in vivo detection of apoptosis. Nat Med 5:1323–1327
14. Hakumaki JM, Brindle KM (2003) Techniques: visualizing apoptosis using nuclear magnetic resonance. Trends Pharmacol Sci 24:146–149
15. Harada K, Honmou O, Liu H, Bando M, Houkin K, Kocsis JD (2007) Magnetic resonance lactate and lipid signals in rat brain after middle cerebral artery occlusion model. Brain Res 1134:206–213
16. Hashimoto R, Hough C, Nakazawa T, Yamamoto T, Chuang DM (2002) Lithium protection against glutamate excitotoxicity in rat cerebral cortical neurons: involvement of NMDA receptor inhibition possibly by decreasing NR2B tyrosine phosphorylation. J Neurochem 80:589–597
17. Houkin K, Nakayama N, Kamada K, Noujou T, Abe H, Kashiwaba T (1998) Neuroprotective effect of the free radical scavenger MCI-186 in patients with cerebral infarction: clinical evaluation using magnetic resonance imaging and spectroscopy. J Stroke Cerebrovasc Dis 7:315–322
18. Huppi PS, Lazeyras F (2001) Proton magnetic resonance spectroscopy ((1)H-MRS) in neonatal brain injury. Pediatr Res 49:317–320
19. Juranek I, Baciak L (2009) Cerebral hypoxia–ischemia: focus on the use of magnetic resonance imaging and spectroscopy in research on animals. Neurochem Int 54:471–480
20. Kim KS, Park SJ, Lim KH, Kim EJ, Lee JH, Pi SY (2000) In vivo ^{1}H MR spectroscopic analysis of apoptosis in hypoxic-ischemic newborn rats. Proc Intl Soc Mag Reson Med 8:1090
21. Kim YR, van Meer MP, Tejima E, Murata Y, Mandeville JB, Dai G, Chuang DM, Rosen BR, Lo EH (2008) Functional MRI of delayed chronic lithium treatment in rat focal cerebral ischemia. Stroke 39:439–447
22. Kreis R, Hofmann L, Kuhlmann B, Boesch C, Bossi E, Huppi PS (2002) Brain metabolite composition during early human brain development as measured by quantitative in vivo 1H magnetic resonance spectroscopy. Magn Reson Med 48:949–958
23. Li H, Li Q, Du X, Sun Y, Wang X, Kroemer G, Blomgren K, Zhu C (2011) Lithium-mediated long-term neuroprotection in neonatal rat hypoxia–ischemia is associated with antiinflammatory effects and enhanced proliferation and survival of neural stem/progenitor cells. J Cereb Blood Flow Metab 31:2106–2115
24. Li Q, Li H, Roughton K, Wang X, Kroemer G, Blomgren K, Zhu C (2010) Lithium reduces apoptosis and autophagy after neonatal hypoxia–ischemia. Cell Death Dis 1:e56
25. Lindskog M, Kogner P, Ponthan F, Schweinhardt P, Sandstedt B, Heiden T, Helms G, Spenger C (2003) Noninvasive estimation of tumour viability in a xenograft model of human neuroblastoma with proton magnetic resonance spectroscopy (1H MRS). Br J Cancer 88:478–485
26. Lorenz JM, Wooliever DE, Jetton JR, Paneth N (1998) A quantitative review of mortality and developmental disability in extremely premature newborns. Arch Pediatr Adolesc Med 152:425–435
27. Mikhailenko VM, Philchenkov AA, Zavelevich MP (2005) Analysis of 1H NMR-detectable mobile lipid domains for assessment of apoptosis induced by inhibitors of DNA synthesis and replication. Cell Biol Int 29:33–39
28. Nonaka S, Chuang DM (1998) Neuroprotective effects of chronic lithium on focal cerebral ischemia in rats. Neuroreport 9:2081–2084
29. Nonaka S, Hough CJ, Chuang DM (1998) Chronic lithium treatment robustly protects neurons in the central nervous system against excitotoxicity by inhibiting *N*-methyl-D-aspartate receptor-mediated calcium influx. Proc Natl Acad Sci U S A 95:2642–2647
30. Omata N, Murata T, Takamatsu S, Maruoka N, Mitsuya H, Yonekura Y, Fujibayashi Y, Wada Y (2008) Neuroprotective effect of chronic lithium treatment against hypoxia in specific brain regions with upregulation of cAMP response element binding protein and brain-derived neurotrophic factor but not nerve growth factor: comparison with acute lithium treatment. Bipolar Disord 10:360–368
31. Palmer C, Vannucci RC, Towfighi J (1990) Reduction of perinatal hypoxic–ischemic brain damage with allopurinol. Pediatr Res 27:332–336
32. Peden CJ, Rutherford MA, Sargentoni J, Cox IJ, Bryant DJ, Dubowitz LM (1993) Proton spectroscopy of the neonatal brain following hypoxic–ischaemic injury. Dev Med Child Neurol 35:502–510
33. Pi SY, Kim KS, Yoon KH, Yoon HS, Lim KH, Kim EJ, Lee JH (2000) Investigation of hypoxic-ischemic injured newborn rat brain by in vivo ^{1}H MR spectroscopy. Proc Intl Soc Mag Reson Med 8:1093
34. Rice JE 3rd, Vannucci RC, Brierley JB (1981) The influence of immaturity on hypoxic–ischemic brain damage in the rat. Ann Neurol 9:131–141
35. Serrai H, Senhadji L, Wang G, Akoka S, Stroman P (2003) Lactate doublet quantification and lipid signal suppression using a new biexponential decay filter: application to simulated and 1H MRS brain tumor time-domain data. Magn Reson Med 50:623–626
36. Straiko MM, Young C, Cattano D, Creeley CE, Wang H, Smith DJ, Johnson SA, Li ES, Olney JW (2009) Lithium protects against anesthesia-induced developmental neuroapoptosis. Anesthesiology 110:862–868
37. Strbian D, Tatlisumak T, Ramadan UA, Lindsberg PJ (2007) Mast cell blocking reduces brain edema and hematoma volume and improves outcome after experimental intracerebral hemorrhage. J Cereb Blood Flow Metab 27:795–802
38. Swanson RA, Morton MT, Tsao-Wu G, Savalos RA, Davidson C, Sharp FR (1990) A semiautomated method for measuring brain infarct volume. J Cereb Blood Flow Metab 10:290–293
39. Vannucci RC, Vannucci SJ (1997) A model of perinatal hypoxic–ischemic brain damage. Ann N Y Acad Sci 835:234–249
40. Vannucci RC, Vannucci SJ (2005) Perinatal hypoxic–ischemic brain damage: evolution of an animal model. Dev Neurosci 27:81–86
41. Xu J, Culman J, Blume A, Brecht S, Gohlke P (2003) Chronic treatment with a low dose of lithium protects the brain against ischemic injury by reducing apoptotic death. Stroke 34:1287–1292
42. Yan XB, Hou HL, Wu LM, Liu J, Zhou JN (2007) Lithium regulates hippocampal neurogenesis by ERK pathway and facilitates recovery of spatial learning and memory in rats after transient global cerebral ischemia. Neuropharmacology 53:487–495
43. Yan XB, Wang SS, Hou HL, Ji R, Zhou JN (2007) Lithium improves the behavioral disorder in rats subjected to transient global cerebral ischemia. Behav Brain Res 177:282–289
44. Young C, Straiko MM, Johnson SA, Creeley C, Olney JW (2008) Ethanol causes and lithium prevents neuroapoptosis and suppression of pERK in the infant mouse brain. Neurobiol Dis 31:355–360