Thioperamide treats neonatal hypoxic-ischemic encephalopathy by postsynaptic H1 receptors

Feiyong Jia, Lin Du, Yunpeng Hao, Shicheng Liu, Ning Li, Huiyi Jiang

Division of Pediatric Neurorehabilitation, Department of Pediatrics, Second Part of First Hospital of Jilin University, Changchun 130031, Jilin Province, China

Research Highlights

(1) The mechanism underlying the ability of thioperamide, a selective histamine H3 receptor gonist, to improve neonatal hypoxic-ischemic encephalopathy was investigated to determine if this compound could be a novel therapy for this condition.
(2) A combined application of thioperamide with H1 and H2 receptor antagonists showed that the action of increased brain histamine was mediated through postsynaptic H1 receptors.

Abstract

Thioperamide, a selective histamine H3 receptor antagonist, can increase histamine content in the brain, improve brain edema, and exert a neuroprotective effect. This study aimed to examine the mechanism of action of thioperamide during brain edema in a rat model of neonatal hypoxic-ischemic encephalopathy. Our results showed that thioperamide significantly decreased brain water content and malondialdehyde levels, while significantly increased histamine levels and superoxide dismutase activity in the hippocampus. This evidence demonstrates that thioperamide could prevent oxidative damage and attenuate brain edema following neonatal hypoxic-ischemic encephalopathy. We further observed that changes in the above indexes occurred after combined treatment of thioperamide with the H1 receptor antagonist, pyrilamine, and the H2 receptor antagonist, tidine. Experimental findings indicated that pyrilamine reversed the effects of thioperamide; however, cimetidine had no significant influence on the effects of thioperamide. Our present findings suggest that thioperamide can increase brain histamine content and attenuate brain edema and oxidative damage by acting in combination with postsynaptic H1 receptors in a rat model of neonatal ic-ischemic encephalopathy.

Key Words

neural regeneration; thioperamide; histamine; histamine receptor; antagonist; cimetidine; pyrilamine; neonatal hypoxic-ischemic encephalopathy; brain edema; hippocampus; malondialdehyde; roxide dismutase; grants-supported paper; neuroregeneration

INTRODUCTION

Neonatal hypoxic-ischemic encephalopathy is a devastating condition for which effective therapeutic treatments are still unavailable[1-3]. There is a high risk for long term neurological sequelae, such as cerebral palsy, psychomotor retardation, and visual or auditory handicaps leading to long-term healthcare costs[4-6]. Brain edema is known to be triggered within hours after cerebral ischemic injuries[7-8]. Diuretics, such as glycerol and mannitol, are usually applied to patients with brain edema following cerebral ischemia[9]. Besides drug therapy, whole bo-

dy hypothermia for neonatal hypoxic-ischemic encephalopathy strongly attenuates cerebral edema[10-12]. However, adverse effects, such as infection, bleeding tendency, and arrhythmia, are problematic[13].

Histamine acts as a neurotransmitter or neuromodulator in the brain, and plays a major role in the pathogenic progression after cerebral ischemia[14-16]. Extracellular histamine increases gradually after ischemia, possibly coming from mast cells or histaminergic neurons[17]. Histamine promotes recovery of neurological function, and alleviates neuronal damage and infarct volume after ischemia, with the H1–3 receptors all being involved[18]. Further studies suggest that histamine alleviates excitotoxicity, suppresses the release of glutamate and dopamine, and inhibits inflammation and glial scar formation[19-23]. Histamine may also affect cerebral blood flow by targeting vascular smooth muscle cells, and promoting neurogenesis. Moreover, endogenous histamine is an essential mediator in cerebral ischemic tolerance. Because of its multiple actions affecting neurons, glia, vascular cells, and inflammatory cells, histamine is likely to be an important target in cerebral ischemia[24-26]. However, important questions surrounding the molecular aspects and pathophysiology of histamine, and related agents in cerebral ischemia, remain to be answered to form a solid scientific basis for therapeutic application. A previous study has shown that histamine H3 receptor antagonists can reduce ischemia-induced cerebral edema in adult rats[27]. However, the role of histamine in a rat model of neonatal hypoxic-ischemic encephalopathy has currently not been reported. On the basis of previous studies regarding the role of histamine in immature epileptic mice[28-30], we hypothesize that increasing brain histamine by using an histamine H3 antagonist will exert a protective role through postsynaptic H1 receptors. Through establishing a rat model of neonatal hypoxic-ischemic encephalopathy and using antagonists against histamine receptors H1–3, the role of brain histamine in neonatal hypoxic- ischemic encephalopathy and its mechanism of neuronal protection may be elucidated.

RESULTS

Quantitative analysis of experimental animals

A total of one hundred and twenty newborn rats modeling neonatal hypoxic-ischemic encephalopathy were successfully established and grouped into four groups: the model group (intraperitoneal injection of normal saline), the H3 group (intraperitoneal injection of H3 receptor antagonist thio- peramide), the H3 + H1 group (intraperitoneal injection of thioperamide and H1 receptor antagonist, pyrilamine), and the H3 + H2 group (intraperitoneal injection of thioperamide and H2 receptor antagonist, cimetidine), with thirty rat pups in each group. The rat pups were subdivided for further determinations at 6, 24 and 72 hours after neonatal hypoxic-ischemic encephalopathy had been established.

Effect of thioperamide on brain water content in neonatal hypoxic-ischemic encephalopathy rats

Results showed that brain water content decreased significantly in both the H3 group and H3 + H2 group when compared with the model group ($P < 0.05$). Brain water content was significantly higher in the H3 + H1 group than in the H3 group ($P < 0.05$); however, no significant difference was found between the H3 and H3 + H2 groups ($P > 0.05$). Meanwhile, brain water content in all groups peaked at 24 hours, then gradually decreased at 72 hours (Figure 1).

Effect of thioperamide on histamine content in the hippocampus of neonatal hypoxic-ischemic encephalopathy rats

Hippocampal histamine content was detected by high performance liquid chromatography. Results showed that the histamine content in the hippocampus was significantly higher in both the H3 and H3 + H2 groups compared with the model group ($P < 0.05$). Histamine content was also significantly higher in the H3 group compared with the H3 + H1 group ($P < 0.05$); however, no significant difference was found between the

H3 + H2 group and the H3 group (P > 0.05).

Statistical results at different time points showed a significant decrease of hippocampal histamine content at 24 hours in all groups when compared with those at 6 hours (P < 0.05). There was no significant difference in each group at 24 and 72 hours (Figure 2).
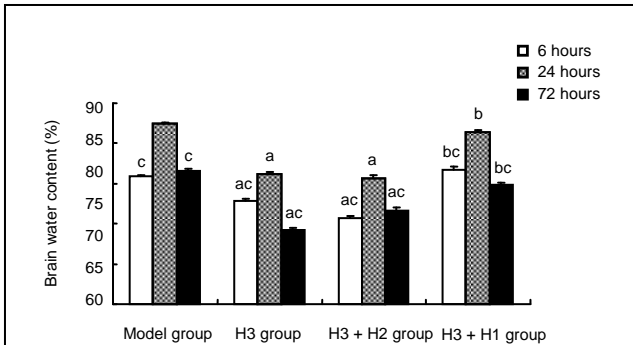

Figure 1   Effect of H3 receptor antagonist thioperamide on brain water content in neonatal hypoxic-ischemic encephalopathy rats.

Data are expressed as mean ± SD. Ten rats were used in each group at each time point. [a]P < 0.05, *vs.* model group; [b]P < 0.05, *vs.* H3 group using analysis of variance; [c]P < 0.05, *vs.* 24 hours in each group using the Student's *t*-test. Results showed that intraperitoneal injection of thioperamide reduced brain edema. The H1 receptor antagonist, pyrilamine, reversed the effects of thioperamide in the H3 + H1 group; however, the H2 receptor antagonist, cimetidine, had no significant influence on the effects of thioperamide. Brain water content (%) = (wet weight – dry weight)/wet weight × 100%.
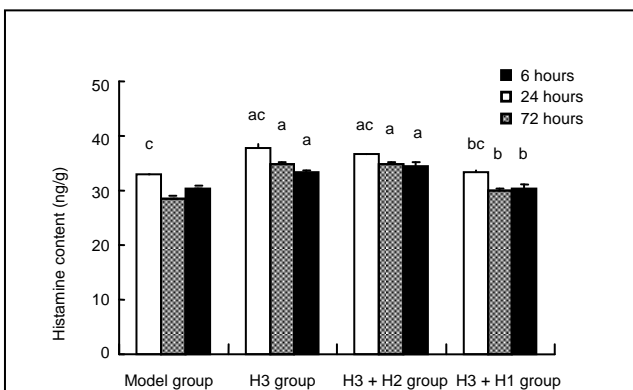

Figure 2   Effect of H3 receptor antagonist thioperamide on hippocampal histamine content in neonatal hypoxic-ischemic encephalopathy rats.

Data are expressed as mean ± SD. Ten rats were used in each group at each time point. [a]P < 0.05, *vs.* model group; [b]P < 0.05, *vs.* H3 group using analysis of variance; [c]P < 0.05, *vs.* 24 hours in each group using the Student's *t*-test. Results showed that intraperitoneal injection of thioperamide can increase hippocampal histamine content. The H1 receptor antagonist, pyrilamine, reversed the effects of thioperamide in the H3 + H1 group; however, the H2 antagonist, cimetidine, had no significant influence on the effects of thioperamide.

Effect of thioperamide on brain malondialdehyde content in neonatal hypoxic-ischemic encephalopathy rats

Results showed that malondialdehyde content in the brain was significantly decreased in the H3 group and H3 + H2 groups compared with the model group (P < 0.05). There was no significant difference in malondialdehyde content between the H3 + H2 group and the H3 group (P > 0.05); however, malondialdehyde in the H3 + H1 group was significantly higher than that in the H3 group (P < 0.05). Statistical results at different time points showed a significant increase at 24 hours in all groups compared with those at 6 and 72 hours (P < 0.05). Histamine content began to decrease at 72 hours (Figure 3).
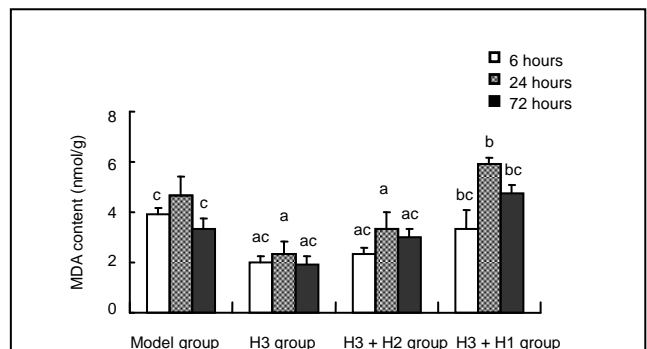
Figure 3   Effect of thioperamide on brain malondialdehyde (MDA) content in neonatal hypoxic-ischemic encephalopathy rats.

Data are expressed as mean ± SD. Ten rats were used in each group at each time point. [a]P < 0.05, *vs.* model group; [b]P < 0.05, *vs.* H3 group using analysis of variance; [c]P < 0.05, *vs.* 24 hours in each group using the Student's *t*-test. Results showed that intraperitoneal injection of thioperamide can decrease MDA content. The H1 receptor antagonist, pyrilamine, reversed the effects of thioperamide in the H3 + H1 group; however, the H2 receptor antagonist, cimetidine, had no significant influence on the effects of thioperamide.

Effect of thioperamide on brain superoxide dismutase activity in neonatal hypoxic-ischemic encephalopathy rats

Results showed that brain superoxide dismutase content was significantly higher in the H3 and H3 + H2 groups compared with the model group (P < 0.05). There was no significant difference in superoxide dismutase content between the H3 + H2 and the H3 groups (P > 0.05); however, superoxide dismutase content in the H3 + H1 group was significantly lower than that in the H3 group (P < 0.05). Results at different time points revealed a significant decrease in superoxide dismutase content at

24 hours, and a slight increase at 72 hours ($P < 0.05$; Figure 4).

Figure 4 Effect of H3 receptor antagonist thioperamide on brain superoxide dismutase (SOD) activity in the neonatal hypoxic-ischemic encephalopathy rats.

Data are expressed as mean ± SD. Ten rats were used in each group at each time point. [a]$P < 0.05$, *vs.* model group; [b]$P < 0.05$, *vs.* H3 group using analysis of variance; [c]$P < 0.05$, *vs.* 24 hours in each group using the Student's *t*-test. Results showed that intraperitoneal injection of thioperamide can increase SOD activity. The H1 receptor antagonist, pyrilamine, reversed the effects of thioperamide in the H3 + H1 group; however, the H2 antagonist, cimetidine, had no significant influence on the effects of thioperamide.

DISCUSSION

Neonatal hypoxic-ischemic encephalopathy is the most common disorder of inpatient newborns, and an important factor causing death and sequelae within the central nervous system in children[31-35]. Studies have shown that arachidonic acid oxidation involved with lipoxygenase and cyclooxygenase is the initial stage for neuronal damage after cerebral hypoxia[36-39]. Arachidonic acid oxidation leads to overproduction of oxygen free radicals, excitatory amino acids, nitrogen monoxide and inflammatory factors[40-44]. Lipid peroxidation and ion pump damage in the cell membrane lead to the influx of Na^+, Ca^{2+} and water[45-46]. The aforementioned changes cause neuronal edema, apoptosis and necrosis.

Previous studies have shown that cerebral ischemia in immature rats can lead to extensively severe brain damage in several days[47-53]. The inflammatory reaction and the immature neurons may create brain and blood-brain-barrier damage, and T lymphocytes, polymorphonuclear granulocytes and microglia take part in the inflammatory response during focal cerebral ischemia. During development, brain histamine and the number of mast cells change dramatically. Mast cells can be found in the cerebral choroid plexus in embryonic rats

and increase gradually after birth as a histamine source. Brain histamine is high in 15-day-old embryonic rats, but then decreases. However, the amount of histamine is increased after birth and reaches its peak at 2 weeks of age[54]. Histamine plays a significant role in cerebral neuronal proliferation and growth.

In our present study, four indexes have been used for assessing neuronal damage in the brain. First, hypoxic-ischemic injury leads to dysfunction of the blood-brain barrier and edema of brain tissue. Cerebral water content measurement is used for detecting the severity of brain swelling and can be used as a means to indirectly reflect extent of brain tissue damage. Second, histamine plays a role in neuronal protection during the process of cerebral ischemia; therefore, hippocampal histamine content measurement was used in our study. Third, malondialdehyde is the product of oxygen free radicals and lipid peroxidation of polyunsaturated fatty acids in the cell membrane. Malondialdehyde content parallels that of oxygen free radicals; therefore, malondialdehyde content measurement can be used as a substitute of oxygen free radicals that does not harm neurons. Fourth, O^{2-} is the initial output in the process of active oxygen production, and superoxide dismutase plays a catalytic role in the dismutation reaction of the super-oxygen anion, so the measurement of superoxide dismutase activity can be used for assessing oxidation damage in neurons.

Histamine, in addition to other neurotransmitters, exerts its physiological effects by altering cellular excitability by binding its specific receptors in target cells. Until now, four histamine receptors, H1–4, have been shown to be involved in mediating histamine actions[55-58]. The H3 receptor, an autoreceptor, modulates synthesis and release of histamine in a negative feedback fashion. The H3 receptor is highly sensitive and can be activated at a relatively low concentration (two orders of magnitude) of histamine[59]. In the present study, the H3 receptor antagonist, thioperamide, was used in a rat model of neonatal hypoxic-ischemic encephalopathy. Increased cerebral histamine content, decreased brain water content, lowered malondialdehyde and enhanced superoxide dismutase levels were found in the H3 group when compared with the model group, and a statistical difference was seen between these two groups. These results demonstrate that severity of brain damage in the H3 group was relatively mild. Our data therefore shows that H3 receptor antagonists play an important role in neuronal protection. This is in accordance with previous reports[60-61].

Previous studies into histamine H2 receptor blockade in ischemic brain are contradictory[62-65]. Some studies have shown that H2 receptor antagonists play a protective role against cerebral edema, whereas other reports demonstrate that H2 receptor antagonists aggravate cerebral injuries induced by neonatal hypoxic-ischemic encephalopathy. Moreover, combining H2 receptor antagonists and catecholamines led to more serious damage[62-65]. Our data have shown that cimetidine has no influence on the neuroprotective effect of thioperamide in neonatal hypoxic-ischemic encephalopathy rats.

Our results have shown that there is a pronounced increase in brain water content in the H3 + H1 group compared with the H3 group. Malondialdehyde content was also statistically higher in the H3 + H1 group than the H3 group. Furthermore, compared with the H3 group, the superoxide dismutase level was significantly decreased in the H3 + H1 group. These data revealed that an H1 receptor antagonist can block the protective effects of an H3 receptor antagonist in this animal model. H3 receptor antagonists play a protective role in neuronal damage induced by hypoxia and ischemia. Pyrilamine, as an H1 receptor antagonist, can block these protective effects and aggravate injuries induced by hypoxia and ischemia. We deduce that increased brain histamine caused by an H3 receptor antagonist exerts neuronal protective effects through postsynaptic H1 receptors.

Central nervous system histamine is one of the earliest neurotransmitters or neuromodulators in systemic development. Studies have shown that brain histaminergic neurons in the central nervous system are matured in rats by postnatal days 14 to 28. The earlier development of the histaminergic system compensates for the delayed appearance of other protective neuronal systems, such as the N-methyl-D-aspartic receptor complex and γ-aminobutyric acid receptor. Brain histamine plays an important role in normal development of an individual nervous system[66-68]. Our results have shown that both the H3 and H3 + H2 groups demonstrate similar effects in our encephalopathic rats with regard to brain water content, malondialdehyde and, hippocampal histamine levels, and superoxide dismutase activity. H3 receptor antagonists, therefore, play a role in neuronal protection in this animal model. The use of H1 receptor antagonist, pyrilamine, reversed the protective function of thioperamide.

In all, histamine H3 receptor antagonists have a protec-

tive effect on damaged neurons in a rat model of neonatal hypoxic-ischemic encephalopathy through postsynaptic H1 receptor. This study provides a theoretical basis for further clinical research surrounding the use of H3 receptor antagonists in neonatal hypoxic-ischemic encephalopathy.

MATERIALS AND METHODS

Design
A randomized, controlled animal study.

Time and setting
Experiments were performed at the Laboratory of Neurology, the First Hospital of Jilin University, China, from March 2009 to July 2010.

Materials
One hundred and twenty Wistar rat pups, aged 7 days, were purchased from the Experimental Animal Center of Bethune Medical College of Jilin University, China (license No. SCXK (Ji) 2008-0003). All procedures were in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, formulated by the Ministry of Science and Technology of China[69].

Methods
Establishment of neonatal hypoxic-ischemic encephalopathy model
The rat model of neonatal hypoxic-ischemic encephalopathy was established according to the Rice method with some modifications[70]. Ambient temperature was maintained between 37°C and 37.5°C. A hypoxic cabinet was placed in a thermostatic water bath. Unilateral common carotid artery ligation was performed in 7-day-old postnatal rats. Four hours later, the pups were exposed to 8% oxygen at 37°C for 3.5 hours. The rat pups that developed spontaneous levorotation were included in further experiments (Figure 5).

Drug administration
Rats in the H3 group were intraperitoneally injected with histamine H3 receptor antagonist thioperamide (T123: 10 mg; Sigma, St. Louis, MO, USA) at 5 mg/kg (freshly dissolved in saline at the concentration of 1 mg/mL[71], 2 hours prior to injection) immediately after hypoxia. Rat pups in the H3 + H1 group were intraperitoneally injected with histamine H1 receptor antagonist pyrilamine (5 mg/kg, initial concentration of 1 mg/mL; P5514: 25 g; Sigma), and thioperamide (5 mg/kg) was administered 30 minutes later. Experimental animals in the H3 + H2

group were intraperitoneally injected with histamine H2 receptor antagonist cimetidine (100 mg/kg, starting concentration of 20 mg/mL; C4522: 25 g; Sigma) 1 hour prior to thioperamide (dose as above). Rats in the model group were treated using 0.1 mL of saline.
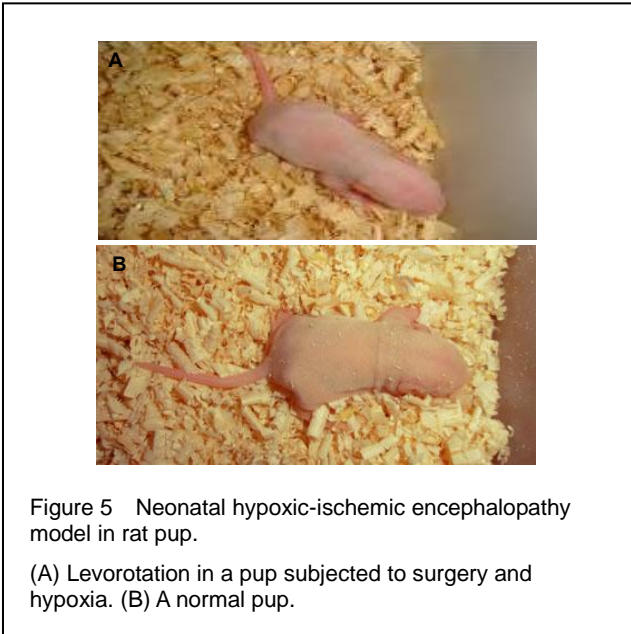

Figure 5 Neonatal hypoxic-ischemic encephalopathy model in rat pup.

(A) Levorotation in a pup subjected to surgery and hypoxia. (B) A normal pup.

Brain tissue sampling
Animals were sacrificed *via* prompt decapitation (on ice). Brain samples (including brain stem and parts of the spinal cord) were dissected and stored in wide-mouth bottles filled with 10% formalin at 4°C.

Determination of brain water content
The water content of brain tissue was determined using the wet and dry mass method[72]. The left cerebral hemispheres were removed and weighed immediately after dissection (wet weight) and then dried in a vacuum oven (ZK072, Shanghai Laboratory Instrument Works Co., Ltd., Shanghai, China) at 100°C for 48 hours. The dried brain was re-weighed. The percentage of the water content was calculated as (wet weight – dry weight)/wet weight × 100%.

Measurement of brain histamine content
Hippocampi were promptly dissected away, on ice, following decapitation. Tissue was then stored at –80°C until assayed. Brain tissue was weighed and homogenized in 3% perchloric acid. The homogenate was centrifuged at 15 000 × *g* for 20 minutes at 4°C to obtain a clear supernatant. After filtration (0.22 μm), histamine was analyzed fluorometrically with o-phthalaldehyde (Lianyungang Runze Chemical Co., Ltd., Lianyungang city, Jiangsu Province, China) after separation on a high performance liquid chromatography system (Shimadzu

Corporation, Kyoto, Japan). The histamine level was determined by the standard sample curve.

Measurement of brain malondialdehyde content
Brain tissue was weighed after surface water was blotted away using filter paper. The right cerebral hemispheres were homogenized in 10 times saline volume using a tissue homogenizer. The homogenate was centrifuged at 3 500 r/min for 10 minutes at 4°C. Malondialdehyde content was measured using the sulfate phenobarbital method[73]. Malondialdehyde, as one of the degradation products of hyperperoxylated lipid, was condensed with 2-thiobarbituric acid to produce a red output that had a maximum absorbing peak at 532 nm. The malondialdehyde measurement was performed using a malondialdehyde detection kit (Nanjing Jiancheng Bioengineer Institute, Nanjing, Jiangsu Province, China). Malondialdehyde level was calculated using the following equation: malondialdehyde content (nmol/g) = (absorbance value of sample – absorbance value of sample blank)/(absorbance value of standard – absorbance value of standard blank)[74].

Determination of brain superoxide dismutase activity
Superoxide dismutase enzyme activity of the right cerebral hemispheres was determined using a superoxide dismutase kit (Nanjing Jiancheng Bioengineering Institute). Xanthine and xanthine oxidase were used to generate superoxide anion radicals which reacted with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride quantitatively to form a red formazan dye. Superoxide dismutase inhibited the reaction by converting the superoxide radical to oxygen[73]. Absorbance was measured at 505 nm on a Cecil 1021 UV/visible spectrophotometer (Guangzhou Huarui Chemical Instrument Co., Ltd., Guangzhou, Guangdong Province, China) for 30 seconds after the addition of xanthine oxidase as a start reagent and 3 minutes after reaction as duplicate samples. Superoxide dismutase activity was calculated using the following equation: superoxide dismutase activity (U/mg) = (absorbance value of the control – absorbance value of the sample)/absorbance value of the control/50% × (volume of reaction solution/tissue volume (mL)/protein content of tissue (mg/mL).

Statistical analysis
Data was expressed as mean ± SD. One-way analysis of variance was used to test significance between different groups. SPSS 14.0 software (SPSS, Chicago, IL, USA) was used for statistical analyses. Student's *t*-test was used to analyze data at different time points of each group. A $P < 0.05$ value was considered statis-

tically significant.

REFERENCES

[1] Khurshid F, Lee KS, McNamara PJ, et al. Lessons learned during implementation of therapeutic hypothermia for neonatal hypoxic ischemic encephalopathy in a regional transport program in Ontario. Paediatr Child Health. 2011; 16(3):153-156.

[2] Ni X, Yang ZJ, Wang B, et al. Early antioxidant treatment and delayed hypothermia after hypoxia-ischemia have no additive neuroprotection in newborn pigs. Anesth Analg. 2012;115(3):627-637.

[3] Lynch NE, Stevenson NJ, Livingstone V, et al. The temporal evolution of electrographic seizure burden in neonatal hypoxic ischemic encephalopathy. Epilepsia. 2012; 53(3):549-557.

[4] Mwaniki MK, Atieno M, Lawn JE, et al. Long-term neurodevelopmental outcomes after intrauterine and neonatal insults: a systematic review. Lancet. 2012;379(9814): 445-452.

[5] Martinez-Biarge M, Diez-Sebastian J, Kapellou O, et al. Predicting motor outcome and death in term hypoxic-ischemic encephalopathy. Neurology. 2011;76(24): 2055-2061.

[6] Allemand F, Reale F, Sposato M, et al. Perinatal Hypoxic-Ischemic Encephalopathy: epileptic and paretic outcome at one year of age. Ital J Pediatr. 2009;35(1):14.

[7] Chen W, Ostrowski RP, Obenaus A, et al. Prodeath or prosurvival: two facets of hypoxia inducible factor-1 in perinatal brain injury. Exp Neurol. 2009;216(1):7-15.

[8] Ferrari DC, Nesic OB, Perez-Polo JR. Oxygen resuscitation does not ameliorate neonatal hypoxia/ischemia-induced cerebral edema. J Neurosci Res. 2010;88(9): 2056-2065.

[9] Yasuhara T, Hara K, Maki M, et al. Mannitol facilitates neurotrophic factor up-regulation and behavioral recovery in neonatal hypoxic-ischemic rats with human umbilical cord blood grafts. J Cell Mol Med. 2010;14(4):914-921.

[10] Shankaran S, Pappas A, McDonald SA, et al. Childhood outcomes after hypothermia for neonatal encephalopathy. N Engl J Med. 2012;366(22):2085-2092.

[11] Joolay Y, Harrison MC, Horn AR. Therapeutic hypothermia and hypoxic ischemic encephalopathy: opinion and practice of pediatricians in South Africa. J Perinat Med. 2012;40(4):447-453.

[12] Jacobs SE, Morley CJ, Inder TE, et al. Whole-body hypothermia for term and near-term newborns with hypoxic-ischemic encephalopathy: a randomized controlled trial. Arch Pediatr Adolesc Med. 2011;165(8):692-700.

[13] Grände PO, Romner B. Osmotherapy in brain edema: a questionable therapy. J Neurosurg Anesthesiol. 2012; 24(4):407-412.

[14] Adachi N, Liu K, Ninomiya K, et al. Reduction of the infarct size by simultaneous administration of L-histidine and diphenhydramine in ischemic rat brains. Resuscitation.

2011;82(2):219-221

[15] Hu WW, Chen Z. Role of histamine and its receptors in cerebral ischemia. ACS Chem Neurosci. 2012;3(4):238-247.

[16] Akhtar M, Pillai K, Vohora D. Effect of thioperamide on oxidative stress markers in middle cerebral artery occlusion model of focal cerebral ischemia in rats. Hum Exp Toxicol. 2008;27(10):761-767.

[17] Lindsberg PJ, Strbian D, Karjalainen-Lindsberg ML. Mast cells as early responders in the regulation of acute blood-brain barrier changes after cerebral ischemia and hemorrhage. J Cereb Blood Flow Metab. 2010;30(4): 689-702.

[18] Benarroch EE. Histamine in the CNS: multiple functions and potential neurologic implications. Neurology. 2010; 75(16):1472-1479.

[19] Dai H, Fu Q, Shen Y, et al. The histamine H3 receptor antagonist clobenpropit enhances GABA release to protect against NMDA-induced excitotoxicity through the cAMP/protein kinase A pathway in cultured cortical neurons. Eur J Pharmacol. 2007;563(1-3):117-123.

[20] Vizuete ML, Merino M, Venero JL. Histamine infusion induces a selective dopaminergic neuronal death along with an inflammatory reaction in rat substantia nigra. J Neurochem. 2000;75(2):540-552.

[21] Flik G, Dremencov E, Cremers TI, et al. The role of cortical and hypothalamic histamine-3 receptors in the modulation of central histamine neurotransmission: an in vivo electrophysiology and microdialysis study. Eur J Neurosci. 2011;34(11):1747-1755.

[22] Ellender TJ, Huerta-Ocampo I, Deisseroth K, et al. Differential modulation of excitatory and inhibitory striatal synaptic transmission by histamine. J Neurosci. 2011;31 (43):15340-15351.

[23] Sakai K, Takahashi K, Anaclet C, et al. Sleep-waking discharge of ventral tuberomammillary neurons in wild-type and histidine decarboxylase knock-out mice. Front Behav Neurosci. 2010;4:53.

[24] Lozada A, Munyao N, Sallmen T, et al. Postischemic regulation of central histamine receptors. Neuroscience. 2005;136(1):371-379.

[25] Adachi N. Cerebral ischemia and brain histamine. Brain Res Brain Res Rev. 2005;50(2):275-286.

[26] Hiraga N, Adachi N, Liu K, et al. Suppression of inflammatory cell recruitment by histamine receptor stimulation in ischemic rat brains. Eur J Pharmacol. 2007;557(2-3): 236-244.

[27] Irisawa Y, Adachi N, Liu K, et al. Alleviation of ischemia-induced brain edema by activation of the central histaminergic system in rats. J Pharmacol Sci. 2008;108(1):112-123.

[28] Jia F, Kato M, Dai H, et al. Effects of histamine H(3) antagonists and donepezil on learning and mnemonic deficits induced by pentylenetetrazol kindling in weanling mice. Neuropharmacology. 2006;50(4):404-411.

[29] Kukko-Lukjanov TK, Lintunen M, Jalava N, et al. Involvement of histamine 1 receptor in seizure susceptibility and neuroprotection in immature mice. Epilepsy Res.

2010;90(1-2):8-15.

[30] Yokoyama H, Onodera K, Iinuma K, et al. Proconvulsive effects of histamine H1-antagonists on electrically-induced seizure in developing mice. Psychopharmacology (Berl). 1993;112(2-3):199-203.

[31] Lee BS, Woo CW, Kim ST, et al. Long-term neuroprotective effect of postischemic hypothermia in a neonatal rat model of severe hypoxic ischemic encephalopathy: a comparative study on the duration and depth of hypothermia. Pediatr Res. 2010;68(4):303-338.

[32] Ghi T, Giunchi S, Pilu G, et al. Neonatal hypoxic-ischemic encephalopathy in apparently low risk pregnancies: retrospective analysis of the last five years at the University of Bologna. J Matern Fetal Neonatal Med. 2010;23(6): 516-521.

[33] Itoo BA, Al-Hawsawi ZM, Khan AH. Hypoxic ischemic encephalopathy. Incidence and risk factors in North Western Saudi Arabia. Saudi Med J. 2003;24(2):147-153.

[34] Butt TK, Farooqui R, Khan MA. Risk factors for hypoxic ischemic encephalopathy in children. J Coll Physicians Surg Pak. 2008;18(7):428-432.

[35] Pisani F, Orsini M, Braibanti S, et al. Development of epilepsy in newborns with moderate hypoxic-ischemic encephalopathy and neonatal seizures. Brain Dev. 2009; 31(1):64-68.

[36] Rink C, Khanna S. Significance of brain tissue oxygenation and the arachidonic acid cascade in stroke. Antioxid Redox Signal. 2011;14(10):1889-1903.

[37] Muralikrishna Adibhatla R, Hatcher JF. Phospholipase A2, reactive oxygen species, and lipid peroxidation in cerebral ischemia. Free Radic Biol Med. 2006;40(3):376-387.

[38] Hoda MN, Singh I, Singh AK, et al. Reduction of lipoxidative load by secretory phospholipase A2 inhibition protects against neurovascular injury following experimental stroke in rat. J Neuroinflammation. 2009;6:21.

[39] Farooqui AA, Ong WY, Horrocks LA. Inhibitors of brain phospholipase A2 activity: their neuropharmacological effects and therapeutic importance for the treatment of neurologic disorders. Pharmacol Rev. 2006;58(3):591-620.

[40] Cho KJ, Seo JM, Kim JH. Bioactive lipoxygenase metabolites stimulation of NADPH oxidases and reactive oxygen species. Mol Cells. 2011;32(1):1-5.

[41] Zheng Y, Brash AR. Formation of a cyclopropyl epoxide via a leukotriene A synthase-related pathway in an anaerobic reaction of soybean lipoxygenase-1 with 15S- hydroperoxyeicosatetraenoic acid: evidence that oxygen access is a determinant of secondary reactions with fatty acid hydroperoxides. J Biol Chem. 2010;285(18):13427-13436.

[42] Ruel J, Chabbert C, Nouvian R, et al. Salicylate enables cochlear arachidonic-acid-sensitive NMDA receptor responses. J Neurosci. 2008;28(29):7313-7323.

[43] Müller C, Gardemann A, Keilhoff G, et al. Palmitate protects hepatocytes from oxidative stress and triacylglyceride accumulation by stimulation of nitric oxide synthesis in the presence of high glucose and insulin concentration. Free Radic Res. 2010;44(12):1425-1434.

[44] Choi SH, Langenbach R, Bosetti F. Genetic deletion or pharmacological inhibition of cyclooxygenase-1 attenuate lipopolysaccharide-induced inflammatory response and brain injury. FASEB J. 2008;22(5):1491-1501.

[45] Strbian D, Kovanen PT, Karjalainen-Lindsberg ML, et al. An emerging role of mast cells in cerebral ischemia and hemorrhage. Ann Med. 2009;41(6):438-450.

[46] Mattila OS, Strbian D, Saksi J, et al. Cerebral mast cells mediate blood-brain barrier disruption in acute experimental ischemic stroke through perivascular gelatinase activation. Stroke. 2011;42(12):3600-3605.

[47] Wang LW, Tu YF, Huang CC, et al. JNK signaling is the shared pathway linking neuroinflammation, blood-brain barrier disruption, and oligodendroglial apoptosis in the white matter injury of the immature brain. J Neuroinflammation. 2012;9:175.

[48] Huang Z, Song L, Wang C, et al. Hypoxia-ischemia upregulates TRAIL and TRAIL receptors in the immature rat brain. Dev Neurosci. 2011;33(6):519-530.

[49] Ohshima M, Tsuji M, Taguchi A, et al. Cerebral blood flow during reperfusion predicts later brain damage in a mouse and a rat model of neonatal hypoxic-ischemic encephalopathy. Exp Neurol. 2012;233(1):481-489.

[50] Shin WJ, Gwak M, Baek CH, et al. Neuroprotective effects of lithium treatment following hypoxic-ischemic brain injury in neonatal rats. Childs Nerv Syst. 2012;28(2):191-198.

[51] Lai PC, Huang YT, Wu CC, et al. Ceftriaxone attenuates hypoxic-ischemic brain injury in neonatal rats. Biomed Sci. 2011;18:69.

[52] Wixey JA, Reinebrant HE, Spencer SJ, et al. Efficacy of post-insult minocycline administration to alter long-term hypoxia-ischemia-induced damage to the serotonergic system in the immature rat brain. Neuroscience. 2011;182: 184-192.

[53] Weis SN, Schunck RV, Pettenuzzo LF, et al. Early biochemical effects after unilateral hypoxia-ischemia in the immature rat brain. Int J Dev Neurosci. 2011;29(2):115-120.

[54] Wolburg H, Paulus W. Choroid plexus: biology and pathology. Acta Neuropathol. 2010;119(1):75-88.

[55] Saligrama N, Noubade R, Case LK, et al. Combinatorial roles for histamine H1-H2 and H3-H4 receptors in autoimmune inflammatory disease of the central nervous system. Eur J Immunol. 2012;42(6):1536-1546.

[56] Teuscher C, Subramanian M, Noubade R, et al. Central histamine H3 receptor signaling negatively regulates susceptibility to autoimmune inflammatory disease of the CNS. Proc Natl Acad Sci U S A. 2007;104(24):10146-10151.

[57] Lu C, Diehl SA, Noubade R, et al. Endothelial histamine H1 receptor signaling reduces blood-brain barrier permeability and susceptibility to autoimmune encephalomyelitis. Proc Natl Acad Sci U S A. 2010;107(44):18967-18972.

[58] Tiligada E, Kyriakidis K, Chazot PL, et al. Histamine pharmacology and new CNS drug targets. CNS Neurosci

Ther. 2011;17(6):620-628.

[59] Li L, Li G, Sheng S, et al. Substantial reaction between histamine and malondialdehyde: a new observation of carbonyl stress. Neuro Endocrinol Lett. 2005;26(6):799-805.

[60] Cheng Z, He W, Zhou X, et al. Cordycepin protects against cerebral ischemia/reperfusion injury in vivo and in vitro. Eur J Pharmacol. 2011;664(1-3):20-28.

[61] The Ministry of Science and Technology of the People's Republic of China. Guidance Suggestions for the Care and Use of Laboratory Animals. 2006-09-30.

[62] Sakai N, Onodera K, Maeyama K, et al. Effects of thioperamide, a histamine H3 receptor antagonist, on locomotor activity and brainhistamine content in mast cell-deficient W/Wv mice. Life Sci. 1991;48(25):2397-2404.

[63] Chen Z, Sakurai E, Hu W, et al. Pharmacological effects of carcinine on histaminergic neurons in the brain. Br J Pharmacol. 2004;143(5):573-580.

[64] Patnaik R, Mohanty S, Sharma HS. Blockade of histamine H2 receptors attenuate blood-brain barrier permeability, cerebral blood flow disturbances, edema formation and cell reactions following hyperthermic brain injury in the rat. Acta Neurochir Suppl. 2000;76:535-539.

[65] Németh L, Deli MA, Falus A, et al. Cerebral ischemia reperfusion-induced vasogenic brain edema formation in rats: effect of an intracellular histamine receptor antagonist. Eur J Pediatr Surg. 1998;8(4):216-219.

[66] Molina-Hernández A, Díaz NF, Arias-Montaño JA. Histamine in brain development. J Neurochem. 2012;122(5):872-882.

[67] Wada H, Inagaki N, Itowi N, et al. Histaminergic neuron system: morphological features and possible functions. Agents Actions Suppl. 1991;33:11-27.

[68] Wada H, Inagaki N, Itowi N, et al. Histaminergic neuron system in the brain: distribution and possible functions. Brain Res Bull. 1991;27(3-4):367-370.

[69] Rice JE, Vannucci RC, Brierley JB. The influence of immaturity on hypoxicischemic brain damage in the rat. Ann Neurol. 1981;9(2):131-141.

[70] Zhong J, Zhao L, Du Y, et al. Delayed IGF-1 treatment reduced long-term hypoxia-ischemia-induced brain damage and improved behavior recovery of immature rats. Neurol Res. 2009;31(5):483-489.

[71] Jia F, Kato M, Dai H, et al. Effects of histamine H(3) antagonists and donepezil on learning and mnemonic deficits induced by pentylenetetrazol kindling in weanling mice. Neuropharmacology. 2006;50(4):404-411.

[72] Liu H, Yang M, Qiu GP, et al. Aquaporin 9 in rat brain after severe traumatic brain injury. Arq Neuropsiquiatr. 2012;70(3):214-220.

[73] Bhutia Y, Ghosh A, Sherpa ML, et al. Serum malondialdehyde level: Surrogate stress marker in the Sikkimese diabetics. J Nat Sci Biol Med. 2011;2(1):107-112.

[74] Reddy MK, Labhasetwar V. Nanoparticle-mediated delivery of superoxide dismutase to the brain: an effective strategy to reduce ischemia-reperfusion injury. FASEB J. 2009;23(5):1384-1395.