Journal of Hainan Medical University

http://www.hnykdxxb.com

Influence of hypothermia combined with erythropoietin on serum neurological function indexes in newborns with severe hypoxic ischemic encephalopathy

Hua Tian✉, Xiao−Li Huang, Li−Jun Zhang

Department of Pediatrics, the Central Hospital of Enshi Autonomous Prefecture Hubei Province, Enshi, Hubei, 445000

ARTICLE INFO

Article history:
Received 11 May 2017
Received in revised form 17 May 2017
Accepted 25 May 2017
Available online 24 Jun 2017

Keywords:
Hypoxic ischemic encephalopathy
Newborn
Hypothermia
Erythropoietin
Neurological function

ABSTRACT

Objective: To study the influence of hypothermia combined with erythropoietin (EPO) on serum neurological function indexes in newborns with severe hypoxic ischemic encephalopathy (HIE). **Methods:** A total of 48 cases of newborns with severe hypoxic ischemic encephalopathy in our hospital were enrolled and divided into control group and observation group according to random number table, 24 cases in each group. On the basis of conventional treatment, patients in control group were treated with mild hypothermia, and those in observation group were treated with mild hypothermia combined with EPO. Serum nerve injury indexes, neurological function indexes and nerve apoptosis indexes were compared between two groups before and after treatment. **Results:** Before treatment, differences in the levels of nerve injury indexes, neurological function indexes and nerve apoptosis indexes were not statistically significant between two groups. After treatment, serum nerve injury indexes NSE and S-100B levels of observation group were lower than those of control group, neurolocial function indexes BDNF, NGF, IGF-1 and GH levels of observation group were higher than those of control group, and nerve apoptosis indexes sFas and sFasL levels of observation group were lower than those of control group. **Conclusion:** Mild hypothermia combined with EPO can reduce the neurological damage and inhibit neuronal apoptosis in children with severe HIE.

1. Introduction

Hypoxic ischemic encephalopathy (HIE) is the cerebral hypoxia/ischemia in the newborn caused by a variety of perinatal factors, children with severe brain injury, children with severe HIE can show coma, muscular tone flabbiness, convulsion and other severe performances, and the clinical mortality is high[1,2]. Correcting hypoxemia, electrolyte metabolism disorder and metabolic acidosis, increasing cerebral energy supply and so on are all conventional methods for HIE treatment, mild hypothermia therapy has also been successful applied in HIE treatment, but there are still many children with severe HIE who are not significantly improved after treatment[3]. Erythropoietin (EPO) is considered to be the most promising drug for prevention and control of HIE, which has the biological effects such as anti-oxidation, anti-inflammation and anti-apoptosis, can exert therapeutic value for multiple pathological links of neuronal damage in the course of the HIE, and helps to promote the recovery of neural function. In this study, it was used together with hypothermia for the treatment of children with severe HIE, and the changes in serum neurological function indexes were discussed.

2. Subjects and methods

2.1 Subjects

A total of 48 newborns that were born and diagnosed with severe hypoxic ischemic encephalopathy in our hospital between June 2013 and May 2016 were enrolled, and the families of the children signed the consent form. According to the random number table, the included children were divided into control group and observation group (n=24). Control group included 13 male cases and 11 female cases, the birth weight was 2.98-3.49 kg and (3.16±0.27) kg in average, and the gestational age at birth was 36-40 weeks and

✉Corresponding author: Hua Tian, Department of Pediatrics, the Central Hospital of Enshi Autonomous Prefecture Hubei Province, Enshi, Hubei, 445000.
Tel: 13886757773
Fund Project: Key Projects of Health and Family Planning Commission of Hubei Province in 2015 No: WJ2015MA122.

(38.62±1.05) weeks in average; observation group included 12 male cases and 12 female cases, the birth weight was 2.91-3.42 kg and (3.13±0.25) in average, and the gestational age at birth was 35-40 weeks and (38.47±1.01) weeks in average. The two groups of newborns were not statistically different in distribution of gender, birth weight and gestational age at birth (P>0.05), and the hospital ethics committee approved the study.

2.2 Diagnostic criteria

(1) With history of perinatal asphyxia; (2) in coma, abolition of reflex, and the muscular tone loss/disappearance; (3) craniocerebral CT showing diffuse low-density shadow, loss of gray matter and white matter boundary, and might be accompanied by subarachnoid hyperemia and intraventricular hemorrhage; (4) increase in serum creatine phosphokinase BB isozyme.

2.3 Therapy

Both groups received conventional clinical therapy for severe neonatal hypoxic ischemic encephalopathy, including correcting hypoxemia/hypercapnia, rectifying hypotension, providing enough glucose for energy, correcting metabolic acidosis, controlling convulsions, reducing intracranial pressure, etc. Control group of children, based on conventional treatment, received mild hypothermia therapy, specifically as follows: the ice cap of mild hypothermia instrument (Zhuhai King Tontown Medical Instrument Co., LTD., the article number of HCY-200) was put on children's head, nasopharyngeal temperature represented the temperature of the skull base, it was maintained at 34 ℃, and the treatment lasted for 72 h. After mild hypothermia therapy, children were naturally re-warmed, and the temperature reached 36 ℃ after 6 h. Observation group of children, on the basis of conventional treatment, received hypothermia combined with EPO treatment, specifically as follows: recombinant EPO injection (Roche Diagnostics GmbH, approved by J20090058) 200 IU/kg, 3 times per week, for continuous 7 d of treatment. Hypothermia therapy was done synchronously, and the specific method and treatment time were the same as those for the control group.

2.4 Observation indexes

Before and after treatment, 1.0 mL of peripheral venous blood was extracted from the children, anti-coagulated and then centrifuged at low velocity to get supernatant and cryopreserve it for testing. Enzyme linked immunosorption assay (ELISA) was used to detect the levels of nerve injury indexes in serum, including neuron-specific enolase (NSE) and S-100B protein (S-100B). Serum levels of neural function indexes were determined by ELISA, including brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), insulin growth factor 1 (IGF-1) and growth hormone (GH). ELISA was used to measure the nerve apoptosis indicators in serum, including soluble Fas (sFas) and soluble FasL (sFasL).

2.5 Statistical processing

Statistical software was SPSS 21.0, and the statisticians were with professional statistical knowledge and researcher qualification. Nerve injury indexes, neurological function indexes and nerve apoptosis indexes and other measurement data were in terms of mean ± standard deviation, and comparison between groups was by grouping t test. P<0.05 was the standard of statistical significance in differences in the study.

3. Results

3.1 Nerve injury indexes

Comparison of serum nerve injury indexes NSE (μg/mL) and S-100B (μg/L) levels between two groups of children before and after treatment was as follows: before treatment, differences in serum NSE and S-100B levels were not statistically significant between two groups (P>0.05); after treatment, serum NSE and S-100B levels of both groups were lower than those before treatment, serum NSE and S-100B levels of observation group were lower than those of control group, and differences were statistically significant within group before and after treatment as well as between groups after treatment (P<0.05), shown in Table 1.

3.2 Neurological function indexes

Comparison of serum neurological function indexes BDNF (ng/L), NGF (μg/L), IGF-1 (ng/mL) and GH (ng/mL) levels between two groups of children before and after treatment was as follows: before treatment, differences in serum BDNF, NGF, IGF-1 and GH levels were not statistically significant between two groups (P>0.05); after treatment, serum BDNF, NGF, IGF-1 and GH levels of both groups

Table 1.

Comparison of serum nerve injury index levels before and after treatment.

Groups	n	NSE		S-100B	
		Before treatment	After treatment	Before treatment	After treatment
Control group	24	40.27±5.18	29.37±3.42*	1.18±0.22	0.84±0.09*
Observation group	24	40.19±4.95	21.64±2.88*	1.17±0.21	0.62±0.07*
T		0.219	9.283	0.152	5.479
P		＞0.05	＜0.05	＞0.05	＜0.05

Note: compared with same group before treatment, *P＜0.05.

Table 2.

Comparison of serum neurological function index levels before and after treatment.

Groups	*n*	BDNF		NGF		IGF-1		GH	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control group	24	1 203.18±159.38	1 539.28±183.62*	102.17±14.53	121.64±15.94*	23.84±3.11	41.26±5.18*	21.27±3.04	40.56±5.23*
Observation group	24	1 212.65±143.82	1 893.26±200.37*	103.48±13.79	143.28±16.74*	23.75±3.09	58.09±7.42*	21.45±3.01	57.84±7.19*
T		0.271	20.381	0.143	12.183	0.164	9.483	0.284	10.273
P		>0.05	<0.05	>0.05	<0.05	>0.05	<0.05	>0.05	<0.05

Note: compared with same group before treatment, *$P<0.05$.

were higher than those before treatment, serum BDNF, NGF, IGF-1 and GH levels of observation group were higher than those of control group, and differences were statistically significant within group before and after treatment as well as between groups after treatment ($P<0.05$), shown in Table 2.

3.3 Nerve apoptosis indexes

Comparison of serum nerve apoptosis indexes sFas and sFasL levels between two groups of children before and after treatment was as follows: before treatment, differences in serum sFas and sFasL levels were not statistically significant between two groups ($P>0.05$); after treatment, serum sFas and sFasL levels of both groups were lower than those before treatment, serum sFas and sFasL levels of observation group were lower than those of control group, and differences were statistically significant within group before and after treatment as well as between groups after treatment (P<0.05), shown in Table 3.

4. Discussion

Mild hypothermia therapy adopts physical methods to reduce patients' body temperature to the expected level, it has been widely applied in HIE and other nerve injury diseases and achieved affirmative effect at present, but hypothermia therapy alone is not enough to completely reverse nerve injury of children with severe HIE, and there is still severe cerebral organic damage in some children. EPO is a hormonal matter that is secreted by the kidneys and the liver, its basic function is to promote the formation of red blood cells and it has been successfully applied in patients with nephrotic anemia[4,5]. In recent years, studies have found that EPO not only has the biological activities of anti-inflammation, anti-oxidation and anti-apoptosis, but can also promote neural development, protect neurological function, induce angiogenesis and so on through the erythropoietin receptor (EPOR) specifically distributed in brain tissue[6,7]. At present, there is not much clinical research on EPO for children with severe HIE, hypothermia combined with EPO was used in the treatment of children with severe HIE in our hospital in this research, and the changes in serum neurological function index contents was used as the breakthrough point to discuss its curative effect.

There is neuronal ischemic hypoxic injury after the occurrence of HIE, the substances specifically existing within the cells can go outside of cells and enter into the peripheral blood through the damaged blood brain barrier, and they are sensitive markers for nerve injury[8]. NSE exists in neurons and neuroendocrine cells, its content is little in human peripheral blood and fluctuates slightly under physiological condition, the NSE is massively released into the blood after brain injury, it is considered to be one of the most reliable indicators for early judgment of nerve cell damage, and its content is consistent with nerve damage[9,10]. S-100B exists in astrocytes in the central nervous system and is the glial marker protein, and many studies have confirmed that the serum S-100B content increases in traumatic brain injury, cerebral infarction, cerebral hemorrhage and other cerebral diseases[11]. In the study, serum NSE and S-100B content were compared between two groups of children before and after treatment, and it was found that the serum NSE and S-100B contents of two groups decreased after treatment, but the NSE and S-100B contents of observation group decreased more obviously after treatment, and it indicates that that EPO therapy can effectively reduce the degree of brain injury in children with severe HIE, which is speculated to be directly related to the effect of EPO on reducing nerve cell apoptosis and promoting the regeneration of nerve cells.

The nerve damage indexes can quantifiably reflect the extent of brain damage, and certain neurofunctional indicators can also be used as reliable mediums for the diagnosis of neurological function and the evaluation of prognosis of severe HIE. BDNF and NGF are with neurotrophic function and can promote the growth of axons, serum BDNF and NGF contents decrease in children with HIE,

Table 3.

Comparison of serum nerve apoptosis index levels before and after treatment (μg/L).

Groups	*n*	sFas		sFasL	
		Before treatment	After treatment	Before treatment	After treatment
Control group	24	19.37±2.85	12.54±1.76*	5.28±0.71	3.72±0.45*
Observation group	24	19.29±2.74	7.94±0.85*	5.26±0.69	2.17±0.28*
T		0.218	8.932	0.114	6.342
P		>0.05	<0.05	>0.05	<0.05

Note: compared with same group before treatment, *$P<0.05$.

and the specific decrease extent is closely related to the illness severity[12,13]. IGF-1 is a single polypeptide hormone synthesized by the liver and helps to promote fetal growth and development, and study has shown that IGF-1 has a protective effect on HIE injury and can reduce the ischemia hypoxia damage to the nervous system by inhibiting nitric oxide toxicity, preventing the nerve cell apoptosis and other ways[14]. GH is secreted by the hypothalamus system and directly involved in the growth of the fetus, study shows that ischemia hypoxia can cause neonatal temporary or permanent hypothalamus-pituitary system damage, so its ability to secrete GH declines, and serum GH levels in children are lower[15]. It was found in the study that serum BDNF, NGF, IGF-1 and GH levels of observation group after treatment were higher than those of control group, indirectly proving that adding EPO treatment can effectively reduce the brain damage in children with severe HIE and furthest recover their central nervous system function.

Neuron apoptosis is the most serious consequence in HIE, severe neuron apoptosis can cause irreversible nerve damage in children, and it is also the leading cause of their early death[16,17]. Fas system is an important component of the cell-regulated apoptosis signaling pathways, Fas is a member of tumor necrosis factor receptor superfamily, and its combination with ligand FasL can activate a variety of molecules of downstream caspase family, promote apoptosis signaling cascade activation and amplification, and eventually cause apoptosis by activating caspase-3. sFas and sFasL are the soluble components of Fas and FasL, and serum sFas and sFasL levels are directly related to the activity of neuronal apoptosis[18,19]. It was found in the study that serum sFas and sFasL levels of observation group after treatment were lower than those of control group, indicating that adding EPO treatment can reduce the neuron apoptosis activity in children with severe HIE, and contribute to the protection on the nerve cells.

To sum up, hypothermia combined with EPO therapy helps to reduce nerve injury and inhibit neuron apoptosis in children with severe HIE, it is an ideal compatibility therapy, and it is worthy of popularization and application in clinical practice in the future.

References

[1] Huang Weiqing, Peng Xiaoming, Chang Shuting, Xiao Yong, Yan Weiqun, Zhang Fan. Severe neonatal hypoxic-ischemic encephalopathy: clinical features and follow-up of 123 cases. *Chin J Neonatol* 2016; **32**(2): 120-123.

[2] Elshorbagy HH, Azab AA, Kamal NM, Barseem NF, Bassiouny MM, Elsayed MA, et al. Value of electroencephalographic monitoring in newborns with hypoxic-ischemic encephalopathytreated with hypothermia. *J Pediatr Neurosci* 2016; **11**(4): 309-315.

[3] Chen Lang-hui. Head hypothermia therapy time window on neonatal moderately severe hypoxic ischemic encephalopathy curative effect observation. *J Pediatr Pharm* 2014; **20**(5): 28-31.

[4] Zhu Jing, Guo Aili, Zhang Nan, Qin Mingming, Liu Lijuan, Zhu Weiwei. Effect of erythropoietin on hypoxic ischemic encephalopathy in neonates. *J Shandong Univ (Health Science)* 2016; **54**(4): 60-64.

[5] Massaro AN, Campbell HE, Metzler M, Al-Shargabi T, Wang Y, du Plessis A, et al. Effect of temperature on heart rate variability in neonatal icu patients with hypoxic-ischemic encephalopathy. *Pediatr Crit Care Med* 2017; **18**(4): 349-354.

[6] Pei Xue-Mei, Gao Ran, Zhang Guo-Ying, Lin Ling, Wan Sheng-Ming, Qiu Su-Qing. Effects of erythropoietin on serum NSE and S-100B levels in neonates with hypoxic-ischemic encephalopathy. *Chin J Contemp Pediatr* 2014; **16**(7): 705-708.

[7] Rogers EE, Bonifacio SL, Glass HC, Juul SE, Chang T, Mayock DE, et al. Erythropoietin and hypothermia for hypoxic-ischemic encephalopathy. *Pediatr Neurol* 2014; **51**(5): 657-662.

[8] Chaparro-Huerta V, Flores-Soto ME, Merin Sigala ME, Barrera de León JC, Lemus-Varela ML, Torres-Mendoza BM, et al. Proinflammatory cytokines, enolase and s-100 as early biochemical indicators of hypoxic-ischemic encephalopathy following perinatal asphyxia in newborns. *Pediatr Neonatol* 2017; **58**(1): 70-76.

[9] Pei Xuemei, Gao Ran, Zhang Wenwu, Zhang Guoying, Wan Shengming. Single sialic acid four hexose ganglioside on hypoxic ischemic encephalopathy in children with the influence of serum NSE, S-100B. *Chin J Pract Nerv Dis* 2015; **18**(4): 64-67.

[10] Yang L, Li D, Chen S. Hydrogen water reduces NSE, IL-6, and TNF-α levels in hypoxic-ischemic encephalopathy. *Open Med (Wars)* 2016; **11**(1): 399-406.

[11] Wang Shi, Li Zhi-chao. Diagnosis and prognosis value of serum S100B protein and neuron-specific enolase in hypoxic-ischemic encephalopathy. *Chin J Child Health Care* 2015; **23**(1): 90-93.

[12] Ye Wu-cheng, Wang Jun-xia, Ye Peng, He Bo. Evaluation of serum indexes after monosialogangl ioside injection combined with conventional treatment of neonatal hypoxic ischemic encephalopathymonosialogangl ioside injection. *J Hainan Med Univ* 2017; **23**(4): 556-559.

[13] Ren Xue-jian, Zhao Qin, Wang Shuang. Lumbar spine fractures with neurological damage with the change of the serum levels of cytokines and nerve function index and significance. *Hainan Med J* 2015; **26**(4): 571-574.

[14] Xu T, Xu NG, Yang ZH, Wan YZ, Wu QL, Huang KB. Neuroprotective effects of electroacupuncture on hypoxic-ischemic encephalopathy in newborn rats are associated with increased expression of GDNF-RET and protein kinase B. Chin J Integr Med 2016; 22(6): 457-466.

[15] Xu T, Li W, Liang Y, Yang Z, Liu J, Wang Y, et al. Neuroprotective effects of electro acupuncture on hypoxic-ischemic encephalopathy in newborn rats Ass. *Pak J Pharm Sci* 2014; **27**(6 Suppl): 1991-2000.

[16] Zhao Yong-Dong. Effect of Danshen injection on serum sFas and sFasL in patients with acute cerebral infarction. *Chin J Gerontol* 2015; **35**(19): 5457-5460.

[17] Hua C, Ju WN, Jin H, Sun X, Zhao G. Molecular chaperones and hypoxic-ischemic encephalopathy. *Neural Regen Res* 2017; **12**(1): 153-160.

[18] Du Chunliang, Li Na, Bai Ximin, Zhao Jinan, Zhao Yunjie. Influences of Danshenchuanxiongqin injection on acute brain injury patients and the levels of serum HMGB1, NO, Fas. *Chin J Difficult Complicated Cases* 2016; **15**(9): 916-920.

[19] Hua C, Ju WN, Jin H, Sun X, Zhao G. Molecular chaperones and hypoxic-ischemic encephalopathy. *Neural Regen Res* 2017; **12**(1): 153-160.