European Review for Medical and Pharmacological Sciences 2015; 19: 3955-3960

Influence of one-year neurologic outcome of treatment on newborns with moderate and severe hypoxic-ischemic encephalopathy by rhuEP0 combined with ganglioside (GM_1)

X.-Y. ZHU, M.-Y. YE, A.-M. ZHANG, W.-D. WANG, F. ZENG, J.-L. LI, F. FANG

Department of Pediatrics, Xiangyang Hospital, Hubei University of Medicine, Xiangyang, Hubei, China

Xiaoyan Zhu and *Mingyang Ye* must be considered first authors

Abstract. – OBJECTIVE: To observe the one-year neurologic prognostic outcome of newborns with moderate and severe hypoxic-ischemic encephalopathy (HIE) who received recombinant human erythropoietin (rhuEPO) combined with exogenous monosialotetrahexosylganglioside (GM_1) treatment to provide new guidelines for clinical treatment.

PATIENTS AND METHODS: Seventy-six newborns with moderate and severe HIE were selected from February 2011 to February 2014 in our hospital. This study received the informed consent of our hospital's Ethics Committee and the newborns' guardians. The newborns were divided to an observation group (n = 34 cases) and a control group (n = 42 cases). All newborns underwent hypothermia and conventional treatment for their conditions. The control group received GMI treatment and observation group received rhuEPO combined with GMI treatment. The curative differences and neural behavior from these two groups were compared.

RESULTS: The excellent, efficient proportion and total effective rate of the newborns from the observation group were higher than the control group. The death rate, cerebral palsy and the invalid ratio of the newborns from the observation group were lower than that of the control group. Awareness, muscle tension, primitive reflex and increased intracranial pressure recovery time of the newborns in the observation group were less than those of the control group. The Neonatal Behavior Neurological Assessment (NBNA) score of both groups after the treatment of 7, 14 and 28 days were significantly higher and increased with time ($p < 0.05$). The MDI, PDI and DQ score of newborns from the two groups all increased after treatment of 3, 6 and 12 months than those of before, which increased with time ($p < 0.05$).

CONCLUSIONS: The rhuEPO + GMI treatment in newborns with HIE improves short-term clinical effects and long-term neurological symptoms.

Key Words:

Recombinant human erythropoietin, Exogenous monosialotetrahexosyl ganglioside, Newborns, Hypoxic-ischemic encephalopathy, Neurologic outcome.

Introduction

Neonatal hypoxic-ischemic encephalopathy (HIE), is a severe disease, featured with partial or complete anoxia and decreased cerebral blood flow caused by asphyxia in the perinatal period. Neonatal HIE is a common severe complication caused by newborns' asphyxia and anoxia and is an important reason leading to neurogenesis disorder or even death. Morbidity of HIE in our country is very high, about 6.3-12.4%[1]. However, current clinics cannot explain the pathogenesis of this disease, which focuses on energy failure, theory of oxygen radical and reperfusion injury, theory of calcium overload, toxic effect of excitatory amino acid, and nerve cell apoptosis. Thus, there is a lack of effective therapies[2]. Clinical practice shows that recombinant human erythropoietin (rhuEPO) could reduce central nerve cell apoptosis of the HIE, improving the function of the nervous system. Monosialoganglioside, GM_1 is one of the main species of mammalia gangliosides, with the functions of repairing injured nervous tissue, reducing the release of amino acid and stabilizing cytomembrane[4]. The research founds[5] that the severity of the cerebral injury was related to the low level of gangliosides, and gangliosides are protective for ischemia-reperfusion injury of brain development. How to improve and enhance the prognosis of moderate and severe HIE of newborns is the key point of educational circles to explore. Based on this, this

Corresponding Author: Xiaoyan Zhu, MD; e-mail: 505893788@qq.com

work further analyzes the influence of one-year neurologic outcome prognostic of moderate and severe HIE by the treatment of rhuEPO combined with ganglioside GM_1, providing new thinking for clinical treatment.

Patients and Methods

Patients

Seventy-six newborns with moderate and severe HIE were chosen from February 2011 to February 2014 in our hospital. Diagnostic criteria and clinical grading of the HIE referred to moderate-severe HIE diagnosis and classification criterion made by the Subspecialty Group of Neonatology, Pediatric Society, Chinese Medical Association and new criteria revised by Changsha "Session of National Newborns" in November 2004. This study obtained the informed consent of our hospital's Ethics Committee and the newborns' guardians. The exclusion criteria were as follows: excessive intracranial bleeding; severe anemia; serious infections and breathing difficulties; skull fracture; severe congenital malformations and patients who refused treatment. The newborns were divided to form an observation group (n = 34 cases) and a control group (n = 42 cases), according to a random number table method. The difference from a comparison of gender, gestational age, weight, HIE degree and Apgar score of these two groups did not have any statistical significance ($p > 0.05$), with comparability (Table I).

Research Method

All newborns adopted routine treatment such as antispasmodic, correct acidosis, reduce intracranial pressure, sedation, and oxygen uptake, according to the corresponding guidelines of Chinese Medical Association, combined with mild hypothermia treatment. The specific implementation steps are as follows: inform guardians of the operation necessity and steps and possible complications. Use temperature lowering instrument using the cooling method of semiconductor circulating water (produced by radio factory of Hengyang, Hunan), a special ice cap on the head, temperature sensors in the nasopharynx with the temperature of ice cap adjusted automatically among 5-20°C according to the temperature of nasopharynx. The nasopharynx temperature should be maintained at (34.0 ± 0.2)°C, the rectal temperature should remain above 34.5°C for more 72 hours. Also, newborns in the control group were given rhuEPO treatment, which was provided by Beijing Sihuan Bioengineering. A quantity of 200 u rhuEPO was added to the glucose solution with a concentration of 10% for intravenous drip once on alternate days, three times a week and four weeks as a period of treatment. Observation group was treated by rhuEPO and ganglioside GMl, 20 mg/d GM_1 was added to glucose injection with concentration of 5% for intravenous drip of two hours, once a day and four weeks as a period of treatment.

Evaluation Indexes

Routine blood, reticulocyte, liver function, renal function, electrolyte, blood sugar and blood pressure were checked before and after treatment. The treatment effect of newborns forms two groups were assessed to excellent, effective and invalid. Excellent: clear consciousness within five-day treatment, muscle tone restored normal; effective: clear consciousness within seven-day treatment, muscle tone improved and breathed smoothly; invalid: each index did not improve after treatment. The total effective rate is (excellent+ effective) / total number × 100%.

Neonatal behavior neurological assessment score (NBNA score) was used to evaluate two groups of children's neurological behavior, in room temperature of 22-27°C, quiet and half dark environment to execute the neurological behavior. There are 20 items including behavior

Table I. Comparison of general data of two groups.

Groups	Case	M/F	Weight (kg)	Gestational age (week)	Degree		Apgar score	
					M	S	1 min	5 min
Observation group	34	20/14	3.2 ± 0 .7	39.2 ± 3.5	15	19	3.4 ± 1.1	4.6 ± 1.3
Control group	42	25/17	3.1 ± 0.8	39.4 ± 3.3	17	25	3.2 ± 1.2	4.5 ± 1.4
χ^2/t	–	0.946	0.524	0.231	0.117	0.684	0.291	
p	–	0.402	0.326	0.817	0.731	0.493	0.772	

ability, active and passive muscle tone and so on, with a total score 40 points, checked by fixed physicians after the treatment of 7d, 14d and 28d respectively.

The Bayley Scale of infant development (China urban revision of Hunan Medical University) was used to evaluate the psychomotor development of newborns. The results were expressed by the mental development index (MDI) and the psychomotor development index (PDI), among which, the development index < 70 means that the prognosis is poor, 70-80 is at the limit and ≥ 80 stands for normal with good prognosis.

The psychomotor developmental inspection was as follows: the newborns enrolled into groups were at the Neonatal Unit of our hospital for a follow-up visit after 3, 6, and 12 months (± 7d) since birth. The Children Development Scale used was revised by the Capital Institute of Pediatrics and finished by a child health care physician in our hospital's Inspection Room by blind trial. A score of Developmental Quotient (DQ) < 80 points means neurological abnormality symptoms, such as early cerebral palsy and epilepsy, which belong to poor prognosis.

Statistical Analysis

The SPSS19.0 statistical software (SPSS Inc., Chicago, IL, USA) was used to process the data, mean ± standard deviation (± s) standing for measurement data, with T to detect between groups' comparison, cases or percentage expressing counting data, with χ^2 to detect between groups' comparison; ($p < 0.05$) meant difference was of statistically significant.

Results

Clinical Efficacy Comparison of Two Groups of Newborns

The end point had four cases off, among which, 33 cases in the observation group and 39 cases in the control group. In the observation group, one case died, two cases had cerebral palsy, and in the control group, three cases died, and five had cerebral palsy. The excellent, effective proportion and total effective rate of the newborns from the observation group were higher than that of the control group, and the differences had statistical significance ($p < 0.05$). The death rate, rate of cerebral palsy and the invalid ratio of the newborns from the observation group were lower than that of the control group. They were defined by the evaluation value of Bailey Scale total score, and the poor prognosis differences of both groups had statistical significance ($p < 0.05$) (Table II).

Early Symptoms and Recovery Time Comparison of Two Groups of Newborns

Awareness, muscle tension, primitive reflex and increased intracranial pressure recovery time of the newborns from the observation group in the early stage after treatment were less than those of the control group, and the differences had statistical significance ($p < 0.05$) (Table III).

NBNA Score Comparison of Two Groups of Newborns

The Neonatal Behavior Neurological Assessment (NBNA) score comparison of both two

Table II. Clinical efficacy comparison of two groups of newborns [n (%)].

Groups	n	Excellent	Effective	Invalid	Total effective rate (%)
Observation group	33	11 (33.33)	17 (51.52)	5 (15.15)	84.85
Control group	39	8 (20.51)	19 (48.72)	12 (30.77)	69.23
χ^2	–	3.268	2.657	3.947	4.264
p	–	0.026	0.034	0.013	0.001

Based on the boundary of Bailey scale less than 80 points (please examine) to divide to abnormal children and normal children, the comparison of abnormal children and normal children.

Groups	n	Dec. normal prognosis	Poor prognosis
Observation group	33	11 (33.33)	17 (51.52)
Control group	39	8 (20.51)	19 (48.72)
χ^2		2.345	1.938
p		0.026	0.034

Table III. Symptoms and recovery time comparison of two groups of newborns with HIE in early treatment ($\bar{x}$ ± s, day).

Groups	Awareness	Muscle tension	Primitive reflex	Increased intracranial pressure
Observation group	3.2 ± 1.3	6.3 ± 1.1	6.3 ± 1.2	5.6 ± 1.4
Control group	5.6 ± 2.3	8.9 ± 2.5	8.4 ± 2.1	9.3 ± 1.5
t	3.097	3.356	3.871	3.526
p	0.027	0.025	0.019	0.021

groups of newborns before treatment did not have any statistical significance ($p > 0.05$). After the treatment of 7, 14 and 28 days, the NBNA scores were significantly higher than that of before, and the score increased with time ($p < 0.05$). At the same time, the NBNA score of the observation group was higher than that of the control group after the treatment of 7, 14, and 28 days. The differences had statistical significance ($p < 0.05$) (Table IV).

Bayley Scale Index Comparison of Two Groups of Newborns

The comparison of the Bailey Scale Infant Development Index (MDI, PDI, and DQ) before the treatment did not have any statistical significance ($p > 0.05$). The scores of the above index of the newborns from the two groups all increased after treatment of 3, 6 and 12 months than those of before, which increased gradually with time ($p < 0.05$). The increase in MDI, PDI, and DQ scores of the observation group were more obvious than those of the control group after treatment for 3, 6 and 12 months. The differences had statistical significance ($p < 0.05$) (Table V).

Comparison of rheEP0 Adverse Reactions

A comparison of the two groups of newborns' blood pressure, liver and kidney function and abnormal electrolyte level before and after the treatment was made. The difference had no statistical significance ($p > 0.05$). The two groups of newborns did not have any adverse reactions, such as severe rash, fever, and thrombosis. The hemoglobin and reticulocyte percentage of the two groups of newborns after treatment were higher than those of before. After the comparison between these two groups, the difference had no statistical significance ($p > 0.05$). Platelet levels before and after treatment in both groups had no significant change.

Discussion

Neonatal HIE is the most common nervous system disease of perinatal infants in our country. This can lead to severe disorder of nervous system development, such as hypophrenia, cerebral palsy, spams, and dystaxia. Poor long-term and short-term prognosis is also an important disease of neonatal death[6]. However, there is no fundamental and effective prevention and control measures in the clinic, mostly from the trophic nerve, encephaledema prevention, hypothermia and complication treatment pointing to symptoms. The serious neurological sequel may bring heavy mental and economic burden to the children, their families, and society.

With the continuous research, we find that hemopoietin and gangliosides can improve the clinical effect of newborns with HIE and long-term prognosis. Wang et al[7] showed that after 6 hours of different dose of rhuEPO injections in rats the rhuEPO was found in the rat plasma, cerebrospinal fluid and brain parenchyma, which suggested that EPO can pass through injured blood-brain barrier. The EPO of cerebrospinal fluid and serum of newborns with severe HIE is higher than normal newborns and newborns with

Table IV. NBNA score comparison of two groups of newborns ($\bar{x}$ ± s, d).

Groups	Before treatment	7d	14d	28d
Observation group	26.7 ± 2.3	33.5 ± 1.7	36.2 ± 1.4	39.3 ± 1.5
Control group	27.1 ± 1.9	28.4 ± 1.5	31.2 ± 1.6	33.2 ± 1.3
t	0.347	2.393	2.638	3.257
p	0.902	0.028	0.024	< 0.001

Table V. Bayley scale score comparison of two groups of newborns with HI ($\bar{x} \pm s$, month).

Groups		Observation group	Control group	*t*	*p*
Before treatment	MDI	72.3 ± 4.3	73.2 ± 5.3	0.632	0.414
	PDI	73.8 ± 5.2	74.1 ± 4.5	0.325	0.925
	DQ	71.5 ± 6.6	72.2 ± 5.2	0.367	0.631
After 3 months	MDI	79.2 ± 7.1	75.4 ± 4.6	1.638	0.038
	PDI	80.2 ± 3.8	77.4 ± 4.6	1.526	0.041
	DQ	76.6 ± 4.9	74.9 ± 6.1	1.785	0.036
After 6 months	MDI	81.7 ± 5.5	77.6 ± 9.2	2.301	0.032
	PDI	81.3 ± 5.4	79.6 ± 5.8	2.015	0.034
	DQ	78.5 ± 9.3	76.6 ± 7.2	2.634	0.028
After 12 months	MDI	82.5 ± 3.2	79.8 ± 4.2	2.948	0.026
	PDI	82.5 ± 6.4	79.5 ± 4.8	2.745	0.028
	DQ	79.9 ± 5.4	77.5 ± 4.2	3.124	0.019

mild HIE. This proves that EPO can pass through the injured blood-brain barrier, provide nerve protection and reduce apoptosis[8,9]. Hemopoietin is a kind of protein generated by kidney secretion and erythrocyte adjustment, is also factor to promote hemocyte, which combine with receptor of hemopoietin on red cell membrane to play the function of red blood cell proliferation and differentiation[10,11]. This is a conventional drug to cure neonatal and renal anemia, without any significant adverse reactions. As nerve protection factor, EPO can interdict toxic effect of excitatory amino acid, antiapoptotic effect, antibiosis, antioxidation and angiogenesis induction, promote nerve protective effect, reduce the parenchymal damage and brain edema to protect the nerve. With the deeper studies EPO will become an important protective agent for treating cerebral ischemia injury[12-15].

GM_1 is a significant ganglioside in mammalian nervous tissue, which can pass through blood-brain barrier and play an important function for the growth, differentiation, and regeneration of neuron cell. At the same time, it can also protect the hypoxic-ischemic brain damage[16]. The GM_1 can stabilize the nerve cell membrane, prevent the nerve cell from apoptosis, and inhibit toxicity reaction of excitatory amino acids nerve. Ramirez et al[6] showed that hippocampus endogenous GM_1 of newborn rats significantly reduces through hypoxia-ischemia injury, and the reduction can promote virus and other harmful substances from invading the spinal cord. Therefore, exogenous GM_1 can release the hypoxic-ischemic brain damage[17,18]. The treatment of EPO combined with GMI to cure HIE is a new clinical mode in recent years, which achieved fruitful results. The combination of these two drugs is beneficial for the recovery of the injured brain cells and tissues and protect the nervous system[19,20]. Through this study, we discovered that the excellent, effective proportion and total effective rate of newborns from the observation group were higher than that of the control group. The differences had statistical significance. The death rate, rate of cerebral palsy and the invalid ratio of newborns from the observation group were lower than that of the control group. The differences had statistical significance. Awareness, muscle tension, primitive reflex and increased intracranial pressure recovery time of the newborns from the observation group in the early stage after treatment were less than those of the control group. The differences had statistical significance. The NBNA score of both groups of newborns after treatment of 7, 14 and 28 days were higher, and the scores increased with time. At the same time, the NBNA score of the observation group was higher than those of the control group after the treatment of 7, 14, and 28 days. The differences had statistical significance. The MDI, PDI and DQ score of the newborns from the two groups increased after treatment for 3, 6 and 12 months compared to those of before, which increased gradually with time ($p < 0.05$). Meanwhile, the increase of MDI, PDI, and DQ scores of the observation group were more obvious than those of the control group after treatment for 3, 6 and 12 months. The differences had statistical significance.

Conclusions

The treatment of rhuEPO combined with GM_1 on newborns with moderate and severe

HIE has some efficacy in improving short-term clinical effects and long-term neurological symptoms. This mode is safe and easy to operate and might become an ideal treatment for newborns with HIE.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Wixey JA, Reinebrant HE, Spencer SJ, Buller KM. 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.
2) McJarrow P, Schnell N, Jumpsen J, Clandinin T. Influence of dietary gangliosides on neonatal brain development. Nutr Rev 2009; 67: 451-463.
3) Deng XR, Lin ZL, Chen Q, Qu EL. Clinical effects of neonatal hypoxic-schemic encephalopathy treated with ganglioside and effects of tumor necrosis factor-α and interleukin-6. Chinese Journal of Postgraduate Medicine 2013; 36: 4-7.
4) Marks K, Shany E, Shelef I, Golan A, Zmora E. Hypothermia: a neuroprotective therapy for neonatal hypoxic ischemic encephalopathy. Isr Med Assoc J 2010; 12: 494-500.
5) Pfister RH, Soll RF. Hypothermia for the treatment of infants with hypoxic-ischemic encephalopathy. J Perinatol 2010; 30 Suppl: S82-S87.
6) Ramirez MR, Muraro F, Zylbersztejn DS, Abel CR, Arteni NS, Lavinsky D, Netto CA, Trindade V. Neonatal hypoxia-ischemia reduces ganglioside, phospholipid and cholesterol contents in the rat hippocampus. Neurosci Res 2003; 46: 339-347.
7) Wang Y, Zhang ZG, Rhodes K, Renzi M, Zhang RL, Kapke A, Lu M, Pool C, Heavner G, Chopp M. Post-ischemic treatment with erythropoietin or carbamylated erythropoietin reduces infarction and improves neurological outcome in a rat model of focal cerebral ischemia. Br J Pharmacol 2007; 151: 1377-1384.
8) Rogers EE, Bonifacio SL, Glass HC, Juul SE, Chang T, Mayock DE, Durand DJ, Song D, Barkovich AJ, Ballard RA, Wu YW. Erythropoietin and hypothermia for hypoxic-ischemic encephalopathy. Pediatr Neurol 2014; 51: 657-662.
9) Pei XM, Gao R, Zhang GY, Lin L, Wan SM, Qiu SQ. [Effects of erythropoietin on serum NSE and S-100B levels in neonates with hypoxic-ischemic encephalopathy]. Zhongguo Dang Dai Er Ke Za Zhi 2014; 16: 705-708.
10) Torun YA, Ozdemir MA, Ulger H, Nisari M, Akalin H, Patiroglu T, Ozkul Y, Onal M, Karakukcu M. Erythropoietin improves brain development in short-term hypoxia in rat embryo cultures. Brain Dev 2014; 36: 864-869.
11) El Shimi MS, Awad HA, Hassanein SM, Gad GI, Imam SS, Shaaban HA, El Maraghy MO. Single dose recombinant erythropoietin versus moderate hypothermia for neonatal hypoxic ischemic encephalopathy in low resource settings. J Matern Fetal Neonatal Med 2014; 27: 1295-1300.
12) Al-Salam Z. Erythropoietin may improve the Outcome in Infants with Moderate to Severe Hypoxic Ischemic Encephalopathy. J Clin Neonatol 2013; 2: 8-9.
13) Olgun Y, Kirkim G, Kolatan E, Kiray M, Ba riyanik A, erbetçio lu B, Yilmaz O, Gökmen N, Ellidokuz H, Kumral A, Sütay S. Otoprotective effect of recombinant erythropoietin in a model of newborn hypoxic-ischemic encephalopathy. Int J Pediatr Otorhinolaryngol 2013; 77: 739-746.
14) Andropoulos DB, Brady K, Easley RB, Dickerson HA, Voigt RG, Shekerdemian LS, Meador MR, Eisenman CA, Hunter JV, Turcich M, Rivera C, McKenzie ED, Heinle JS, Fraser CD Jr. Erythropoietin neuroprotection in neonatal cardiac surgery: a phase I/II safety and efficacy trial. J Thorac Cardiovasc Surg 2013; 146: 124-131.
15) Wang YJ, Pan KL, Zhao XL, Qiang H, Cheng SQ. [Therapeutic effects of erythropoietin on hypoxic-ischemic encephalopathy in neonates]. Zhongguo Dang Dai Er Ke Za Zhi 2011; 13: 855-858.
16) Weismann CM, Ferreira J, Keeler AM, Su Q, Qui L, Shaffer SA, Xu Z, Gao G, Sena-Esteves M. Systemic AAV9 gene transfer in adult GM1 gangliosidosis mice reduces lysosomal storage in CNS and extends lifespan. Hum Mol Genet 2015; 10: 168.
17) Yagi-Utsumi M, Kato K. Structural and dynamic views of GM1 ganglioside. Glycoconj J 2015; 32: 105-112.
18) Yanagisawa K. GM1 ganglioside and Alzheimer's disease. Glycoconj J 2015; 32: 87-91.
19) Koga M, Gilbert M, Li J, Yuki N. Complex of GM1- and GD1a-like lipo-oligosaccharide mimics GM1b, inducing anti-GM1b antibodies. PLoS One 2015; 10: e0124004.
20) Sokolova TV, Rychkova MP, Avrova NF. [Protective effect of GM1 ganglioside against toxic action of glutamate on cerebellar granule cells]. Zh Evol Biokhim Fiziol 2014; 50: 399-401.