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Original Article

Monosialotetrahexosylganglioside sodium combined with hyperbaric oxygen on nervous system development and brain physiology in children with hypoxic ischemic encephalopathy

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Abstract: To investigate the short-term and long-term clinical effects of monosialotetrahexosylganglioside sodium (GM1) combined with hyperbaric oxygen in neonatal hypoxic ischemic encephalopathy. A total of 80 children with hypoxic ischemic encephalopathy who were admitted to our hospital from January 2016 to March 2018 were selected and divided into the observation group and control group according to a random alphabet method, with 40 cases in each group. Neuron-specific enolase (NSE) and amplitude-integrated electroencephalogram (aEEG) were monitored after treatment in both groups, and the mental development index (MDI) and the psychomotor development index (PDI) of children were evaluated by the Bayley Scales of Infant Development (BSID) 12 months after discharge. The results showed that there were no significant differences in NSE levels and aEEG scores of children with mild severity between the two groups after treatment ($P > 0.05$). However, in both moderate and severe children, the NSE level and aEEG score in the observation group were significantly lower than that in the control group ($P < 0.05$). There were no statistically significant differences in MDI and PDI scores of children with mild severity between the two groups after 12 months of treatment ($P > 0.05$). However, in both moderate and severe children, the MDI and PDI scores in the observation group were significantly higher than that in the control group ($P < 0.05$). The incidence of adverse reactions in the observation group and the control group were 12.50% and 7.50%, respectively, ($P = 0.640$). The treatment of neonatal Neonatal hypoxic ischemic encephalopathy (HIE) with GM1 combined with hyperbaric oxygen can significantly improve the short-term and long-term nervous system development and brain physiology in children with moderate and severe HIE.

Keywords: Hypoxic ischemic encephalopathy, hyperbaric oxygen, monosialotetrahexosylganglioside sodium, nervous system development

Introduction

Neonatal hypoxic ischemic encephalopathy (HIE) is a brain injury caused by oxygen deprivation to the brain, and is also one of the major factors resulting in neurological impairments such as epilepsy, developmental delay, motor impairment, neurodevelopmental delay, and cognitive impairment [1, 2]. HIE, due to fetal or neonatal asphyxia is a leading cause of death or severe impairment among infants. Given the prevalence and severity of outcomes of HIE, many trials have been conducted to clarify the pathophysiology of neonatal HIE. There is evidence from both animal and human studies that reveal that hypothermia provides a mechanism for neuroprotection reducing the severity of brain injury leading to an improved neurological outcome in many patients [3-5]. Currently, the treatment of neonatal HIE is primarily aimed at improving the metabolic function of damaged neurons, improving cerebral blood flow and brain cell metabolism, maintaining environmental stability and controlling convulsions, etc. Monosialotetrahexosylganglioside sodium (GM1) is a neurotrophic factor

Table 1. Clinical data of children with HIE between the two groups

Parameters	Control group	Observation group	χ^2/t	*P*
Case	40	40		
Gender (n)			0.802	0.370
Male	23	19		
Female	17	21		
Gestational age (week)	38.54±1.32	39.11±1.53	0.667	0.656
Birth weight (kg)	3.26±0.43	3.23±0.51	0.758	0.633
aEEG score	3.96±1.41	4.19±1.49	1.323	0.547
NSE (μg/l)	27.32±11.42	28.25±14.64	1.112	0.596
Severity			0.465	0.793
Mild	16	14		
Moderate	15	18		
Severe	9	8		

extracted from the porcine brain, which exerts good therapeutic effects on central nervous system diseases such as Parkinson's disease, cerebral hemorrhage, cerebral palsy, cerebral atrophy, etc. Monosialotetrahexosylganglioside is a class of neurosphingolipids, usually located on neuronal membranes, and has the capability of inducing endogenous nerve growth factor and promoting repair and development of the nervous system [6]. Currently, GM1 has been gradually applied in the treatment of neonatal HIE [7]. Hyperbaric oxygen therapy is a modality in which pure oxygen is inhaled under in the environment with one more atmospheric pressure. It has become a common therapy for brain injury diseases by increasing oxygen partial pressure, blood and tissue oxygen content, and the effective diffusion distance of oxygen to improve the ischemic hypoxic state of brain tissue [8]. This study investigated the effect of GM1 combined with hyperbaric oxygen in the treatment of neonatal HIE, and discussed the effects on the nervous system development and brain physiology of the affected children, with an aim to provide guidance for clinical treatment.

Materials and methods

Clinical data

Children with HIE who were admitted to the Second Affiliated Hospital of Guizhou University of Traditional Chinese Medicine from January 2016 to March 2018 were selected as study subjects. Inclusion criteria: (1) patients who met the diagnostic criteria for neonatal HIE developed by the Neonatology Group of the Chinese Pediatric Society, Chinese Medical Association; (2) patients who were treated in Second Affiliated Hospital of Guizhou University of Traditional Chinese Medicine within 12 h of birth; (3) birth weight ≥ 2.5 kg; (4) patients without heart, liver and kidney diseases, infections and other serious systemic diseases; (5) patients without history of drug allergy; (6) patients who or whose families signed informed consent forms. Exclusion criteria: (1) patients with congenital anomalies; (2) patients with cranial injury brain malformation or anaemia; (3) patients with chromosomal abnormalities; (4) patients with drug allergy or infection. A total of 80 HIE neonates were included in this study, and the children were divided into an observation group and control group according to a random alphabet method, with 40 cases in each group. There were 42 males and 38 females in total. The gestational age of the children ranged from 37 to 41 weeks, with a mean of 38.83±1.32 weeks. The mean birth weight was 3.25±0.98 kg. There were no significant differences in the sex, gestational age, birth weight, aEEG score, NSE and severity of the children between the two groups ($P > 0.05$), as detailed in **Table 1**. This study was approved by our hospital ethics committee.

Treatment methods

All neonates with HIE were treated according to the neonatal HIE treatment regimen from the HIE Treatment Collaborative Group of Ninth Five-Year Plan Project, and treatment measures included routine symptomatic treatment such as heart rate control, blood pressure control, blood glucose control, maintenance of ventilation, anticonvulsants and electrolyte balance. The control children were treated with hyperbaric oxygen on this basis, and the treatment method was as follows: after the children entered the single oxygen chamber, the temperature of the chamber was set to 25°C, the oxygen content was 80%, the pressure was set to 0.03-0.05 MPa, the rate of ascending

Table 2. NSE levels of children with HIE between the two groups (ng/ml)

Severity	Control group	Observation group	t	P
Mild	5.76±1.46	4.36±1.68	6.754	0.118
Moderate	14.64±3.57	8.79±3.43	14.759	0.022
Severe	20.25±5.36	12.32±3.67	12.653	0.037

Table 3. aEEG scores of children with HIE between the two groups

Severity	Control group	Observation group	t	*P*
Mild	4.33±0.79	4.37±1.01	2.634	0.453
Moderate	3.16±1.15	3.64±1.36	8.477	0.270
Severe	2.35±1.42	3.02±1.74	7.532	0.045

and decreasing pressure was 2.5×10^{-3} MPa/min, the pressurization time was 10-15 min, the pressure was stable for 20-25 min and the decreasing time was 10-15 min. Hyperbaric oxygen therapy was administered once a day in a 10-day cycle, one week apart, 3 cycles in total. The addition of GM1 in the hyperbaric oxygen therapy was administered at a dose of 20 mg in the observation group (Changchun Xiangtong Pharmaceutical Co., Ltd., NMPA No.: H20066833), and the drug was added to 30 ml of 10% glucose injection for intravenous drip once a day in a 2-week cycle for 3 cycles in total.

Neuron-specific enolase (NSE) assay

One ml of venous blood was collected from children and centrifuged to determine NSE level using an enzyme-linked immunoassay (ELISA) (SenBeiJia Biological Technology Co., Ltd.) according to the manufacturer's instructions.

Amplitude-integrated electroencephalogram (aEEG) monitoring

aEEG monitoring was carried out using the NicoletOne 32-lead brain function monitoring instrument and international 10/20 standard electrode placement system, with bilateral central and parietal C3-C4 and P3-P4 as signal acquisition points, F3-F4 as reference electrode. aEEG scoring was performed based on references, with the scores between 0-5. A higher score indicated EEG closer to normal (Burdjalov et al., 2003). The aEEG was monitored for all neonates for 4 h in a natural quiet state upon admission.

Bayley scales of infant development (BSID) evaluation

Neurobehavioral development was evaluated for the children 12 months after discharge from hospital using the Bayley Scales of Infant Development (BSID), and the results were expressed as the mental development index (MDI) and psychomotor development index (PDI).

Statistical analysis

Statistical analysis was performed using SPSS Statistics 17.0 software. Count data were evaluated using the chi-square test. The measurement data were analyzed using the t-test. Data were expressed as mean ± standard deviation, frequency or percentage. $P < 0.05$ suggested a statistically significant difference.

Results

NSE levels in children with HIE between the two groups

The study showed that there was no significant difference in NSE levels of children with mild severity between the two groups after treatment, as shown in **Table 2** ($P > 0.05$). However, in both moderate and severe children, the NSE level in the observation group was significantly lower than that in the control group ($P < 0.05$).

aEEG scores of children with HIE between the two groups

There was no significant difference in aEEG scores of children with mild severity between the two groups after treatment ($P > 0.05$). However, in both moderate and severe HIE children, the aEEG score in the observation group was significantly higher than that in the control group ($P < 0.05$), as detailed in **Table 3**.

BSID scores of children with HIE between the two groups

The mental development index (MDI) and the psychomotor development index (PDI) of children were evaluated by the Bayley Scales of Infant Development (BSID) 12 months after treatment. The result showed that a higher

Table 4. BSID scores of children with HIE between the two groups

BSID	Severity	Control group	Observation group	t	*P*
MDI	Mild	92.56±4.44	94.75±4.85	1.654	0.579
	Moderate	81.53±3.38	85.37±4.42	7.536	0.020
	Severe	73.36±6.35	80.13±5.12	5.798	0.035
PDI	Mild	94.65±3.68	95.22±4.19	2.751	0.468
	Moderate	83.68±3.30	88.12±3.18	6.772	0.033
	Severe	71.54±4.71	79.27±3.73	8.774	0.019

Table 5. Adverse reactions of children with HIE between the two groups

Adverse reactions	Control group	Observation group	χ^2	*P*
Electrolyte abnormalities	1	1	3.389	0.640
Thrombocytopenia	0	1		
Blood glucose abnormalities	1	0		
Infections	2	1		
Arrhythmias	1	0		
Incidence	12.50%	7.50%		

score indicated a better treatment effect. As shown in **Table 4**, there were no statistically significant differences in MDI and PDI scores of children with mild severity between the two groups ($P > 0.05$). However, in both moderate and severe children, the MDI and PDI scores in the observation group were significantly higher than that in the control group ($P < 0.05$).

Adverse reactions in children with HIE between the two groups

The main adverse reactions during treatment of the children include electrolyte abnormalities, thrombocytopenia, blood glucose abnormalities, infections and arrhythmias. As shown in **Table 5**, the incidence of adverse reactions in the observation group and the control group were 12.50% and 7.50%, respectively, and there was no significant difference between the two groups ($\chi^2 = 3.389$, $P = 0.640$).

Discussion

Neonatal HIE may gravely endanger the life and health of newborns. At present, a variety of therapies have been implemented in major hospitals, including sub-hypothermia, hyperbaric oxygen therapy, drug therapy, etc. However, no specific treatment is recommended at present [9]. GM1 is an exogenous ganglioside that is commonly used as a nerve damage repair agent. Since ganglioside is a class of nerve cell membrane components, it can penetrate the blood-brain barrier and bind to nerve cell membranes, maintaining the ionic balance inside and outside the cell and inhibit apoptosis of nerve cells [10]. Some studies pointed out that GM1 can significantly improve motor nerve conduction velocity and sensory nerve conduction velocity in patients with diabetic peripheral neuropathy [9, 10]. After applying GM1 combined with hyperbaric oxygen for traumatic brain epilepsy, researchers found that the clinical symptoms and electroencephalograms of patients with simple partial and total seizure were significantly improved, and the earlier the medication was administered, the better the effect was [11, 12]. Hyperbaric oxygen therapy is a modality in which pure oxygen is inhaled under an environment with one or more atmospheric pressure. At present, it is widely used in cerebrovascular gas embolism, carbon monoxide poisoning, acute spinal cord injury, cerebrovascular spasm and other neurological diseases. Hyperbaric oxygen therapy has an outstanding ameliorative effect on brain tissue edema in hypoxic states by increasing oxygen partial pressure, blood and tissue oxygen content, and an effective diffusion distance of oxygen to improve the ischemic hypoxic state of brain tissue [13]. It was reported in a study that ganglioside combined with hyperbaric oxygen for neonatal HIE was as effective as 85.9%, and greatly shortened the time of primitive reflex and muscle tone in children [14]. Additionally, other treatment options for neonatal HIE include hyperbaric oxygen combined exercise and medium-frequency electrical stimulation [15], which can significantly reduce the incidence of sequelae in children. Neuron-specific enolase (NSE) is regarded as an early biochemical indicator of neonatal brain injury and also an important indicator for diagnosing the extent of neuronal damage and assessing nervous system development [16]. NSE is a soluble protein found in CNS cells and peripheral

nerve tissue, and it is very rare in normal fluids (including blood and cerebrospinal fluid). In the case of neuronal damage and necrosis, the blood-brain barrier is compromised followed by the increase in NSE levels. Hence NSE can be used as a quantitative marker of brain damage [17]. Amplitude-integrated electroencephalogram (aEEG) has high sensitivity and specificity in brain function monitoring, which can sensitively reflect the physiological changes in the brain during the early stages of cerebral ischemia and hypoxia, and is also an effective method to detect clinical seizures and reversible changes in brain function, with high diagnostic value for prognostic evaluation [18]. In children with mild HIE, the brain damage is mainly a state of cerebral edema, which has not yet progressed and therefore most recover completely within a week or so. In this study, the treatment effect of children with different severity of disease was investigated separately. After treatment, there were no significant difference in the aEEG scores and NSE levels of HIE children with mild disease between the two groups, suggesting that the prognosis of neonates with mild HIE was relatively good even without the addition of GM1, namely hyperbaric oxygen alone. However, long-term prognostic assessment and follow-up are still needed to determine their chronic efficacy. Notably, at the end of treatment, in children with moderate and severe HIE, NSE levels were significantly lower in the observation group than in the control group, whereas aEEG scores were significantly higher in the observation group than in the control group. It was shown that compared with hyperbaric oxygen therapy alone, hyperbaric oxygen combined with GM1 had a better therapeutic effect for children with severe HIE, and could significantly improve the nervous system development and brain physiology of the children. This was due to not only the improvement of the ischemic hypoxic state of the brain tissue in children by hyperbaric oxygen therapy, but also the induction of endogenous nerve growth factor secretion and promotion of neurological repair and development by GM1 [19, 20].

Moreover, the mental development index (MDI) and the psychomotor development index (PDI) of children were evaluated by the Bayley Scales of Infant Development (BSID) 12 months after treatment, in order to show the chronic effects of the two therapies. The study demonstrated that there were no statistically significant differences in MDI and PDI scores of children with mild severity between the two groups, however, in both moderate and severe childrden, the MDI and PDI scores in the observation group were significantly higher than that in the control group, which indicated that hyperbaric oxygen therapy had a better chronic therapeutic effect on children with mild HIE. However, the addition of GM1 in the hyperbaric oxygen therapy for moderate and severe children could remarkably improve their neurological development and the level of neurobehavioral development at long-term follow-up. What's more, there was no significant difference in the incidence of adverse reactions between the observation group and the control group in this study. Moreover, the adverse reactions either disappeared on their own or after symptomatic treatment, and no serious adverse reactions were observed, which indicated that the treatment in this study had a better safety profile. However, due to selection bias that occurred when the selection of individuals is not randomized in a wider scope and confounders not been concerned, the outcome could be inaccurate, unreliable or unavailable. More research with wider scope and more accurate variables are needed in the future.

In conclusion, HIE treatment with GM1 combined with hyperbaric oxygen therapy can significantly improve the short-term and long-term nervous system development and brain physiology in children with moderate and severe HIE, which indicates that during clinical treatment, hyperbaric oxygen therapy can be used if the disease severity of the children is mild; the addition of GM1 therapy should be considered if the disease severity of children is moderate or severe.

Disclosure of conflict of interest

None.

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