ORIGINAL ARTICLE

Comparative Effects of Monosialoganglioside *versus* Citicoline on Apoptotic Factor, Neurological Function and Oxidative Stress in Newborns with Hypoxic-Ischemic Encephalopathy

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Abstract

Objective: To determine the comparative effect of monosialoganglioside *versus* citicoline on the content changes of serum apoptotic factors (PDCD5, sFas and sFasL), neurological function indices (BDNF, NSE, S100-β and NGF) and oxidative stress indices (SOD, MDA and GSH-PX) in newborns with hypoxic-ischemic encephalopathy (HIE).

Study Design: An experimental study.

Place and Duration of Study: Emergency Department, Affiliated Children's Hospital of Zhengzhou University, China, from October 2016 to February 2018.

Methodology: A total of 90 newborns with HIE were randomly divided into a treatment group and a control group, with 45 cases in each group. In addition to the conventional treatment, the treatment group was given monosialoganglioside treatment, while the control group was given citicoline treatment. Both groups were treated for 10 days. After treatment, the content differences of serum apoptosis factors (PDCD5, sFas and sFasL), neurological function indices (BDNF, NSE, S100-β and NGF) and oxidative stress indices (SOD, MDA and GSH-PX) were observed in the two groups.

Results: After treatment, the levels of serum PDCD5, sFas, sFasL, MDA, NSE and S100-β in the treatment group were lower than those in the control group (all $p<0.001$). The contents of serum SOD, GSH-PX, BDNF and NGF in the treatment group were higher than those in the control group (all $p<0.001$).

Conclusion: Monosialoganglioside can effectively improve the apoptotic factors, neurological function and oxidative stress indices in newborns and maintain the stability of the internal environment, so it is worthy of promotion and application.

Key Words: *Newborn, Hypoxic-ischemic encephalopathy (HIE), Monosialoganglioside, Citicoline.*

INTRODUCTION

Hypoxic-ischemic encephalopathy (HIE) is mainly caused by intrauterine hypoxia and partial or complete hypoxia caused by perinatal asphyxia, fetal or neonatal brain injury caused by cerebral blood flow reduction or pause.[1,2] HIE is not only a serious threat to the life and health of newborns, but also the most common cause of cerebral palsy, mental retardation, hearing impairment, and epilepsy in children. Itoo *et al*. pointed out that the important associated risk factors of HIE included being a primigravida mother, lack of antenatal care, pregnancy induced hypertension, prolonged 2nd stage of labor, delivery by use of instruments or emergency cesarean section and intrauterine growth retardation.[3] Early, timely and effective treatment can improve the cure rate of children with HIE, reduce the mortality rate of children and improve the prognosis of the nervous system. However, the condition of HIE is complex and serious, and the pathogenesis is not yet completely clear. Therefore, HIE treatment has not achieved break-through progress, and there is no unified effective treatment with the use of drugs and other interventional methods, but mainly based on comprehensive supportive and symptomatic care.[4,5] Medical therapy is an important part of HIE treatment. Therapeutic agents include barbiturates, allopurinol, magnesium sulfate, mannitol, naloxone, dopamine and others isoflourine, allopurinol, erythropoeitin. However, there is no clinical evidence that these drugs can significantly reduce the mortality rate of children with HIE and improve the prognosis of the nervous system.[6,7] How to choose drugs needs to be further studied.

Monosialoganglioside is a widely used drug for the treatment of nervous system diseases. It has a good curative effect on vascular or traumatic central nervous system injury and senile dementia. It can also be used as an enhancer of neurotrophic factors to promote the recovery of neurological function. Clinically, it is mainly used in the treatment of acute ischemic stroke, primary brain stem injury, acute spinal cord injury, and peripheral nerve injury.[8] Animal experiments have also confirmed that monosialoganglioside has neuroprotective effects

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on rats with neonatal hypoxic-ischemic brain damage, which can reduce the occurrence of cerebral edema and convulsions, promote the recovery of brain damage, and increase learning and memory function of aged rats with neonatal hypoxic-ischemic brain damage.[9] Citicoline (CDP-choline; cytidine 5'-diphosphocholine), is a nucleic acid derivative, and it belongs to the precursor substance of phosphatidylcholine and coenzyme biosynthesized by lecithin.[10] Citicoline has been evaluated in animal experiments and human clinical trials that provide evidence of its cholinergic and neuroprotective actions.[11] Some studies have suggested that citicoline can reduce cerebral vascular resistance, increase cerebral blood flow perfusion, reduce cerebrovascular palsy and cerebral edema, and promote the recovery of brain tissue.[12]

The objectives of this study was to determine the comparative effect of monosialoganglioside *versus* citicoline on the content changes of serum apoptotic factors (PDCD5, sFas and sFasL), neurological function indices (BDNF, NSE, S100-β and NGF) and oxidative stress indices (SOD, MDA and GSH-PX) in newborns with hypoxic-ischemic encephalopathy (HIE).

METHODOLOGY

This study was conducted at the Emergency Department, Affiliated Children's Hospital of Zhengzhou University, China, from October 2016 to February 2018. A total of 90 newborns with HIE, meeting the criteria of Diagnostic Basis and Clinical Grading of Neonatal Hypoxic-ischemic Encephalopathy formulated by China in 1996, all grades, were included. The diagnosis of brain damage was confirmed by CT brain examination and it met the diagnostic criteria for HIE, established by the World Health Organization (WHO) reference standard; in absence of other severe congenital organ dysfunction. The family members of children understood the research process and signed the informed consent. Exclusion criteria were congenital cerebral vascular dysfunction; interruption of treatment or family members voluntarily waiving treatment; and incomplete clinical data.

The 90 newborns were randomly divided into a treatment group and a control group by using a random number table, with 45 cases in each group. Both groups were given the conventional basic treatment, including reducing intracranial pressure, controlling convulsions, providing the daily fluid requirement, correcting hypotension, maintaining blood glucose in the normal range, and maintaining water and electrolyte balance. In the control group, 125 mg citicoline was added to 20 mL of 5% glucose injection and infused once a day for 10 days. The treatment group received monosialoganglioside on the basis of routine treatment, *i.e.*, 20 mg monosialoganglioside was added to 20 mL of 5% glucose injection and infused once a day for 10 days. After 10 days of treatment in both the groups, one ml of blood was collected and left to stand at room temperature and centrifuged to remove the supernatant for serological detection. The content of programmed cell death gene 5 (PDCD5), soluble Fas (sFas), and soluble Fas ligand (sFasL) were determined by ELISA double antibody sandwich method. Neurological function indices, *i.e.* brain-derived neurotrophic factor (BDNF), neuron-specific enolase (NSE), S100-β protein (S100-β), and nerve growth factor (NGF) were determined by ELISA. Oxidative stress indices including superoxide dismutase (SOD), malondialdehyde (MDA), and superoxide dismutase (GSH-PX) were determined by spectrophotometer method.

All the data were analysed by the Statistical Package for Social Sciences (SPSS) computer software programme (version 25). The levels of serum apoptotic factors, serum neurological function indices, serum oxidative stress indices were analysed by applying independent sample t-test for the detection of any significant differences between the treatment group and control group. The resulting data was expressed as mean ± SD (standard deviation). A p-value of <0.05 was considered as significant.

RESULTS

Among the 90 newborns, 49 were males (54.44%) and 41 were females (45.56%); gestational age was 35-41 weeks, with the mean gestational age of 38.15 ±2.76 weeks; and birth weight was 2.41-3.92 Kg, with mean birth weight of 2.83 ±0.52 Kg. Disease grading SERNET Grading of HIE was mild in 52 cases (57.78%), moderate in 34 cases (37.78%), and severe in 4 cases (4.44%). The mean gestational age of newborns in the treatment group was 38.06 ±2.95 weeks, and 38.24 ±2.51 weeks in the control group.

After treatment, levels of serum PDCD5, sFas, and sFasL in the treatment group were lower than those in the control group (all p<0.001, Table I). Levels of serum BDNF and NGF in the treatment group were higher than those in the control group (both p<0.001). The levels of NSE and S100-β in the treatment group were lower than those in the control group (both p<0.001, Table II). Levels of serum SOD and GSH-PX in the treatment group were higher than those in the control group (both p<0.001). The content of MDA in the treatment group was lower than that in the control group (p<0.001, Table III).

Table I: Comparison of the levels of serum apoptotic factors after treatment between the two groups.

Parameter	Control group (Mean ± SD) n=45	Treatment group (Mean ± SD) n=45	p-value
PDCD5 (μg/L) n=45	10.64 ±1.66	6.25 ±0.58	<0.001
sFas (μg/L) n=45	18.57 ±2.61	10.16 ±1.69	<0.001
sFasL (μg/L) n=45	4.14 ±1.05	2.07 ±0.52	<0.001

SD=Standard deviation.

Table II: Comparison of the levels of serum neurological function indices after treatment between the two groups.

Parameter	Control group (Mean ± SD) n=45	Treatment group (Mean ± SD) n=45	p-value
BDNF (μg/L) n=45	1396.21 ±169.22	1823.85 ±241.56	<0.001
NSE (μg/L) n=45	14.57 ±1.57	9.34 ±0.54	<0.001
S100-β (μg/L) n=45	2.37 ±0.34	1.18 ±0.57	<0.001
NGF (μg/L) n=45	121.45 ±36.56	146.39 ±24.11	<0.001

SD=Standard deviation.

Table III: Comparison of the levels of serum oxidative stress indices after treatment between the two groups.

Parameter	Control group (Mean ± SD) n=45	Treatment group (Mean ± SD) n=45	p-value
SOD (U/L) n=45	73.51 ±6.03	86.85 ±6.52	<0.001
MDA (mol/L) n=45	8.32 ±0.55	5.74 ±0.49	<0.001
GSH-PX (U/mL) n=45	38.53 ±4.51	53.12 ±5.10	<0.001

SD=Standard deviation.

DISCUSSION

The purpose of clinical treatment of HIE is to maintain internal environment stability, improve damaged neurons, control convulsions, and promote cerebral circulation as much as possible. To find a reasonable and effective therapeutic drug for HIE is the focus of current research and treatment. Monosialoganglioside is neuroprotective agents that are widely found in the cell membrane nervous system of mammalian. Some studies have confirmed that monosialoganglioside has the ability to promote functional recovery of CNS injury caused by various causes, and possesses significant protection against neurodegeneration caused by injury.[13,14] In addition, studies have also found that monosialoganglioside has the ability to protect against excitatory amino acid toxicity; and reduce cell death, and brain edema.[15] The mechanism of protection and repair of damaged nerves by monosialoganglioside mainly manifests in many aspects. Monosialo-ganglioside can promote the growth of nerve cords, activate neurotrophic factors, reduce the death of cerebral neurons, cerebral cortical neurons, hippocampal pyramidal neurons and nerve cells, and inhibit damage to neurons by toxic products.[16] Monosialoganglioside can protect calcium pumps, reduce intracellular calcium overload caused by calcium influx, protect cerebral ischemia, reduce edema, and promote recovery of damaged neuronal structures and functions.[17] In this study, monosialoganglioside was used as adjunctive therapeutics in children with HIE in our hospital. The role of monosialoganglioside was studied from the perspective of serological indicators.

Apoptosis and local neurological impairment caused by ischemia and hypoxia are the basis for the development of HIE. PDCD5, sFas, and sFasL are recognized as pro-apoptotic factors.[18] In this study, the authors found that the levels of serum PDCD5, sFas, and sFasL in the treatment group were lower than those in the control group; which indicated that after treatment, the degree of neuronal apoptosis decreased in the treatment group, and monosialoganglioside had a better anti-apoptotic effect than citicoline.

Some studies have suggested that monosialo-ganglioside can reduce the release of excitatory amino acids such as amino acids which can have a direct killing effect on nerve cells; consequently, it is speculated that monosialoganglioside plays a role in anti-apoptosis of neural cells by this way.[19] Apoptosis of nerve cells will directly cause neurological impairment in children with HIE. Both BDNF and NGF have the function of nourishing nerves and promoting the growth of axons.[20] The levels of BDNF and NGF were significantly decreased in children with HIE, which was a direct sign of neurological impairment in children. NSE and S100-β have very low serological levels in physiological conditions. They exist only in nerve cells and are released into the extracellular space when neuronal damage occurs. They enter the peripheral blood through the blood-brain barrier and the changes in the content are detected. In this study, it was found that the levels of serum BDNF and NGF in the treatment group were higher, and the levels of NSE and S100-β were lower, which suggested that monosialoganglioside had neurotrophic effects of promoting nerve regeneration and reducing nerve cell destruction. This conclusion is also consistent with previous findings.[21]

Intracellular calcium overload in neurons and the resulting damage to oxygen-free radicals have been one of the most important causes of the exacerbation of HIE and the expansion of nerve injury. Effective clearance of oxygen-free radicals in children is one of the keys to HIE therapy.[22] In this study, the content of oxidative stress indices in the two groups was detected. It was found that the level of serum MDA was lower and the levels of SOD and GSH-PX were higher in the treatment group after treatment, which revealed that the oxidation / anti-oxidation balance system in children with HIE was optimized after treatment with monosialoganglioside.

The limitations of this study relate to the sample size and focus on only the biochemical effects. The neurological outcome of the newborns with HIE, and outcome of these neonates and correlation with the lab findings need further exploration and research.

CONCLUSION

Monosialoganglioside can effectively improve the apoptotic factors, neurological function, and oxidative

stress indices in newborns and maintain the stability of the internal environment, so it is worthy of promotion and application.

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