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Effect of mild hypothermia combined with VitC and EPO therapy on target organ damage in children with neonatal asphyxia

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ABSTRACT

Objective: To study the effect of mild hypothermia combined with vitamin C (VitC) and erythropoietin (EPO) therapy on target organ damage in children with neonatal asphyxia. **Methods:** Children with neonatal asphyxia who were treated in Taihe County People′s Hospital between April 2014 and February 2017 were selected and randomly divided into two groups, mild hypothermia group received mild hypothermia combined VitC and EPO therapy, and control group received VitC and EPO therapy. Serum levels of of target organ damage markers, oxidative stress indexes and apoptosis indexes were measured before treatment as well as 3 d and 7 d after treatment. **Results:** 3 d and 7 d after treatment, serum NSE, H-FABP, cTnI, CysC, MDA, Caspase-3, PDCD5, sFas and sFasL levels of both groups of children were significantly lower than those before treatment while TAS, SOD, GSH and Bcl-2 levels were significantly higher than those before treatment, and serum NSE, H-FABP, cTnI, CysC, MDA, Caspase-3, PDCD5, sFas and sFasL levels of mild hypothermia group were significantly lower than those of control group while TAS, SOD, GSH and Bcl-2 levels were significantly higher than those of control group. **Conclusion:** Mild hypothermia combined with VitC and EPO therapy can reduce the target organ damage of children with neonatal asphyxia by inhibiting oxidative stress and apoptosis.

1. Introduction

Neonatal asphyxia is a neonatal disease with high fatality rate and disability rate, and the asphyxia can cause ischemia anoxia and increase the injury to multiple organs in the body[1,2]. Massive oxygen free radical generation in ischemia anoxic condition is an important pathological link causing multiple organ damage, and anti-oxidation is an important means to treat neonatal asphyxia and reduce the viscera damage caused asphyxia[3]. Vitamin C (VitC) and erythropoietin (EPO) are the drugs that protect the cells, the former has direct antioxidant effect and can remove oxygen free radicals and reduce the tissue damage caused by oxidative stress[4], and the latter can be combined with the receptors on the cell membrane to inhibit apoptosis, reduce oxidative stress and inflammatory response, and thereby reduce tissue damage[5]. Mild hypothermia therapy is a physical therapy developed in recent years, which reduces the release of oxygen free radicals, inflammatory factors, excitatory amino acids and other traumatic mediators through physical cooling so as to help reduce tissue damage. The effect of mild hypothermia combined with VitC and EPO therapy on target organ damage in children with neonatal asphyxia was analyzed in the following studies.

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2. Research subjects and methods

2.1 General information of research subjects

Children with neonatal asphyxia who were treated in Taihe County People′s Hospital between April 2014 and February 2017 were selected, all children were in accordance with the diagnostic criteria for neonatal asphyxia, and the children with congenital diseases were excluded. A total of 68 children were enrolled in the study, and the random number method was used to divide them into two groups, each with 34 cases. Mild hypothermia group received mild hypothermia combined VitC and EPO therapy, including 20 male

cases and 14 female cases with gestational age of 37-40 weeks and weight of 3.1-4.2 kg; control group received VitC and EPO therapy, including 19 male cases and 15 female cases with gestational age of 37-41 weeks, and weight of 3.0-4.3 kg. There was no statistically significant difference in general information between the two groups (P>0.05).

2.2 Therapy

Both groups of children received conventional supportive treatment, including maintaining water and electrolyte balance, neuroprotection, nutritional support, etc. Control group received intravenous drip of the solution of 250 mg/kd vitamin C for injection and 20 mL 10% glucose injection, 1 time/d as well as intravenous injection of recombinant human erythropoietin 500 U/kg, 3 times per week; mild hypothermia group received mild hypothermia therapy on the basis of vitamin C and recombinant human erythropoietin therapy, and the method was as follows: the children were put on hypothermia water cushion, the cushion was filled with temperature-variable circulating solution, the anal temperature was measured once every 10 min, and the temperature of hypothermia water cushion was adjusted to make anus temperature rise to 33.5 ℃ in 1 h and last for 3 d; 3 d later, the anus temperature was measured once every 12 h to ensure the anal temperature rise less than 0.5 ℃ per hour .

2.3 Serum index detecting

Before treatment as well as 3 d and 7 d after treatment, 3 mL of peripheral venous blood was collected and centrifuged to separate serum, enzyme-linked immunosorbent assay kit was used to determine serum NSE, H-FABP, cTnI, CysC, Caspase-3, PDCD5, Bcl-2, sFas and sFasL levels, and radioimmunoprecipitation kit was used to determine the contents of MDA, TAS, SOD and GSH.

2.4 Statistical methods

SPSS 17.0 software was used to input and analyze data, measurement data analysis between two groups was by t test and P<0.05 indicated statistical significance in differences.

3. Results

3.1 Target organ injury marker levels

Before treatment as well as 3 d and 7 d after treatment, analysis of target organ injury markers NSE, H-FABP, cTnI and CysC between two groups of children was as follows: serum NSE, H-FABP, cTnI and CysC levels were not significantly different between two groups of children before treatment; 3 d and 7 d after treatment, serum NSE, H-FABP, cTnI and CysC levels of both groups of children were significantly lower than those before treatment, and serum NSE, H-FABP, cTnI and CysC levels of mild hypothermia group were significantly lower than those of control group.

3.2 Oxidative stress index levels

Before treatment as well as 3 d and 7 d after treatment, analysis of oxidative stress indexes MDA (μmol/L), Caspase-3 (U/L), TAS (mmol/L), SOD (U/L) and GSH (U/L) between two groups of children was as follows: serum MDA, Caspase-3, TAS, SOD and GSH levels were not significantly different between two groups of children before treatment; 3 d and 7 d after treatment, serum MDA and Caspase-3 levels of both groups of children were significantly lower than those before treatment while TAS, SOD and GSH levels were significantly higher than those before treatment, and serum MDA and Caspase-3 levels of mild hypothermia group were significantly lower than those of control group while TAS, SOD and GSH levels were significantly higher than those of control group.

Table 1.

Changes in target organ injury markers before and after treatment (ng/mL).

Groups	n	Time	NSE	H-FABP	cTnI	CysC
Mild hypothermia group	34	Before treatment	62.31±7.79	30.49±5.58	1.58±0.20	2.42±0.32
		3 d after treatment	32.52±4.26^{*a}	17.65±2.03^{*a}	0.77±0.09^{*a}	1.77±0.20^{*a}
		7 d after treatment	21.42±2.57^{*a}	11.24±1.46^{*a}	0.45±0.06^{*a}	1.32±0.15^{*a}
Control group	34	Before treatment	62.89±7.76	31.02±5.27	1.62±0.21	2.48±0.36
		3 d after treatment	47.63±6.61^{a}	24.25±3.26^{a}	1.14±0.15^{a}	2.02±0.27^{a}
		7 d after treatment	35.42±4.49^{a}	17.68±2.03^{a}	0.87±0.10^{a}	1.67±0.19^{a}

*: comparison of indexes between mild hypothermia group and control group after treatment, P<0.05; ^{a}: comparison of indexes within group between before and after treatment, P<0.05.

Table 2.

Changes in oxidative stress indexes before and after treatment.

Groups	n	Time	MDA	Caspase-3	TAS	SOD	GSH
Mild hypothermia group	34	Before treatment	5.62±0.77	3.82±0.46	523.5±67.6	68.62±7.86	75.51±9.34
		3 d after treatment	3.31±0.46^{*a}	2.31±0.32^{*a}	731.2±78.9^{*a}	87.61±9.86^{*a}	96.72±11.26^{*a}
		7 d after treatment	2.18±0.28^{*a}	1.77±0.20^{*a}	774.5±88.5^{*a}	98.33±10.35^{*a}	114.25±13.32^{*a}
Control group	34	Before treatment	5.58±0.72	3.88±0.48	527.6±64.6	69.11±8.24	76.12±8.92
		3 d after treatment	4.29±0.56^{a}	3.14±0.42^{a}	633.1±76.8^{a}	75.65±8.92^{a}	84.52±10.25^{a}
		7 d after treatment	3.42±0.45^{a}	2.72±0.34^{a}	698.3±80.3^{a}	82.31±8.95^{a}	98.52±11.27^{a}

*: comparison of indexes between mild hypothermia group and control group after treatment, P<0.05; ^{a}: comparison of indexes within group between before and after treatment, P<0.05.

Table 3.

Changes in apoptosis indexes before and after treatment (ng/mL).

Groups	*n*	Time	PDCD5	Bcl-2	sFas	sFasL
Mild hypothermia group	34	Before treatment	23.41±3.52	6.41±0.77	17.85±2.03	13.26±1.78
		3 d after treatment	13.22±1.56[*a]	11.25±1.32[*a]	10.28±1.44[*a]	8.39±1.03[*a]
		7 d after treatment	9.39±1.03[*a]	15.63±1.78[*a]	7.64±0.89[*a]	6.64±0.77[*a]
Control group	34	Before treatment	23.66±3.72	6.52±0.79	18.01±1.94	13.52±1.72
		3 d after treatment	19.52±2.32[a]	8.89±1.02[a]	14.57±1.76[a]	10.78±1.32[a]
		7 d after treatment	14.48±1.78[a]	10.25±1.34[a]	10.38±1.38[a]	8.85±0.93[a]

[*]: comparison of indexes between mild hypothermia group and control group after treatment, $P<0.05$; [a]: comparison of indexes within group between before and after treatment, $P<0.05$.

3.3 Apoptosis index levels

Before treatment as well as 3 d and 7 d after treatment, analysis of apoptosis genes PDCD5, Bcl-2, sFas and sFasL between two groups of children was as follows: serum PDCD5, Bcl-2, sFas and sFasL levels were not significantly different between two groups of children before treatment; 3 d and 7 d after treatment, serum PDCD5, sFas and sFasL levels of both groups of children were significantly lower than those before treatment while Bcl-2 levels were significantly higher than those before treatment, and serum PDCD5, sFas and sFasL levels of mild hypothermia group were significantly lower than those of control group while Bcl-2 levels were significantly higher than those of control group.

4. Discussion

Neonatal asphyxia is with high fatality rate and disability rate, and the clinical treatment is quite difficult. The ischemia hypoxia caused by asphyxia will on the one hand, directly affect the aerobic metabolism of normal tissues and cells and reduce energy generation while accelerate anaerobic metabolism and cause lactic acid accumulation, and the continuous accumulation of lactic acid in local tissue can cause acidosis and cell damage. The ischemia hypoxia, on the other hand, can also significantly increase the production of oxygen free radicals and cause the oxidation of lipid, protein and other compositions in cell structure thorough oxidative stress reaction, resulting in cellular structure destruction and functional damage[6]. Antioxidant drugs are commonly used for clinical treatment of neonatal asphyxia, VitC, also known as ascorbic acid, is a kind of non-enzyme antioxidant and can remove oxygen free radicals and reduce oxidative stress damage to the tissue[7]; EPO is a cytoprotective hormone that can not only directly inhibit the generation of free radicals, but also remove free radicals by increasing the activity of antioxidant enzymes[8]. Mild hypothermia treatment is the physical therapy developed in recent years, and it reduces the local temperature to suppress the metabolic activity of tissues and cells, which on the one hand, reduces the oxygen demand and strengthens the tissue tolerance to hypoxia, and on the other hand, also reduces the release of oxygen free radicals, inflammatory factors, excitatory amino acids and other traumatic mediators[9,10].

In the above studies, mild hypothermia therapy was used on the basis of VitC and EPO therapy so as to play the roles of hypothermia on enhancing the tissue tolerance to hypoxia and reducing the release of traumatic mediators. Myocardium, brain and kidney are the common involved target organs in the process of neonatal asphyxia, and the viscera injury caused by ischemia hypoxia can cause the marker molecules in relevant cells to be released into the blood circulation. NSE is an enolase specifically existing in neurons and neuroendocrine cells, and it is involved in the regulation of glycolytic pathways[11]; H-FABP and cTnI are the proteins specifically expressed in myocardial cells, the former participates in the regulation of fatty acid metabolism and the latter participates in the formation of the cytoskeleton[12]; CysC is a small molecule that can freely pass the glomeruli and be reabsorbed and degraded in the renal tubule. The damage of renal function can result in the CysC excretion disorder and the increase in its content[13]. In the study, analysis of the changes in myocardium, brain, kidney and other target organ damage markers indicated that serum NSE, H-FABP, cTnI and CysC levels of both groups of children decreased after treatment, and serum NSE, H-FABP, cTnI and CysC levels of mild hypothermia group after treatment were lower than those of control group. This indicates that VitC and EPO therapy can alleviate the damage of the myocardium, brain, kidney and other target organs in the course of neonatal asphyxia, and the combination of mild hypothermia can further reduce the damage of target organs.

Massive generation of oxygen free radicals and excessive activation of oxidative stress response are the important pathological links in the course of neonatal asphyxia[14]. The lipid in the cell membrane and mitochondrial membrane structure is the composition most vulnerable to the oxygen free radical attack, the oxidation reaction between lipid and oxygen free radicals will generate MDA, and also cause the destruction of the cell membrane and mitochondrial membrane structure; the destruction of the cell membrane structure will directly cause cell function damage, and the destruction of the mitochondrial membrane structure can cause cytochrome C release into the cytoplasm, start the activation of caspase-3 and cause cell death[15]. SOD and GSH are the catalytic enzymes that have antioxidant effects in the body, which can reduce oxygen free radicals to hydrogen peroxide and further reduce the hydrogen peroxide to water, which was eliminated from the body; excessively generated oxygen free radicals in neonatal asphyxia will cause SOD and GSH to be continuously consumed and cause TAS to decrease[16]. In the study, analysis of the changes in oxidative stress products and anti-oxidation indexes showed that serum MDA and Caspase-3 levels of both groups of children significantly decreased while TAS, SOD and GSH levels significantly increased after treatment, and serum MDA and Caspase-3 levels of mild hypothermia group after treatment were significantly lower than those of control group while TAS, SOD and

GSH levels were significantly higher than those of control group. This indicates that VitC and EPO therapy can inhibit the generation of oxygen free radicals and reduce oxidative stress response, and the combination of mild hypothermia can further enhance the antioxidant capacity and inhibit oxidative stress response.

The activation of oxidative stress in neonatal asphyxia is not only directly responsible for the destruction of cell structure and function, but can also initiate the apoptosis mediated by multiple pathways and cause cell damage. PDCD5 is a newly discovered pro-apoptotic molecule in recent years, which can combine with activated Caspase-3 molecules and enhance the pro-apoptotic activity of Caspase-3[17]. Fas/FasL and Bcl-2 are the apoptotic molecules that regulate Caspase-3 activation through the death receptor pathway and mitochondrial pathway respectively, Fas and FasL combination can make Caspase-8 activated through FADD structure domain, and then cause Caspase-3 activation through cascade reaction[18,19]; Bcl-2 can inhibit the release of cytochrome C in mitochondria and inhibit the activation of Caspase-3[20,21]. In the study, analysis of the changes in serum pro-apoptosis and anti-apoptosis molecules showed that serum PDCD5, sFas and sFasL levels of both groups of children significantly decreased while Bcl-2 levels significantly increased after treatment, and serum PDCD5, sFas and sFasL levels of mild hypothermia group after treatment were significantly lower than those of control group while Bcl-2 levels were significantly higher than those of control group. This shows that VitC and EPO therapy can inhibit apoptosis, and the combination of mild hypothermia can further decrease the generation of pro-apoptosis molecules, increase the generation of anti-apoptosis molecules and inhibit apoptosis.

Mild hypothermia combined with VitC and EPO can reduce the target organ damage of children with neonatal asphyxia, and reducing oxygen free radical generation, enhancing antioxidant capacity and inhibiting apoptosis are the molecular pathways for mild hypothermia combined with VitC and EPO to protect the organs.

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