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Study on sildenafil combined with inhalational nitric oxide therapy on the curative effects and serum levels of HIF-1α, ET-1, and calcium in neonatal pulmonary hypertension

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Abstract. – OBJECTIVE: To explore the effect of sildenafil combined with inhalational nitric oxide (NO) therapy on the curative effects and serum levels of hypoxia-inducible factor (HIF)-1α, endothelin-1 (ET-1), and calcium in persistent pulmonary hypertension of the newborn (PPHN).

PATIENTS AND METHODS: Eighty-six patients with neonatal pulmonary hypertension treated in Xuzhou Children's Hospital from March 2015 to February 2016 were randomly divided into the observation group and control group, treated with sildenafil and sildenafil combined with inhalational NO, respectively. The clinical efficacy of newborns in the two groups was compared. Fraction of inspiration O_2 (FiO_2), Oxygen Index (OI), blood oxygen partial pressure (PaO_2), blood oxygen saturation (SpO_2), and pulmonary arterial pressure of newborns in the two groups were compared before treatment and 2 h, 12 h, and 24 h after treatment. The serum levels of HIF-1α, ET-1, and calcium of patients in the two groups were compared before treatment and 3, 5, 7 days after treatment.

RESULTS: The total effective rate of the observation group (95.34%) was significantly higher than that of the control group (74.41%) (p<0.05). After treatment, FiO2, OI, and pulmonary arterial pressure of patients in the two groups decreased, and the decrease in the observation group was significantly lower than in the control group (p<0.05). After treatment, PaO2 and SpO_2 of patients in the observation group were higher than those of the control group. The levels of HIF-1α and ET-1 of patients in the two groups decreased and were significantly lower in the observation group compared with the control group. The levels of calcium of patients in the two groups increased and were significantly higher in the observation group than the control group (p<0.05).

CONCLUSIONS: Sildenafil combined with inhalational NO therapy for neonatal pulmonary hypertension can quickly improve oxygenation, effectively reduce pulmonary arterial hypertension, and is worthy of clinical application.

Key Words:
Sildenafil, Nitric oxide, Inhalation, Neonates, Pulmonary hypertension.

Introduction

Persistent pulmonary hypertension of the newborn (PPHN) is a common critical illness of the lung with an extremely high fatality rate of around 50%[1]. Various pathogenic factors (pulmonary vasospasm, pneumonia, respiratory distress syndrome, asphyxia, inhalation of amniotic fluid and meconium, and urinary tract infection during pregnancy) lead to dysfunction of resistance and pressure in the pulmonary circulation after birth, which causes the pulmonary artery pressure to increase and surpass the arterial pressure in the general circulation, thus, causing blood shunting from right to left, resulting in severe cyanosis, respiratory failure, cardiac insufficiency, acidosis, and other symptoms[2,3]. Nitric oxide (NO) is an alternative pulmonary vasodilatory substance. It can prevent the collapse of pulmonary alveoli, and reduce resistance in the pulmonary vasculature. It is a gold standard for the treatment of PPHN[4]. Sildenafil can improve the local microcirculation of newborns, and effectively contain and prevent damage to the lungs[5]. In this study, sildenafil was combined with inhalational NO for the treatment of PPHN, and desirable results were obtained.

Patients and Methods

Patients

Eighty-six newborns with PPHN admitted to the Neonatology Department of Xuzhou Chil-

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dren's Hospital from March 2015 to February 2016 were included in this study. Inclusion criteria: (1) According to the standards defined by the World Health Organization, pulmonary arterial systolic pressure was > 4.0 kPa[6]; (2) Clinical manifestations included cyanosis, acceleration of respiratory rate, peripheral branches of the pulmonary artery appearing slender and the right ventricle expanded by X-ray examination; (3) Parents signed the informed consent forms. Exclusion criteria: (1) Pneumothorax and hemorrhagic disorders; (2) Congenital heart disease. The patients were randomly and equally divided into the observation group and control group, with 43 cases each. The baseline parameters of the two groups of newborns are shown in Table I. The study was approved by the Ethics Committee of Xuzhou Children's Hospital.

Treatment

The secretions in the oral cavity and nasal cavity of newborns were cleared up. The newborns in the control group were treated with NO inhalation. Newborns were placed in an incubator to receive mechanical ventilation with high-frequency oscillation (frequency: 1000-2000 times/min). The low discharge placed NO with a concentration of 800 ppm after decompression into the output loop of the respirator. The initial value was 10 ppm. Patients were observed every 15 min. If the inhalation of newborns was invalid, the dose was increased gradually by 5 ppm each time, without exceeding 20 ppm. When newborns had inhaled effectively for 12 h, the dose was reduced by 5 ppm every 30 min, while maintaining the ideal level of arterial partial pressure of oxygen (PaO_2). The dose was finally reduced to 6 ppm. When the demand for oxygen was < 50%, inhalation was stopped. In addition to the aforementioned treatment, newborns in the observation group were given citric acid sildenafil (Pfizer Pharmaceutical, New York, NY, USA, approval number: GYZZ H20020526) by nasal feeding, 1.0 mg/kg every 6 h, until the pulmonary artery pressure fell to the normal level.

Measurement of Indexes

The dynamic pulmonary artery pressure was measured three times with a color digital Doppler diasonograph (GE Vivid Type 7, New York, NY, USA) to obtain the mean. Blood was collected from the left radical artery before treatment, and 2 h, 4 h, and 24 h after treatment for blood gas analysis to determine the levels of a fraction of inspired oxygen (FiO_2), PaO_2, and oxygen saturation (SpO_2). A total of 3 ml of peripheral venous blood was extracted 3, 5, and 7 days after treatment. The supernatant was collected after centrifugation for 10 min at 1006.2 × g to measure the levels of hypoxia-inducible factor (HIF)-1α and endothelin-1 (ET-1) in serum by enzyme-linked immunosorbent assay (ELISA). Furthermore, 2-4 ml of femoral venous blood was collected to determine the serum calcium level using original Beckman kits (Beckman Coulter Limited, Brea, CA, USA) with the ion selective electrode method.

Evaluation Criteria

The curative effects of the two groups of newborns were compared. The evaluation criteria were as follow: (1) Significant: post-treatment, the various clinical symptoms, and signs of newborns were alleviated, and all indexes were markedly improved; (2) Effective: post-treatment, shortness of breath, cyanosis, groans, and other clinical symptoms and signs were alleviated, and the indexes of the pulmonary artery were improved; (3) Ineffective: post-treatment, no improvement or deterioration was observed in symptoms and signs of newborns. The total effective rate = the significant rate + the effective rate.

The FiO_2, PaO_2, and SpO_2 of the two groups of newborns were analyzed at different time points including before treatment and 2 h, 12 h, and 24 h after treatment. The pulmonary artery pressure was monitored at different time points with the Doppler method by determining the rate of backflow in continuous Doppler, and obtaining V, the value of tricuspid regurgitation, among which the pulmonary artery pressure = 4 × V2 + 5 mmHg. The oxygenation index (OI) of the two groups of newborns was calculated according to the formula: OI = Mean airway pressure (MAP) × FiO_2 × 100 ÷ PaO_2.

Before the treatment and at 3, 5, and 7 days after treatment, 3 ml of peripheral venous blood was extracted from the two groups of newborns. The supernatant was collected after centrifugation to measure the levels of HIF-1α and ET-1 in serum by ELISA. Simultaneously, 2-4 ml of femoral venous blood was collected, and the serum calcium level was determined with the ion-selective electrode method.

Statistical Analysis

SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) was used for data analysis. FiO_2, PaO_2, SpO_2, MAP, OI, HIF-1α, ET-1 and calcium levels

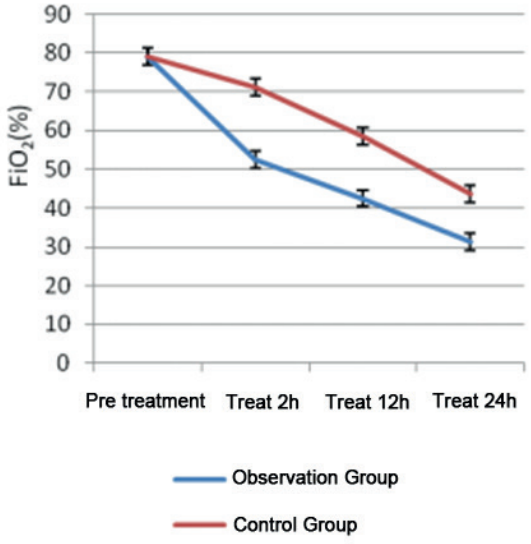

Figure 1. The trend of FiO_2 levels in the two groups of newborns.

are presented as mean ± standard deviation, and comparisons were by *t*-test. Curative effect is presented as percentage (%), and comparisons were by rank sum test. $p<0.05$ was taken as statistically significant.

Results

The curative effects of the two groups of newborns were compared post-treatment. The total effective rate (95.74%) in newborns of the observation group was higher than that (74.47%) in the control group. The difference was statistically significant ($p<0.05$) (Table II).

FiO_2 in the two groups of newborns at different time points was compared. The FiO_2 levels in the two groups were not significantly different ($p>0.05$). The FiO_2 levels in the observation group were significantly lower than those in the control group ($p<0.05$) post-treatment (Table III).

At 2 h after treatment, the FiO_2 levels in both groups of newborns began to decline. Over time, the FiO_2 levels continued to decrease, and the de-

Table I. Comparison of baseline parameters of the two groups of newborns.

Parameter	Control group n=43	Observation group n=43	t/χ^2	*p*
Newborn sex (male/female)	21/22	18/25	0.188	0.665
Age of the newborn (d)	7–28	5–28		
Average age of the newborn (d)	14.18±6.25	14.36±6.31	0.133	0.894
Weight (kg)	2.84±0.32	2.85±0.53	0.106	0.915
Severe pneumonia [n (%)]	13 (30.23)	15 (34.88)	0.053	0.818
Inhalation of amniotic fluid and meconium [n (%)]	11 (25.58)	9 (20.93)	0.065	0.798
Bronchopulmonary dysplasia [n (%)]	9 (20.93)	8 (18.60)	0.001	0.999
Hyaline membrane disease [n (%)]	7 (16.28)	6 (13.95)	0.001	0.999
Premature [n (%)]	3 (6.97)	5 (11.62)	0.138	0.711

Table II. Comparison of the curative effects in the two groups of newborns.

Group	Cases (N)	Significant	Effective	Ineffective	Total effective
Observation group	43	37 (14.89)	4 (8.51)	2 (4.26)	41 (95.74)
Control group	43	19 (19.15)	13 (14.89)	11 (25.53)	32 (74.47)

Note: The curative effects of the two groups of newborns underwent rank sum test, among which Z=4.051, $p=0.0001$.

Table III. Comparison of FiO_2 between the two groups of newborns before and after treatment.

Group	Cases	Pre-treatment (%)	2 h after treatment (%)	12 h after treatment (%)	24 h after treatment (%)
Observation treatment	43	79.05±3.47	52.45±2.46	42.48±2.56	31.27±2.33
Control group	43	79.13±3.15	71.18±2.43	58.53±2.37	43.56±2.74
t		0.112	35.520	30.169	22.407
p		0.911	<0.0001	<0.0001	<0.0001

Table IV. Comparison of gas blood indexes between the two groups of newborns before and after treatment.

Group	PaO_2 (mmHg)				SpO_2 (%)			
	Pre-treatment	Treat 2h	Treat 12h	Treat 24h	Pre-treatment	Treat 2h	Treat 12h	Treat 24h
Observation group	35.25±3.56	54.25±3.42	61.34±4.52	67.25±6.34	46.17±2.25	65.27±3.32	74.26±4.46	81.37±5.36
Control group	34.67±3.46	48.28±3.35	54.53±4.37	60.26±6.52	45.64±2.78	51.36±3.47	63.65±4.74	71.58±5.54
t	0.766	8.177	7.102	4.326	0.972	18.993	11.192	8.328
p	0.445	<0.0001	<0.0001	0.0001	0.334	<0.0001	<0.0001	<0.0001

Table V. Comparison of pulmonary artery pressure before and after treatment in the two groups of newborns (mmHg).

Group	Cases	Pre treatment	2 h after treatment	2 h after 1treatment	24 h after treatment
Observation group	43	63.27±4.45	46.25±3.46	29.34±3.48	15.27±2.05
Control group	43	63.43±4.23	58.18±3.42	39.53±3.37	35.56±3.36
t		0.171	16.080	13.794	33.803
p		0.864	<0.0001	<0.0001	<0.0001

cline in the observation group was more evident than in the control group ($p<0.05$) (Figure 1).

The analysis and comparison of gas blood indexes at different time points between the two groups showed that before treatment, the differences between the two groups were not significant ($p>0.05$). Post-treatment, SpO_2, and PaO_2 in the observation group were superior to those in the control group, and the differences were statistically significant ($p<0.05$) (Table IV).

At 2 h after treatment, the gas-blood indexes of the two groups of newborns began to increase. Over time, the indexes continued to increase. Moreover, the increase in the observation group was more significant than in the control group ($p<0.05$) (Figures 2 and 3).

The pulmonary artery pressure of the two groups of newborns at different time points was compared. No significant difference was observed in the pulmonary artery pressure of the two groups before treatment ($p>0.05$). Post-treatment, the pulmonary artery pressure in the observation group decreased more quickly than in the control group, and the difference was statistically significant ($p<0.05$) (Table V).

At 2 h after treatment, the pulmonary artery pressure in the two groups began to decrease. Over the course of treatment, the pulmonary artery pressure continued to decrease. Furthermore, the decline in newborns of the observation group

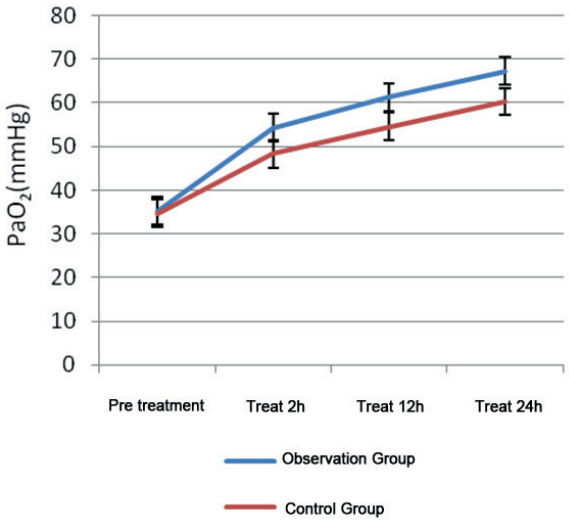

Figure 2. PaO_2 of the two groups of newborns at different time points.

was more significant than that in the control group ($p<0.05$) (Figure 4).

The OI of the two groups of newborns at different time points was compared. There was no significant difference in OI between the two groups of newborns before treatment ($p>0.05$). The decrease of OI level in newborns of the observation group was more significant than in the control group ($p<0.05$) post-treatment (Table VI).

Table VI. Comparison of OI pre-treatment and post-treatment in the two groups of newborns (mmHg).

Group	Cases	Pre treatment	2 h after treatment	12 h after treatment	24 h after treatment
Observation group	43	36.17±3.35	17.65±3.27	11.32±2.43	8.29±2.76
Control group	43	36.28±3.14	22.46±3.42	16.51±2.36	13.53±2.26
t		0.157	6.666	10.047	9.632
p		0.875	<0.0001	<0.0001	<0.0001

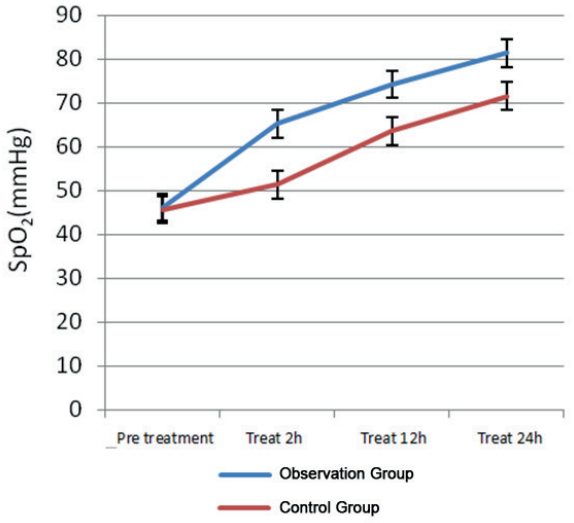

Figure 3. SpO_2 of the two groups of newborns at different time points.

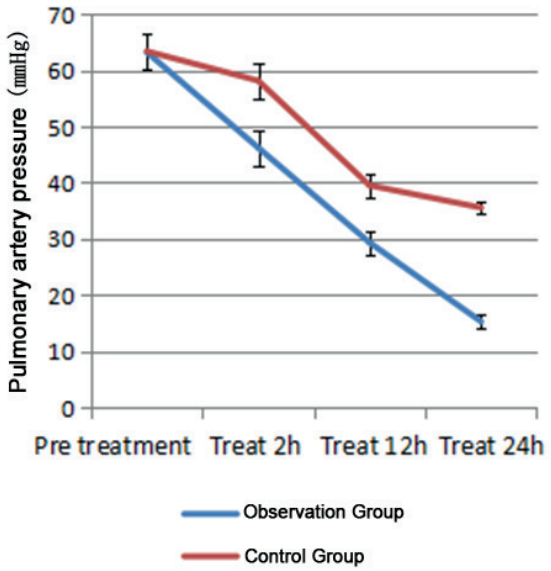

Figure 4. Pulmonary artery pressure of the two groups of newborns at different time points.

The serum levels of HIF-1α and ET-1 in the two groups of newborns at different time points were compared. The levels of HIF-1α and ET-1 before treatment were not significantly different ($p>0.05$). Post-treatment, the levels of HIF-1α and ET-1 in newborns of the observation group were lower than those in the control group, and the differences were statistically significant ($p<0.05$) (Table VII).

On the third day after treatment, the levels of HIF-1α and ET-1 in both groups of newborns began to decrease. Over time, the levels continued to decrease. Moreover, the decline in the observation group was more significant than that in the control group ($p<0.05$) (Figures 5 and 6).

Serum calcium levels of the two groups of newborns at different time points were compared.

Table VII. Comparison of HIF-1α and ET-1 (pg/ml) pre-treatment and post-treatment in the two groups of newborns.

	HIF-1α				ET-1			
Group	Pre treatment	Day 3	Day 5	Day 7	Pre treatment	Day 3	Day 5	Day 7
Observation group	883.25±93.46	654.25±43.46	431.35±34.52	287.27±24.36	329.38±34.62	175.17±22.35	89.16±11.45	51.27±5.38
Control group	884.78±96.73	718.18±43.35	584.73±33.37	389.16±34.56	323.46±33.23	231.46±22.46	163.35±11.72	89.48±5.56
t	0.074	6.829	20.948	15.802	0.809	11.649	26.692	32.385
p	0.941	<0.0001	<0.0001	<0.0001	0.421	<0.0001	<0.0001	<0.0001

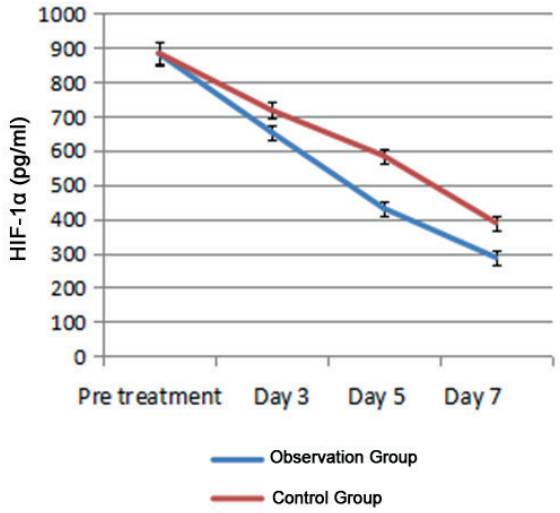

Figure 5. The levels of HIF-1α in the two groups of newborns.

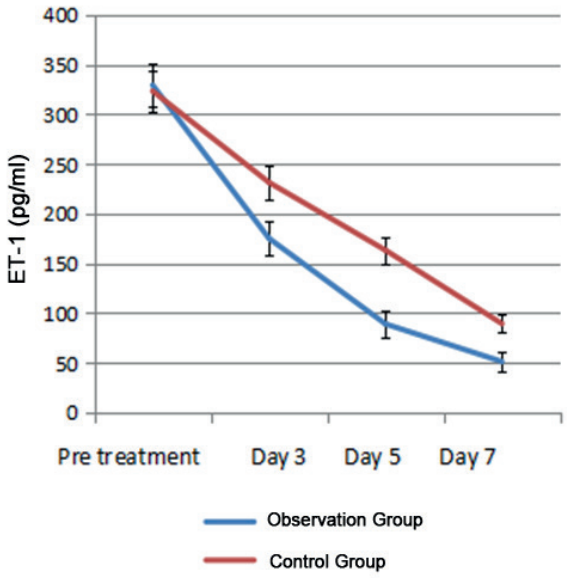

Figure 6. The levels of ET-1 in the two groups of newborns.

The levels before treatment were found to have no significant difference ($p>0.05$). Post-treatment, the levels in newborns of the observation group increased more significantly than in the control group ($p<0.05$) (Table VIII and Figure 7).

On the third day after treatment, the serum calcium levels in the two groups of newborns began to increase. Over the course of treatment, the levels continued to increase. Moreover, the increase in the observation group was more significant than that in the control group ($p<0.05$) (Figure 7).

Discussion

Currently, there is no recognized understanding of the pathogenesis of PPHN. It is believed that PPHN begins with dysfunction caused by small pulmonary artery injury. This leads to abnormalities in vascular cell factors and active substances and results in the imbalance of equilibrium of angiokinetic factors. It begins with the contraction of pulmonary blood vessels. Next, pathological changes of pulmonary vascular walls and pulmonary vascular remodeling occur, ultimately resulting in PPHN[7,8].

The traditional therapy for PPHN involves vasodilators combined with mechanical ventilation for inhalation of high concentration oxygen. However, this has no desirable curative effects and has many side effects[9]. NO inhalation is widely applied as a gold standard for treatment. Using high-frequency oscillation ventilation in inhalation therapy can cause NO to disperse to vascular smooth muscle cells. The levels of soluble guanylic acid cyclase increase, allowing the pulmonary vasculature to relax, lowering the resistance in the pulmonary vasculature, and effectively improving the general and pulmonary circulations. However, once treatment is discontinued, the disease can recur, and NO may suppress platelet aggregation. Therefore, it is necessary to treat PPHN with comprehensive therapy including drugs[10,11]. Sildenafil, an active inhibitor of phosphodiesterase, can relax the muscle cells of newborns, and expand pulmonary blood vessels,

Table VIII. Comparison of serum calcium levels of the two groups of newborns pre-treatment and post-treatment (mmol/l).

Group	Cases	Prior treatment	Day 3	Day 5	Day 7
Observation group	43	1.25±0.16	1.65±0.28	1.94±0.29	2.13±0.11
Control group	43	1.27±0.14	1.38±0.25	1.53±0.25	1.89±0.12
t		0.617	4.717	7.022	9.668
p		0.539	<0.0001	<0.0001	<0.0001

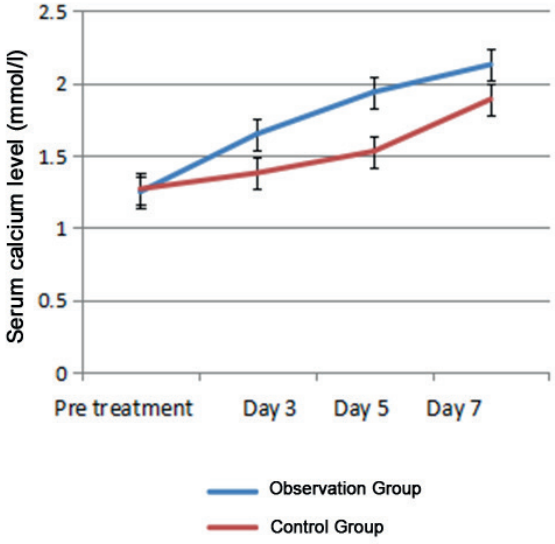

Figure 7. Serum calcium levels in the two groups of newborns.

thereby lowering resistance in blood vessels to improve the local microcirculation[12]. Our results showed that the total effective rate among newborns in the observation group was significantly higher than in the control group, and the improvements in gas blood indexes and pulmonary artery pressure were superior to those in the control group ($p<0.05$).

HIF-1α is a phosphorylated protein. The functional subunit of HIF-1 continues to be expressed in anoxia and plays a crucial regulatory role in oxygen-poor reactions. Its level increases significantly in hypoxic lung tissue[13]. Relevant studies showed[14] that under hypoxic conditions, HIF-1 is produced and participates in hypoxia-induced responses to adapt to hypoxia, thus regulating angiogenesis, angiokinesis, and vascular remodeling. The levels of HIF-1α in newborns with PPHN are generally much higher than in healthy newborns. Through treatment, the volume of blood flow in the lung tissue of newborns increased. Hypoxia was improved to a certain extent. The concentration of HIF-1α declines rapidly, thus decreasing the contraction of the pulmonary artery and reducing pulmonary artery pressure[15]. We showed that on the third day after treatment, the serum levels of HIF-1α in newborns decreased significantly, and on the seventh day, the levels approached normal values. The decrease in the observation group was superior to that in the control group ($p<0.05$).

According to relevant studies[16], ET is the most active endogenous vasoconstrictor currently identified. Lung tissue is a target of ET-1 and is important for secretion and metabolism. With the deterioration of PPHN, the levels of ET-1 in the serum of newborns continued to increase. By activating the division of growth factors directly and then promoting the synthesis and secretion of growth factors, ET-1 causes pulmonary vasoconstriction and participates in pulmonary vascular remodeling. It is therefore important in the occurrence and development of PPHN[17]. After treatment, the anoxia of newborns was alleviated. Sildenafil caused persistent relaxation of the pulmonary vasculature, and had an increasing effect against ET-1, thus reducing the serum levels relatively quickly, as well as reducing the pulmonary pressure. Our results showed that on the third day after treatment, the levels of ET-1 in the two groups of newborns began to decrease. Over the course of treatment, the levels continued to decrease, and evident improvements were observed on the seventh day after treatment. Furthermore, the decrease in the observation group was superior to that in the control group ($p<0.05$).

During the occurrence and development of PPHN, calcium ions facilitate the contraction of smooth muscle cells[18]. In normal cells, the majority of calcium exists in the nucleus and organelles. The calcium ion content is extremely low. In newborns with PPHN, the inflow of high levels of extracellular calcium leads to the overload of intracellular calcium, and the injury and necrosis of pulmonary vascular endothelial cells, thereby destroying the regulatory mechanism of various vasomotor factors and forming the vicious circle of pulmonary hypertension[19]. Related studies have shown that the extracellular total calcium level parallels the level of ionized calcium. Thus, the decrease of serum calcium in newborns indicated the decrease of ionized calcium[20]. Treatment can activate the dissoluble guanylic acid cyclase and suppress the inflow of calcium ions, thus regaining and improving the serum calcium level of newborns.

Conclusions

We showed that sildenafil combined with NO inhalation has good curative effects for PPHN. It can effectively improve the blood gas indexes, and reduce pulmonary hypertension. The sample size in this study was limited. It is, therefore, necessary to conduct further studies on safety and other aspects with larger sample sizes.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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