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Effect of nitric oxide inhalation combined with high-frequency oscillatory ventilation on the prognosis of neonatal severe hypoxemia

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Summary

Objective: The current study aimed to analyze the short-term and long-term curative effects of nitric oxide (NO) inhalation combined with high-frequency oscillatory ventilation (HFOV) on neonatal severe hypoxemia. *Materials and Methods:* A total of 98 neonates meeting the inclusion criteria were retrospectively analyzed. The control group comprised of 48 neonates and the NO inhalation group consisted of 50 neonates. In the control group, conventional mechanical ventilation was replaced by HFOV. In the experimental group, NO inhalation combined with HFOV was performed. The death rates within 28 days, mechanical ventilation and oxygen therapy time, and complications in both groups were observed. The survivors in both groups were followed up for 18 months for neural development evaluation. *Results:* The treatment group showed a significantly lower death rate and noticeably shorter mechanical ventilation and oxygen therapy time than the control group (8% vs. 22.9% with $t = 4.20$ and $p < 0.05$; 5.84 ± 3.36 days vs. 8.05 ± 5.48 days with $t = 2.42$ and $p < 0.05$; and 8.02 ± 4.31 days vs. 12.45 ± 5.14 days with $t = 4.63$ and $p < 0.001$). They did not show significant differences with regards to the complications and the incidences of cerebral palsy, hearing and visual impairments, and severe nervous damage ($p > 0.05$). *Conclusion:* NO inhalation combined with HFOV significantly decreases the death rate of neonates with severe hypoxemia and reduces their mechanical ventilation and oxygen therapy time. It does not increase early adverse effects or affect long-term neurodevelopment.

Key words: prognosis; inhaled nitric oxide; high-frequency oscillatory ventilation; neonates.

Introduction

Nitric oxide (NO) belongs to an endothelium-derived relaxing factor which plays an important role in regulating angiectasis. NO inhalation dilates pulmonary vascular smooth muscle selectively. For this reason, it has been applied as a new treatment method for severe hypoxemia complicated pulmonary hypertension in neonates [1-4]. Recently, this method has also been applied in the treatment of neonatal respiratory failure, but has not been supported from Meta analysis [5-8]. NO inhalation began to be utilized for persistent pulmonary hypertension of newborns in the 1990s [2, 9, 10]. Numerous studies have proven that it rapidly improves oxygenation, regulates the ventilation/perfusion ratio in the lungs, and improves pulmonary circulation; furthermore, it shows a noticeable curative effect on neonatal severe hypoxemia complicated pulmonary hypertension without resulting in noticeable side effects and influencing systemic circulation, and significantly reduces deaths and the use of extracorporeal membrane oxygenation (ECMO) [1, 2]. However, most of these studies utilized NO inhalation combined with conventional mechanical ventilation. Moreover, they focused on the observation of early oxygenation improvement [1, 2, 11, 12]. Reports on the effect of NO inhalation combined with high-frequency oscillatory ventilation (HFOV) are rare. The effect of NO inhalation on neonatal long-term prognosis remains controversial [13, 14].

This study aimed to observe the influence of NO combined with HFOV on hypoxemia in full-term and near full-term newborns, as well as their complications and 18-month follow-up outcomes.

Materials and Methods

Subjects

Neonates with severe hypoxemia that received treatment in the neonatal intensive care unit of Zhengzhou Children's Hospital between January 2008 and October 2011 were enrolled. The inclusion criteria were as follows: 1) gestational age > 35 weeks; 2) under conventional mechanical ventilation, fraction of inspired oxygen (FiO_2) ≥ 0.8, mean airway pressure (MAP) > 0.98 KPa for no less than two hours, and SpO_2 0.98 KPa < 85% or oxygenation index (OI) ≥ 25 ($OI = MAPcmH_2O \times FiO_2 \times 100/PaO_2mmHg$) [1, 2, 12]. The exclusion criteria included: 1) thrombocytopenia (< 50×10^9/l) and hemorrhagic tendency (activated partial thromboplastin time > 72 seconds); 2) complex congenital heart disease; and 3) respiratory failure caused by pneumatothorax and congenital malformation, severe intracranial hemorrhages, multiple organ failure, and so on [14].

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Zhengzhou

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Table 1. — *General data of both groups.*

	Treatment group (50)	Control group (48)	t/x²	*p*
Gestational age (w)	38.3 ± 1.6	38.8 ± 1.8	1.45	> 0.05
Weight (g)	3320 ± 593	3395 ± 602	0.62	> 0.05
Male	37 (74%)	36 (75%)	0.01	0.91
CMV time of origin (h)	7.2 ± 4.5	8.1 ± 5.2	0.91	> 0.05
Primary disease				
Idiopathic PPHN	8	7	0.04	0.85
Severe asphyxia	7	9	0.40	0.52
III–IV RDS	17	15	0.08	0.77
MAS	11	12	0.12	0.73
Pneumonia	4	5	0.17	0.68
Others	3	1	0.96	0.33
PS application	19	17	0.07	0.79

Table 2. — *Early indices of both groups.*

Index	Treatment group (46)	Control group (37)	t/x²	*p*
Mechanical ventilation duration (d)	5.84 ± 3.36	8.05 ± 5.48	2.42	< 0.05
Oxygen therapy duration (d)	8.02 ± 4.31	12.45 ± 5.14	4.63	< 0.001
Frequent hemorrhoid	5	7	1.07	0.29
Pulmonary hemorrhage	4	6	1.09	0.29
NEC	3	3	0.08	0.78
Severe IVH	2	4	1.28	0.26

Table 3. — *Follow-up outcomes of both groups at 18 months.*

Index	Treatment group (39)	Control group (32)	t/x²	*p*
CP-n (%)	9 (23.1)	10 (31.2)	0.60	0.44
Moderate and severe CP-n (%)	2 (5.12)	2 (6.25)	0.04	0.84
Hearing disorders-n (%)	3 (7.61)	2 (6.25)	0.14	0.71
Visual disorders-n (%)	4 (10.26)	5 (15.6)	2.06	0.15
PDI	90.7 ± 15.1	92.2 ± 14.8	0.42	> 0.05
MDI	80.4 ± 19.3	83.9 ± 18.5	0.77	> 0.05
NDI-n (%)	13 (33.3)	10 (31.2)	0.40	0.52

Children's Hospital. Written informed consents were obtained from the guardians of all the participants.

Retrospective analysis was performed. A total of 48 neonates satisfying the inclusion criteria between January 2008 and December 2009 comprised control group. Conventional mechanical ventilation was replaced by HFOV after enrollment. Fifty neonates satisfying the inclusion criteria between January 2010 and October 2011 comprised treatment group. After recruitment, they underwent HFOV combined with NO inhalation. Both groups were diagnosed and treated routinely according to the methods for primary diseases.

Treatment methods

NO gas for treatment use was subpackaged in special aluminum alloy cylinders with a NO concentration of 1,000 ppm and a NO_2 concentration ≤ ten ppm. It was introduced into the gas supply pipe of a breathing apparatus via a special mass flow controller . Sampling was performed proximal to the Y-shaped site of the pipe for monitoring the concentrations of NO and NO_2 using an electrochemical NO/NO_2 concentration detector. The original, also the highest, concentration of NO was 20 ppm [2]. $SpO_2 \geq 90\%$ was maintained and FiO_2 was gradually downregulated. When FiO_2 was ≤ 0.5, the concentration of NO was downregulated to five ppm. If SpO_2 did not improve, the original concentration of NO was maintained. When NO was five ppm and SpO_2 was ≥ 90%, the concentration of NO was downregulated every six to 12 hours with one ppm per regulation. Normally, NO was withdrawn after 48-72 hours of inhalation [7].

Early observation indices

Cranial ultrasounds were performed before treatment and at three days after NO inhalation, and blood gas analyses were performed before treatment and four, 24, 48, and 72 hours after treatment, respectively. In the NO group, the concentration of methemoglobin < 3% and that of NO_2 < two ppm were carefully monitored. Mechanical ventilation time, oxygen therapy time, the incidences of frequent hemorrhoid (pneumatothorax and pulmonary interstitial emphysema), pulmonary hemorrhage, necrotizing enterocolitis (NEC) and severe intracranial hemorrhage (IVH), and the fatality rates within 28 days in the two groups were recorded.

Follow-ups

Both groups were regularly followed up after discharge. Intervention treatment was given according to developmental conditions. The incidences of cerebral palsy (CP), audition, visual acuity, and neural development were evaluated at 18 months. Audition was tested using brain stem auditory evoked potentials and neural development was evaluated using Bayley scales of infant development (physical development index (PDI) and mental development index (MDI)). Neurodevelopmental disorders were considered when any of the following criteria was met: moderate or severe CP, MDI < 70, PDI < 70, visual disorder, and hearing disorder that necessitated audio helpers [13].

Statistical analysis

Data were analyzed using SPSS 18.0 software. Measurement data were presented as means ± deviation of means () and *t*-tested. For enumeration data, chi-square, corrected chi-square, or exact probability tests were performed. A $p < 0.05$ was considered statistically significant.

Results

General data

A total of 98 neonates were recruited in this study, including 50 in the treatment group and 48 in the control group. The general data of the two groups were summarized in Table 1. No significant difference with regard to maternal educational levels was observed between the groups ($p > 0.05$).

Early outcomes

In the treatment group, 46 patients improved or healed and four died with a death rate of 8%. In the control group, 37 improved or healed and 11 died with a death rate of 22.9%. The two groups showed a significant difference in the death rates ($t = 4.20$, $p < 0.05$). Other early indices were also compared. With the exclusion of the deaths, all the patients in both groups were discharged. The mechanical ventilation time, oxygen therapy time, and the incidences of

frequent hemorrhoid, pulmonary hemorrhage, NEC, and IVH were compared between the groups (Table 2).

Follow-up outcomes

In the treatment group, 40 of the 46 discharged patients completed the 18 months of follow-ups and six were lost. One patient died after discharge. In the control group, 32 of the 37 discharged patients completed the 18 months of follow-ups, and five were lost. No death occurred. The survivals of the patients completing follow-ups are summarized in Table 3.

Discussion

Neonatal severe hypoxemia is a neonatal critical disease which is primarily manifested by pulmonary hypertension concomitant with severe asphyxia, meconium aspiration syndrome, severe respiratory distress syndrome, and severe pneumonia. Pulmonary hypertension has an incidence of 1/500 and an approximate mortality rate of 10–50%; even among the survivors, 7–20% suffers from various degrees of sequelae [9]. Although conventional mechanical ventilation has saved numerous neonates, which can achieve a survival rate up to 70% in China [15], it requires very strict ventilation conditions for breathing machines, and tends to cause volemic and biological lung injury, leading to a series of clinical complications and pulmonary and cerebral development disorders [16, 17]. Moreover, pulmonary venous vasodilator drugs fail to decrease pulmonary vascular resistance and often cause a decrease in systemic circulation pressure, consequently affecting overall treatment effectiveness [17].

NO is subject to an endothelium-derived relaxing factor. Its inhalation selectively acts on small resistance vessels in the lungs to relax vascular smooth muscle. This effect dilates vessels, reduces pulmonary vascular resistance and arterial pressure, increases the blood flow volume of the lungs, rapidly improves the pulmonary ventilation-perfusion ratio, and increases blood oxygen concentration [10, 11]. NO has a half-life period of one to five seconds. Inhaled NO during this period binds with hemoglobin immediately after entering circulation. After renal metabolism, it is excreted in the forms of nitrate and nitrite (NO2- / NO3-) with urine, without influencing peripheral vascular tension and resulting in noticeable side effects [1, 2]. However, the vasodilatation effect of inhaled NO requires sufficient lung inflation to guarantee the gas to reach high-resistance pulmonary arteries. Or conversely, unsatisfactory lung inflation and insufficient pulmonary alveolus recruitment may affect the curative effect of inhaled NO, which is the most common reason of NO treatment failure as well [17]. HFOV rapidly improves ventilation by resorting to a very high frequency and an extremely small tidal volume (close to or lower than that in the anatomical dead space). It prevents pulmonary injury caused by conventional mechanical ventilation. Furthermore, the oscillation frequency of the high-frequency breathing machine is in accordance with the resonance frequency of the human lungs. Under this condition, the resistance of small airways drops to the minimum, thereby facilitating gas to move in and out of pulmonary alveoli. In addition, the micro pressure produced by the resonance of the pulmonary alveoli themselves allows alveolar gas to generate movements, which benefits the diffusion and exchange of the gas. The high-efficiency alveolar recruitment effect of HFOV endows HFOV in combination of NO inhalation with a more excellent effect than NO inhalation combined with conventional mechanical ventilation [17]. Compared with pressure/controlled ventilation combined with NO inhalation, HFOV combined with NO inhalation markedly reduces FiO_2 and increases the PO_2/FiO_2 ratio eight hours after treatment [18]. NO inhalation combined with conventional mechanical ventilation has no effect on the death rate; compared with this method, its combination with HFOV shows a significant difference in the death rate (1/8 vs. 5/16) [15]. This finding was also evidenced by this study: NO inhalation combined with HFOV significantly reduced deaths, as well as mechanical ventilation and oxygen therapy time.

The side effects caused by NO inhalation are primarily reflected by its influence on the generation of NO_2 and methemoglobin and the agglomeration of platelets as well as the increase in the risk of hemorrhage [1, 2, 13]. NO at a concentration of 20 ppm to 40 ppm is safe and does not lead to noticeable side effects [13, 17]. In this study, the cases were strictly selected and then closely monitored during treatment. The given inhaled NO concentration was controlled no higher than 20 ppm. The results did not show significant difference in terms of the incidence rates of frequent hemorrhoid, pulmonary hemorrhage, NEC, and severe IVH between the treatment and control groups, which is consistent with that reported by other scholars [1, 2, 17]. This finding proved that 20 ppm NO inhalation is safe.

Recent studies have found that the vasodilatation effect of inhaled NO is not limited to the lungs [19]. Animal experiments have discovered that it also dilates the arteries in the cerebral ischemic regions, whereby it increases local blood flow supply and improves reperfusion injury and neural prognosis after ischemia [19-22], rather than to affect normal cerebral blood flow [19]. It achieves curative effect on adult cerebral stroke [22]. However, whether it has an effect on neonatal neural development remains uncertain [2, 13, 14, 23]. Konduri *et al.* conducted a multi-centered study in which 234 neonates with severe hypoxemia were followed up for 18-24 months and found that compared with the control group, the NO inhalation group does not show significant differences in the incidences of neural development disability (25% vs. 27%) and hearing impairment (24% vs. 23%) and the MDI but a noticeably higher PDI [13. However, this finding is in disagreement with that reported in another multi-centered, randomized, undoubled-blind control study by Field *et al.*: no statistical differences with regard to the death rate, growth development, movement disorders, hearing, and visual acuity of one-year-old neonates are observed between the treatment and control groups [14]. This study is single-

centered and in it, prognoses were evaluated by child health care practitioners that did not participate in early treatment. The results did not show significant differences in CP, hearing and visual impairments, and severe nerve damage between the treatment and control groups ($p > 0.05$). Although the control group had slightly higher PDI and MDI than the treatment group, the differences were not significant. These findings indicate that NO inhalation does not affect the neural development of neonates with severe hypoxemia at 18 months; it has no cerebral protective effect, either. They are consistent with those concluded by Field et *al.* but differ somewhat from those obtained by animal experiments [14, 19-22]. The underlying reasons may be as follows. First, most models supporting the cerebral injury-alleviating effect of NO were adult animal cerebral ischemia models [19-22]. The conclusions drawn in these studies may not be applicable to developing brains. Therefore, animal models corresponding to neonatal cerebral development levels should be chosen in future studies. Second, the sample sizes were still small.

This study has some limitations. First, it was single-centered. To obtain as many as possible patients' data, retrospective analyses had to be performed. Second, the case number for long-term prognostic evaluation was small, which might influence the result judgment of this study. To overcome these limitations, multi-centered, randomized studies should be conducted and more cases should be observed to evaluate the effect of NO inhalation on long-term neonatal prognosis in the future.

To draw a conclusion, NO inhalation combined with HFOV noticeably improves the early prognosis of neonates', reduces their death rate, and shortens their mechanical ventilation and oxygen therapy time without the support of the ECMO technique. It does not increase early complications or affect neonatal long-term neural development. Therefore, it is worth extending to clinical practice.

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