Effect of phototherapy on blood endothelin and nitric oxide levels: clinical significance in preterm infants

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Background: **Phototherapy may have an adverse effect on the hemodynamics of preterm infants, and endothelin (ET) and nitric oxide (NO) are both the powerful vasoactive substances. This study was designed to observe the effect of phototherapy on blood levels of ET and NO in preterm infants.**

Methods: **Sixty-four preterm infants with hyperbilirubinemia requiring phototherapy were studied. Among them, 31 patients were born at 32-36 weeks' gestational age (GA), and 33 patients were ≤32 weeks GA. Control group included 26 full-term infants with hyperbilirubinemia requiring phototherapy. All patients were treated with continuous phototherapy for 24 hours. Blood samples were collected before and after phototherapy. The amount of ET in the blood samples was determined by radioimmunoassay, and NO levels were determined using nitrate reductase. Heart rate, respiratory rate, apnea, and mean arterial blood pressure (MABP) were monitored regularly (defined interval: hourly, 4 hours, etc) during phototherapy.**

Results: **Blood ET levels measured after 24 hours of phototherapy were higher than the pretreatment values, as were blood NO levels measured after 12 hours and 24 hours of phototherapy. Both increases were statistically significant ($P<0.05$) in the GA≤32 weeks group. In the GA>32 weeks group, blood NO levels measured after 24 hours of phototherapy were higher than the pretreatment values; these changes were also statistically significant ($P<0.05$). In the GA≤32 weeks group, heart rate increased and the MABP decreased during phototherapy. The changes after 24 hours of phototherapy compared to the pretreatment values were statistically significant. A few episodes of apnea occurred during phototherapy in the GA≤32 weeks group. This was significantly higher than that in the other two groups.**

Conclusions: **Under phototherapy, blood levels of ET and NO were significantly higher in preterm infants, especially in preterm infants of ≤32 weeks GA.**

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Key words: **endothelins; hyperbilirubinemia; nitric oxide; phototherapy**

Introduction

Hyperbilirubinemia, ie, jaundice occurs frequently in ill preterm infants. In contrast to full-term infants, jaundice in preterm infants tends to be more severe and carries a higher risk of developing bilirubin encephalopathy. Etiological treatment, including infection prevention, correcting acidosis, etc is representative nowadays, but it is more important that phototherapy is given to newborns with jaundice as early as possible to prevent bilirubin encephalopathy, and more treatment options are required for small gestation age infants.[1,2]

Phototherapy has become the safest and most effective treatment for hyperbilirubinemia in newborns,[3] but it is not entirely free from risk, particularly in very low-birth-weight (VLBW) preterm infants. Recent studies have focused on the side effects of phototherapy, such as riboflavin deficiency, blood calculus decrease, hemolysis, change of neonatal behavior and immune function.[4] Continuous phototherapy may lead to sudden death from pulmonary hemorrhage. Li et al[5] reported 3 (2 cases of VLBW) of 257 preterm infants with hyperbilirubinemia requiring phototherapy. Others[6-10] demonstrated that phototherapy increased blood flow velocity to the cerebrum and periphery, but decreased blood flow to the kidneys and the mesentery. Cardiac output was also reduced and the re-opening of the ductus arteriosus was observed. Thus, the safety of phototherapy in preterm infants has been investigated, particularly in relation to hemodynamic control.

ET and NO are the strongest vasoconstrictor and

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vasodilator respectively. Their effects are completely opposing, and under physiological conditions they regulate angiostasis and blood flow. In some pathological conditions, however, the dynamic balance between ET and NO is disturbed, causing changes in hemodynamics that might lead to severe clinical symptoms. Buisson et al[11] have discovered that the main cause of neuron damage is excessive NO production. In addition, it will lead to hemodynamic changes of cerebral blood flow and dysfunction of blood brain barrier, that is, cerebral blood flow decreases significantly at onset, then develops to hyperperfusion,[12] disturbs the function of blood brain barrier, and increases the permeability,[13] resulting in encephaledema eventually.

This study aimed to understand the effect of phototherapy on blood ET and NO in hyperbilirubinemic preterm infants with stable vital signs, to observe changes in vital signs during phototherapy, to assess the safety of phototherapy in preterm infants, and to guide phototherapy in preterm infants.

Methods

From September 2003 to November 2004, 90 newborns receiving phototherapy for high unconjugated bilirubinemia in the First and Second Hospitals affiliated to Jinan University and in Shunde Maternal and Child Health Hospital in Foshan city were enrolled in the study. Patients with stable vital signs were included. The patients were excluded with diseases of the brain, liver, gut or heart, persistent pulmonary hypertension, congenital malformations and hypertension. Newborns treated with muscle relaxants, diuretics, or single blue light were also excluded. The indications for phototherapy were in agreement with the *2000 Icterus Neonatorum Intervention Guidelines* recommended by the Group of Newborns, Division of Pediatrics, Chinese Medical Association.[14] The guidelines are consistent with those published by the American Academy of Pediatrics (AAP).[15]

In 31 patients of gestational age (GA) >32 weeks, the male:female ratio was 15:16 and mean age was 3.74 ±1.7 days, while in 33 patients of GA ≤32 weeks, the male:female ratio was 18:15 and the mean age was 3.79 ±1.7 days. Twenty-six full-term infants were enrolled in the control group, with the male:female ratio of 15:11 and mean age of 4.67±2.3 days.

Routine treatment including phenobarbital, oral medication of nikethamide enzyme and fluid replacement therapy was given. Continuous 24-hour diprosopia illumination was administered to newborns with hyperbilirubinemia (The citrus neonatorum heal-box was produced by Ningbo Daiwei Medical Equipment Company, China). The light was placed 35-40 cm above the patient, and the vertical illumination area was 30 × 60 cm^2. Two milliliters of venous blood was collected before phototherapy and after 12 and 24 hours of phototherapy. 15 μl of 7.5% EDTA and 20 μl aprotinin were added to 1 ml of venous blood. The plasma was separated and ET level was determined using radioimmunoassay. Another 1 ml of venous blood was separated and NO level was determined using nitrate reductase. Heart rate (HR), respiration rate, apnea, mean arterial blood pressure (MABP) and other common side-effects such as fever, skin rash, and diarrhea associated with phototherapy were subsequently monitored. The plasma samples for ET determination were stored in a -70ºC refrigerator until analysis. Samples were defrosted either at room temperature or by re-warming in cold water, then centrifuged for 5 minutes at 3000 rpm and 4ºC. The resulting supernatant was used to determine ET levels. The radioimmunoassay kits were obtained from the BeiMian DongYa Biotechnology Institute, China and the manufacturer's instructions were followed strictly.

The same procedure was used for NO determination in stored plasma samples. The NO nitrate reductase kits were bought from the Nanjing Jiancheng Biotechnology Institute, China. The procedures were performed according to the manufacturer's recommendations.

Statistical analysis

SPSS 10.0 statistical package was used for statistical analysis. The data were compared using single-factor ANOVA of completely randomized design and Student's *t* test for independent samples. Rates were compared using the Chi-square test. The data were presented as means ± SD. A significant difference was defined as $P<0.05$ using two-tailed test.

Results

Changes of plasma ET levels in the course of phototherapy

Blood ET levels increased in the course of phototherapy for preterm infants of GA ≤32 weeks ($P<0.05$). There was no significant difference between ET levels before and after 12 hours of phototherapy ($P>0.05$), but a statistically significant difference was observed between levels determined after 12 and 24 hours of phototherapy ($P<0.05$). There was no statistical difference in plasma ET levels at baseline and after 12 and 24 hours of phototherapy in full-term infants of GA ≥37weeks and preterm infants of GA >32 weeks ($P>0.05$) (Table 1).

Changes of plasma NO levels in the course of phototherapy

In preterm infants of GA ≤32 weeks, the amount of NO in blood rose with the duration of phototherapy. The increases after 12 and 24 hours of phototherapy were statistically significant ($P<0.05$). In preterm infants of GA >32, the NO level after 24 hours but not after 12 hours was significantly higher than the baseline level ($P<0.05$). No statistically significant changes were observed in NO levels after 12 and 24 hours of phototherapy in full-term infants of GA ≥37 weeks ($P<0.05$) (Table 2).

Changes of NO:ET ratio in the course of phototherapy

The NO:ET ratio increased during the course of phototherapy in preterm infants of GA ≤32 weeks ($P<0.05$). There was no statistical significance in the other 2 groups (Table 3).

Changes of HR in the course of phototherapy

HR of each group had a tendency to rise with time of phototherapy but a statistical significance was observed only in preterm infants of GA ≤32 when baseline HR was compared with HR at 12 and 24 hours after phototherapy ($P<0.05$) (Table 4).

Changes of MABP in the course of phototherapy

The value of MABP decreased in the course of phototherapy in the preterm infants of GA ≤32 weeks, but a statistical significance was seen after 24 hours of phototherapy ($P<0.05$). There was no significant change in MABP in the other 2 groups ($P>0.05$) (Table 5).

The scatter gram shows the negative correlation of MABP with a rising NO:ET ratio; Pearson's product-moment correlation coefficient shows $r=-0.647$, $P=0.0005$ after 24 hours of phototherapy for the preterm infants of GA ≤32 weeks (Fig.).

Table 1. Blood endothelin levels at different times of phototherapy in full-term and preterm infants (means ± SD, ng/L)

Infants	*n*	Before phototherapy	After 12-hour phototherapy	After 24-hour phototherapy	*F*	*P*
Full-term	26	69.77±17.44	70.38±16.69	71.37±16.48	0.059	0.942
Gestational age >32 weeks	31	59.51±10.85	60.25±10.24	63.83±10.79	1.846	0.164
Gestational age ≤32 weeks	33	47.95±12.28	52.32±12.09*	62.67±14.61†,‡	11.090	0.000

Compared to before phototherapy, *: $P>0.05$, †: $P<0.05$; compared to after 12-hour phototherapy, ‡: $P<0.05$.

Table 2. Blood nitric oxide levels at different times of phototherapy in full-term and preterm infants (means ± SD, μmol/L)

Infants	*n*	Before phototherapy	After 12-hour phototherapy	After 24-hour phototherapy	*F*	*P*
Full-term	26	67.84±13.74	68.98±14.17	70.26±13.46	0.200	0.819
Gestational age >32 weeks	31	57.33±12.38	58.24±11.39*	64.63±11.06†,‡	4.096	0.020
Gestational age ≤32 weeks	33	52.35±9.82	64.31±10.20†	87.4±10.353†,‡	101.979	0.000

Compared to before phototherapy, *: $P>0.05$, †: $P<0.05$; compared to after 12-hour phototherapy, ‡: $P<0.05$.

Table 3. Blood NO:ET ratio at different times of phototherapy in full-term and peterm infants (means ± SD)

Infants	*n*	Before phototherapy	After 12-hour phototherapy	After 24-hour phototherapy	*F*	*P*
Full-term	26	1.020±0.290	1.024±0.285	1.029±0.280	0.006	0.994
Gestational age >32 weeks	31	0.987±0.247	0.993±0.254	1.042±0.249	0.434	0.649
Gestational age ≤32 weeks	33	1.148±0.327	1.281±0.307*	1.460±0.342†,‡	7.615	0.001

Compared to before phototherapy, *: $P>0.05$, †: $P<0.05$; compared to after 12-hour phototherapy, ‡: $P<0.05$.

Table 4. Heart rate at different times of phototherapy in full-term and preterm infants (means ± SD, rates/min)

Infants	*n*	Before phototherapy	After 12-hour phototherapy	After 24-hour phototherapy	*F*	*P*
Full-term	26	130.81±7.07	131.62±8.38	133.04±8.15	0.533	0.589
Gestational age >32 weeks	31	135.77±8.69	137.10±8.91	140.97±9.64	2.683	0.074
Gestational age ≤32 weeks	33	142.88±10.25	147.24±7.94*	155.97±9.20†,‡	17.406	0.000

Compared to before phototherapy, *: $P>0.05$, †: $P<0.05$; compared to after 12-hour phototherapy, ‡: $P<0.05$.

Table 5. Mean arterial blood pressure at different times of phototherapy in full-term and preterm infants (means ± SD, mmHg)

Infants	*n*	Before phototherapy	After 12-hour phototherapy	After 24-hour phototherapy	*F*	*P*
Full-term	26	60.42±7.06	59.65±5.37	59.10±5.10	0.331	0.719
Gestational age >32 weeks	31	56.35±7.21	55.10±6.72	54.28±8.34	0.654	0.522
Gestational age ≤32 weeks	33	51.70±10.07	49.89±9.70*	44.11±10.63†,‡	5.030	0.008

Compared to before phototherapy, *: $P>0.05$, †: $P<0.05$; compared to after 12-hour phototherapy, ‡: $P<0.05$.

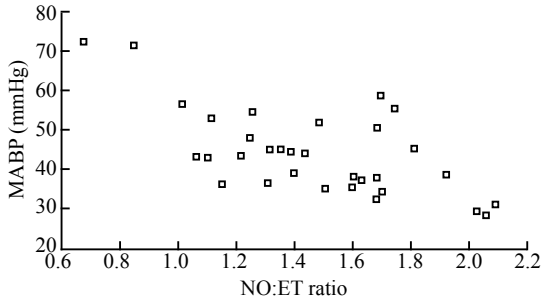

Fig. A scatter gram of NO:ET ratio in blood and MABP after 24 hours of phototherapy in the gestational age ≤32 weeks group. MABP: mean arterial blood pressure.

Table 6. The episodes of apnea before and after phototherapy in each group of infants

Infants	*n*	Before phototherapy	After 24-hour phototherapy
Full-term	26	0	0
Gestational age >32 weeks	31	0	5
Gestational age ≤32 weeks	33	0	14

The episodes of apnea in the course of phototherapy

No apnea was observed in the studied infants before phototherapy. But 14 episodes of apnea were seen in the preterm infants of GA ≤32 weeks during 24 hours of phototherapy. This was comparable to 5 episodes in the preterm infants of GA >32 weeks and none in the full-term infants (Table 6).

Discussion

Previous studies showed that hemodynamic changes in preterm infants as a result of phototherapy were related to the vasoactive substance NO.[16,17] It has been shown that light has the function of relaxing blood vessels (photo relaxation). Some photo-aesthesia materials induced by light in blood vessel can cause the production of NO, which in turn stimulates an increase in cGMP, resulting in smooth muscle relaxation and dilatation of blood vessels.[18]

ET and NO are vasoactive substances with opposing effects. They regulate blood vessel tension and hence maintain the blood flow under physiological conditions. There is a feedback regulation between them: the release of NO will be promoted when ET combines with its B receptor at blood vessel endothelium,[19] and NO can inhibit the release of ET contrarily. In pathological conditions, however, the dynamic equilibrium between NO and ET is upset, leading to changes in hemodynamics and clinical symptoms. A study[20] showed that ET would increase and NO would decrease in RDS patients. This is an important factor leading to lung arterial pressure. Another study[21] showed NO increased in patients with primary hypotension. The NO and ET levels in the observation groups were obviously higher than those in the control group. Balance index (NO:ET) increased compared to the control group. There was a close negative correlation between blood pressure and NO, ET, NO:ET. Researchers considered that NO and ET may contribute to the morbidity of primary hypotension. The effect of phototherapy on circulating NO levels remains unclear. Since regional blood flow is affected by NO and ET, it is important to study the two opposing mechanisms. In our study, the hemodynamic changes were observed in blood NO and ET levels in the course of phototherapy for a period of 24 hours.

In our study, the shorter GA and the longer duration of phototherapy, the higher increase of plasma ET level. Phototherapy has a similar effect on the plasma NO levels in preterm infants, especially those of GA ≤32 weeks. The longer duration of phototherapy, the higher increase of the NO level in their blood. After phototherapy, the NO:ET ratio showed a tendency to increase because of a relatively higher increase in NO compared with ET. This disturbed the dynamic balance between them, leading to a dilatation of blood vessels and a subsequent drop in blood pressure, which might be responsible for the opening of the ductus arteriosus.[10,22]

Impact of phototherapy on vital signs of preterm infants

As Doppler ultrasound is not commonly available in newborn nurseries, changes of vital signs are used as surrogate markers for hemodynamic stability. One study[16] found that HR would increase and blood pressure would fall after 6 hours of phototherapy in the full-term infants, although these changes were not statistically significant. But the clinical data are rare in preterm infants. In our study, HR was increased and MABP was decreased in the course of phototherapy in all the groups. These changes were statistically significant after 24 hours of phototherapy in infants of GA ≤32 weeks. In the full-term infants and infants of GA >32 weeks, the changes in HR and MABP after 24 hours of phototherapy were not statistically significant. This finding suggested that phototherapy had a greater effect on the vital signs of infants of lower GA in our study.

A negative linear correlation between the NO:ET ratio and MABP in infants ≤32 weeks GA

Phototherapy has an impact on ET and NO levels in plasma of preterm infants, particularly preterm infants and those of lower GA. The longer the duration of phototherapy, the higher the rise in plasma ET and NO levels. The lower the GA and the longer

the duration of phototherapy, the higher the NO:ET ratio and the greater the potential for de-stabling the hemodynamic equilibrium. Our findings question the safety of continuous phototherapy for a 24-hour period in preterm infants of low GA. This is of particular importance in sick preterm infants who might already be hemodynamically compromised.

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Contributors: Liu GS contributed to the design of the study and to futher drafts. Wu H analyzed the data and wrote the first draft. Wu BQ, Huang RZ, Zhao LH and Wen Y collected and analyzed the data.

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