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.


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
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.\cite{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 loss of blood brain barrier, and increases the permeability,\cite{13} resulting in encephalodema 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 2000 Icterus Neonatorum Intervention Guidelines recommended by the Group of Newborns, Division of Pediatrics, Chinese Medical Association.\cite{14} The guidelines are consistent with those published by the American Academy of Pediatrics (AAP).\cite{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 cm². 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 -70°C 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 4°C. 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 ≥37 weeks and preterm infants of GA >32 weeks (\( P > 0.05 \)) (Table 1).