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Randomized trial of early erythropoietin supplementation after preterm birth: Iron metabolism and outcome

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ABSTRACT

Background: Excess of iron and oxidant injury shortly after birth may be associated with neonatal morbidities in preterm infants.
Aims: The aim was to determine whether administration of erythropoietin without iron supplementation decreases iron load and morbidity.
Study design and subjects: In a randomized trial, we administered erythropoietin (EPO 250 IU/kg daily during the first 6 days of life) or placebo to 39 preterm infants (BW 700–1500 g, GA ≤ 30.0 weeks).
Outcome measures: The iron status, postnatal morbidities and follow-up at the age of two years were investigated.
Results: In all, 21 EPO- and 18 placebo-treated infants were recruited. A requirement of red blood cell transfusions during first 28 days was similar between the study groups. EPO treatment decreased total serum iron concentration ($p = 0.035$). EPO supplementation had no significant effect on serum transferrin receptors or reactive non-protein-bound iron. There were no differences in neonatal morbidity or in survival without major neurological abnormality at two years of age.
Conclusions: A 6-day course of EPO decreased the iron load in preterm infants. There was no change in reactive, non-protein bound iron plasma levels and no influence on the outcomes during early childhood. Whether the neurocognitive effects of early EPO treatment can be detectable later in childhood remained to be verified.

1. Introduction

Oxidant injury is suggested to play a major role in the pathogenesis in diseases of premature infants [1]. These include respiratory distress syndrome (RDS), bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP) and brain injuries. Oxidant injury measured as the activity of lipid peroxidation is maximal 4 to 7 days after birth. At this age, high oxidant activity is associated with the development of BPD [2].

Iron is an important catalyst for the free radical reactions [3]. Shortly after birth, iron may be available in high quantities as a result of excessive breakdown of the large red blood cell mass and deficiency in iron-binding proteins [4]. The concentration of plasma transferrin, which is the major iron-binding protein, is also low in premature infants [5]. Exceeding the iron-binding capacity of proteins may transform iron as a reactive molecule catalyzing the formation of free oxygen radicals and lipid peroxides [6]. Since several other antioxidant systems, potentially capable of decreasing the toxicity of free iron, are also deficient in preterm infants [7,8], premature infants may be particularly susceptible to iron-catalyzed free radical injury.

Free radicals damage lipids, proteins and DNA, increasing alveolar-capillary permeability by damaging the capillary endothelium and epithelium [9]. As a consequence, susceptibility to high permeability edema and bleeding may increase, leading to inactivation of the surfactant complex in RDS [10] and to intraventricular hemorrhage (IVH) [11].

Our objective, based on an animal study [12], was to evaluate whether erythropoietin (EPO) without iron supplementation during the first neonatal days decreases severe respiratory morbidity by mobilizing iron from serum and decreasing the reactive free iron. These reactive

Abbreviations: BPD, Bronchopulmonary dysplasia; CP, Cerebral palsy; EPO, Erythropoietin; IVH, Intraventricular hemorrhage; NICU, Neonatal intensive care; OI, Oxygen index; OUH, Oulu University Hospital; PDA, Patent ductus arteriosus; PVL, Periventricular leukomalacia; RBC, Red blood cells; RDS, Respiratory distress syndrome; ROP, Retinopathy of prematurity

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molecules may be involved in oxidant injury and may promote serious morbidity in high-risk preterm infants. In the present randomized phase 1–2 trial, we studied the effects of EPO (Six doses, each 250 IU/kg during the first 6 days of life) without iron supplementation on serum biomarkers and on the neonatal and 2-year outcomes.

2. Material and methods

2.1. Randomized double-blind study

The EPO dosage of 250 U/kg given intravenously was chosen on the basis of an open phase I study comparing two doses of EPO: 125 and 250 U/kg (Table A1, Appendix). There was evidence of a linear response in erythropoiesis and no adverse effects were detected. The small randomized, double-blinded, placebo-controlled single-center phase II trial was performed next. The newborn infants admitted to the intensive care unit of the Oulu University Hospital (OUH) with a birth weight of 700–1500 g, gestational age of 24 + 0 to 30 + 0 weeks and with intra-arterial and intra-venous catheters, were considered for eligibility. The exclusion criteria were major congenital malformation, early sepsis or congenital infection, thrombocytopenia with a platelet count of < 403/mm^3, neutropenia with an absolute neutrophil count < 500/mm^3, diastolic blood pressure higher than 60 mm Hg, severe shock or hydrops fetalis. The Ethical Committee of OUH approved the study protocol. Written informed consent was obtained from the parents. The infants were enrolled at the neonatal intensive care unit (NICU) of the OUH from March 1998 to May 2000. Fig. 1 shows the number of infants studied during the trial and during the follow-up visits.

The simple randomization was performed using a random number table and the allocation was concealed. The study drug and placebo were put into identical syringes, and the nurses, doctors and study investigators were blinded. The infants were stratified into two weight groups (750–999 g, 1000–1500 g), receiving intravenously either recombinant human erythropoietin (Eprex®) in six doses for six consecutive days, 250 U/kg each, starting the first day after birth, or isotonic saline as a placebo for a period of 30 min. None of the study infants received iron during the first week after birth. Criteria for discontinuation of the study drug were major lethal malformation discovered after trial entry, congenital infection discovered after trial entry, severe shock or neutropenia (< 500/mm^3) and thrombocytopenia (< 40^3/mm^3). The study drug was resumed once neutrophil or platelet counts recovered. Guidelines for red-cell transfusions during the trial were based on existing policy in the NICU (Table A2). Infants who met the transfusion criteria received packed red blood cells.

2.2. Clinical outcomes

The primary outcome was defined as the severity of acute respiratory disease, which was measured as the oxygen index (OI) calculated from the need for supplemental oxygen and mechanical ventilation during first six days of life (OI = mean airway pressure × FiO_2 × 100/PaO_2). The secondary outcomes were the requirement of red blood cell transfusions during the first two weeks of life. The incidence of mild BPD was defined as the need for supplementary oxygen at 28 days. The incidence of moderate to severe BPD was defined as the need for supplementary oxygen at 28 days and at 36 postconceptional weeks [13]. The oxygen saturation target was 90 to 96%. For the diagnosis of retinopathy of prematurity (ROP), the ophthalmoscopic examination was repeated until retinas were mature, and the highest stage of retinopathy was reported. The severity of ROP was graded according to the international classification [14]. A cranial ultrasound was performed during the neonatal period. IVH grade 3–4 [15] and periventricular leukomalacia (PVL) [16] were included as outcomes.

The number of days on assisted ventilation, the use of supplemental oxygen, the use of postnatal corticosteroid treatment for prevention of BPD and treatment of hypotension, and the length of hospital stay were recorded. Nosocomial sepsis was defined as a positive blood culture after day 3 of life. Diagnostic data on hyperglycemia, hypo- or hypertension requiring therapy, patent ductus arteriosus (PDA) treated with prostaglandin inhibitor therapy or surgery, necrotizing enterocolitis (NEC) and intestinal perforations were also recorded. Follow-up in early childhood was performed at 2 years of corrected age. Overall development was evaluated by the pediatrician (T.K.) using the Griffiths Developmental Score [17]. Cerebral palsy (CP) was defined as described [18]. The child's growth characteristics were recorded.

2.3. Laboratory analyses

Blood samples were collected on the following days after birth: 0, 3, 6, 12 and 28. The blood cells and differential counts of leukocytes were analyzed at the OUH central laboratory. The blood specimens were used to analyze complete cell counts and the morphology of red blood cells (RBC) by microscopy, calculation of hypochromic RBCs, plasma iron content, transferrin and ferritin. Serum concentrations of transferrin receptor were analyzed using an immunoturbidimetric method (Orion Diagnostica, Espoo, Finland). Free plasma iron was analyzed as previously described [19].

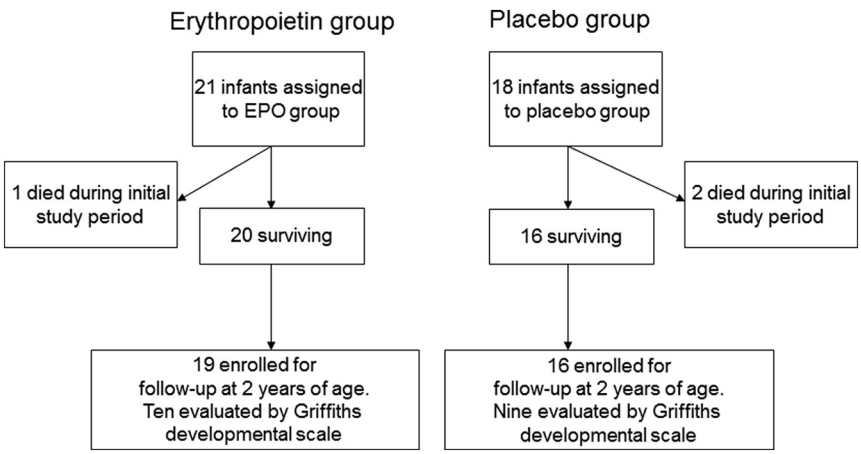

Fig. 1. Study design and follow-up of participants during early and later childhood.