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Effects of early parenteral iron combined erythropoietin in preterm infants
A randomized controlled trial

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Abstract

Backgroud: The aim of the study was to evaluate the effect of early parenteral iron supplementation combined erythropoietin for prevention of anemia in preterm infants.

Methods: In total, 96 preterm infants were randomly assigned to 3 groups: a control group receiving standard parenteral nutrition (group 1: n = 31), an iron-supplemented group (group 2: IS, n = 33), and an iron-supplemented combined erythropoietin group (group 3: IS+EPO, n = 32). The primary objective was to assess hemoglobin (Hb) levels. The secondary objectives included assessment of red blood cell counts (RBC), mean cell volume (MCV), serum iron, ferritin, percentages of reticulocyte (RET), total iron binding capacity (TIBC) and oxidative stress, which was assessed by measuring plasma levels of malondialdehyde and superoxide dismutase at baseline and at 2 weeks. The blood routine indices including Hb, RBC, MCV, and percentages of RET were measured at corrected age of 1 and 3 months.

Results: At 2 weeks of life, the percentages of reticulocyte in group 2 and group 3 were significantly higher than those in group 1 (2.1 ±0.4, 2.5±0.3, and 1.7±0.3, respectively, $P < 0.001$, $P < 0.001$), whereas TIBC were significantly lower than those in group 1 (36.7±4.6, 36.0±4.7, and 41.6±5.2 respectively, $P = 0.011$, $P = 0.006$). There were no significant differences in RBC counts, the levels of hemoglobin, ferritin, malondialdehyde, and superoxide dismutase among the 3 groups at 2weeks of life. RBC, Hb, MCV, body weight, body length, and head circumference at a corrected age of 1 month did not differ among 3 groups. At corrected age of 3months, more infants in the control group had abnormal Hb and MCV levels (Hb levels: 114.3±21.3, 123.7±31.6, and 125.1±21.2, $P = 0.021$, $P = 0.034$, respectively; MCV: 74.1±3.5, 78.3±4.7 and 79.1±5.2, $P = 0.017$, $P = 0.012$, respectively), whereas cases of oral iron, cases of breastfeeding, RBC, body weight, body length, and head circumference were not different among 3 groups.

Conclusion: Early parenteral iron supplementation combined erythropoietin in preterm infants improved the percentages of reticulocyte, decreased total iron binding capacity, and improved the Hb and MCV levels at 3 months of age. Early parenteral iron supplementations with EPO were beneficial for the preterm infants.

Abbreviations: EPO = anemia erythropoietin, Hb = hemoglobin, ID = iron deficiency, MCV = mean cell volume, NICU = neonatal intensive care unit, PN = parenteral nutrition, RBC = red blood cell counts, RET = percentages of reticulocyte, TIBC = total iron binding capacity.

Keywords: anemia, erythropoietin, intravenous iron, parenteral nutrition, preterm infants

1. Introduction

Premature infants are at risk for low iron storage, iron deficiency (ID), and anemia. Erythropoietin (EPO) has been used in preterm infants to prevent and treat anemia, and is usually given 3 times a week as a subcutaneous injection.[1] The iron store is low in preterm infants and rapidly gets depleted during the first 6 to 8 weeks.[2,3] As iron is needed for various tissue functions, iron supplementation is essential for preterm infants. Oral iron therapy is not well tolerated due to gastrointestinal immaturity in preterm infants and hence intravenous iron therapy appears to be an alternative treatment strategy.[4,5] Guidelines on pediatric parenteral nutrition (PN) suggest a dose of 200 μg/kg/d iron,[6–8] but the time of initiation of therapy remains controversial in these infants. In the present study, we aimed to elucidate whether parenteral iron combined erythropoietin (EPO) could improve anemia in preterm infants.

2. Patients and methods

2.1. Patients

We recruited 96 preterm infants with gestational age between 28 and 34 weeks treated in the neonatal intensive care unit (NICU) of the First People's Hospital in Kunshan, Jiangsu University between February 2014 and June 2014. Exclusion criteria were infants with (1) liver and kidney dysfunction; (2) hemolytic

disease of newborns; (3) hemorrhage (gastrointestinal, pulmonary, and intracranial hemorrhage grade III–IV, 24h decline in Hb> 2g/L); (4) blood transfusion; (5) major or life-threatening malformations.[5] Approval was obtained from the Ethics Committee of the First People's Hospital in Kunshan, Jiangsu University, and the study was registered with ClinicalTrials.gov (Identifier: NCT02060851). Parents gave informed consent.

2.2. Randomization

The study was a single-blind, randomized, and controlled clinical trial. The patients were randomly assigned to 1 group in the following manner: treatment cards were imprinted with a unique randomization code and placed in sequentially numbered, opaque envelopes. At the study site, the treatment cards were taken out in the sequential order, and the patients were assigned to the corresponding treatment group based on the randomization number. All investigators, physicians, and nurses involved in patient care and parents were blinded to this assignment. The randomization process was made available only to the pharmacist who supervised the quality of iron sucrose (IS) and the PN preparation. Study infants were followed up until discharge from the hospital. Infants were withdrawn from the trial if severe adverse effects developed or parents withdrew the consent.

2.3. Dosing of study drug

Infants were randomized to 3 groups: (1) group 1—a control group who received standard PN; (2) group 2—an iron-supplemented group (IS); (3) group 3—iron-supplemented combined EPO group (IS+EPO). EPO, 400U/kg, was given twice a week (Monday and Friday). IS, 200μg/kg per day, was given everyday with PN, and the dose was continued until 2 weeks after birth. The patients were treated for 2 weeks, or as long as they received parenteral nutrition. Criteria for discontinuation of drug administration included a systemic adverse reaction to iron such as hypotension, respiratory compromise, rash, or hypertension. All infants received parenteral nutrition from the second day of birth until infants could receive 120mL/kg per day of enteral feedings.

2.4. Nutrition support

Parenteral nutritional support for the study neonates was provided according to published guidelines.[9] The "all-in-one" solution contained lipids (20% lipfundin; B. Braun), amino acids (6% pediatric amino acid), glucose, minerals, trace elements, and vitamins. The infants in group 2 received iron and group 3 received EPO along with PN. All preterm infants were fed standard preterm formula (SPF), NeoSure or Similac, which contained 0.9mg of iron per 100mL. The formula was started with 10mL/kg per day and increased at 10mL/kg per day until 150mL/kg per day. The enteral feeding was stopped when infants developed symptoms of necrotizing enterocolitis (NEC). The amount of enteral nutrition and the symptoms of feeding intolerance were recorded.

2.5. Data collection

The general information, number of ventilator days, length of stays in hospital, number of blood examination in hospital and incidence of NEC and retinopathy of prematurity (ROP) were recorded. The levels of blood sugar and liver and renal function were examined before and after the interventions. Assessment of Hb levels was the primary objective, whereas, the secondary objectives included assessment of RBC count, mean corpuscular volume (MCV), serum iron (SI), transferrin saturation (TS), ferritin, the percentage of reticulocyte count (RET), total iron binding capacity (TIBC), and malondialdehyde (MDA) and superoxide dismutase (SOD) at baseline and 2 weeks after supplementation. RBC count, Hb and MCV levels were measured at corrected ages of 1 month and 3 months. A radio-immunoassay method, chemical method, and an automatic biochemical analyzer was used to test the serum ferritin (DADE BEHRING, Germany), serum iron (automatic biochemical analyzer, 7600, HITACHI), respectively. The extraction method was used to test SOD (SOD Assay Kit, Nanjing built biological science and Technology Co., Ltd) and thiobarbituric acid (TBA) method was used to test MDA (MDA Assay Kit, Nanjing built biological science and Technology Co., Ltd).

2.6. Statistical analyses

The sample size was 21 for each group, which calculated according to 0.05 as type I error of 5%, a power of 80% and 20g/LHb as clinically significant increase. Considering a dropout 20%, we enrolled 26 patients for each group and 78 patients in total. The data was analyzed using SPSS 21.0 for the Windows software, and the results are expressed as mean±SD (x±s) or median and interquartile range. Differences in rates were analyzed by the chi-square test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. General characteristics

Of 96 premature infants enrolled, 91 infants completed the study (30 belonged to the control group, 31 to the IS group, and 30 to the IS+ EPO group). Five infants could not complete the study for the following reasons: 1 in the IS group died because of respiratory failure, and 4 infants (1 in the control group, 1 in the IS group, and 2 in the IS+PN group) were discharged because treatments were discontinued by their parents. There were no significant differences between the baseline characteristics among 3 groups (Table 1).

3.2. Hematological evaluation

The percentages of RET in the IS and IS+EPO groups (2.1±0.4 and 2.5±0.3, respectively) were significantly higher than those in the control group (1.7±0.3) (Table 2). There were no significant differences in the other indicators of anemia, including RBC count, Hb, hematocrit, and MCV among 3 groups.

3.3. Iron storage evaluation

TIBC in the IS and IS+EPO groups (36.7±4.6 and 36.0±4.7, respectively) were significantly lower than those in the control group (41.6±5.2) (Table 2). There were no significant differences in the indicators of iron storage, including SI, SF, and TS, among the 3 groups.

3.4. Comparison of oxidative stress

The levels of MDA and activity of SOD were not significantly different between preintervention and postintervention among the 3 groups (Table 2).

Table 1

Baseline characteristics of study subjects.

	Control (n=30)	IS group (n=31)	IS+EPO (n=30)	P	P*
Sex, M/F	17/13	19/12	16/14	0.7136	0.7952
Gestational age, weeks	30.3±2.1	30.4±1.7	31.3±1.5	0.924	0.876
Birth weight, g	1486±392	1525±502	1490±449	0.567	0.643
Head circumference, cm	28.5±1.8	29.1±2.4	28.6±2.1	0.785	0.967
Day of beginning EN, d	1.1±0.8	0.9±1.2	1.0±1.4	0.135	0.564
Days of PN, d	8.3±1.6	9.2±0.8	8.1±1.3	0.324	0.675
Apgar score	8.5±2.6	8.8±1.4	8.3±2.1	0.653	0.248
Days on ventilator, d	3.5±1.6	3.2±2.1	4.1±1.8	0.162	0.233
Number of blood examination in hospital	8.3±1.5	9.2±1.8	8.7±2.1	0.477	0.368
RBC, $\times 10^9$	5.3±0.6	4.9±0.7	5.1±0.6	0.675	0.876
Hb, g/L	163.1±31.6	161.8±23.6	168.1±22.0	0.846	0.567
HCT, %	52.3±8.3	50.3±6.7	49.9±5.8	0.532	0.438
MCV, fl	95.1±4.7	97.3±4.7	94.2±4.1	0.436	0.367
SI, μmol/L	13.3±4.6	15.6±7.4	14.3±4.3	0.156	0.276
SF, μg/L	219.8±78.5	212.0±63.4	196.6±74.3	0.455	0.265
TIBC, μmol/L	45.3±8.4	43.9±12.5	41.3±6.8	0.654	0.186
TS, %	27.6±9.3	26.6±12.8	30.7±11.7	0.854	0.187
Reticulocyte, %	4.2±0.3	4.4±0.5	3.9±0.4	0.332	0.169
MDA, nmol/g	39.4±18.8	36.9±21.9	38.1±16.3	0.108	0.654
SOD, U	198.0±87.6	231±98.6	245.0±126.7	0.233	0.121

EN = Enteral nutrition, F = female, HCT = hematorit, IS = iron-supplemented group, IS+EPO = iron-supplemented combined EPO group, M = male, MCV = mean cell volume, MDA = malondialdehyde, P = iron-supplemented group versus control group, P* = iron-supplemented combined EPO versus control group, PN = parenteral nutrition, RBC = red blood cell counts, SI = serum iron, SOD = superoxide dismutase, TIBC = total iron binding capacity, TS = transferrin saturation.

3.5. Follow-up results

RBC, Hb levels, MCV levels, body weight, body length, and head circumference at a corrected age of 1 month did not differ among 3 groups. Infants in the control group were significantly more likely to have abnormal Hb and MCV levels at a corrected age of 3 months, whereas RBC, body weight, body length, and head circumference among 3 groups were not significantly different (Table 3). None of the patients developed NEC and ROP during the study period.

4. Discussion

The present study has demonstrated that the percentages of reticulocyte in the iron-supplemented and iron-supplemented with EPO groups of preterm infants were significantly higher after 2 weeks of therapy. TIBC in the iron-supplemented and iron-supplemented with EPO groups were significantly lower than the TIBC in the control group. At 3 months of age, Hb in the iron-supplemented and iron-supplemented with EPO groups was significantly higher than those in the control group, and MCV in iron-supplemented groups were greater than those in the control group. Early parenteral iron supplementation, mostly from day 2 of life, combined with erythropoietin in preterm infants improved reticulocyte counts and decreased total iron binding capacity without influence on other indices, and improved the Hb and MCV levels at 3 months of age. Therefore, early parenteral iron supplementations with EPO were found to be beneficial for the preterm infants.

The study infants tolerated iron well, there was no rash, fever, or other allergic reactions, and the safety of iron dextran has been confirmed.[8,10] Statistical comparison between groups showed that iron did not increase oxidative stress (P> 0.05). However, the number of cases and the study time was limited, and long-term follow-ups could not be performed.

Table 2

Comparison of indicators of postintervention among the 3 groups at 2 weeks.

	Control (n=30)	IS group (n=31)	IS+EPO (n=30)	P	P*
RBC, $\times 10^9$	4.1±0.6	4.0±0.5	4.3±0.7	0.672	0.223
Hb, g/L	138.2±26.2	136.2±20.7	135.2±20.0	0.654	0.176
HCT, %	41.1±8.9	40.4±6.7	40.9±6.9	0.554	0.332
MCV, fl	103.2±3.8	102.7±3.5	104.6±4.3	0.453	0.326
SI, μmol/L	17.6±3.8	17.0±5.0	19.5±2.9	0.656	0.112
SF, μg/L	264.9±83.7	295.1±84.2	289.8±143.9	0.243	0.334
TIBC, μmol/L	41.6±5.2	36.7±4.6	36.0±4.7	0.011	0.006
TS, %	46.2±13.4	47.9±11.5	43.4±12.7	0.425	0.103
Reticulocyte, %	1.7±0.3	2.1±0.4	2.5±0.3	<0.001	<0.001
MDA, nmol/g	33.9±15.0	28.9±9.6	35.1±13.7	0.106	0.345
SOD, U	202.1±83.1	209.7±70.1	175.1±45.8	0.887	0.238

F = female, IS = iron-supplemented group, IS+EPO = iron-supplemented combined EPO group, M = male, MCV = mean cell volume, MDA = malondialdehyde, P = iron-supplemented group versus control group, P* = iron-supplemented combined EPO versus control group, PN = parenteral nutrition, RBC = red blood cell counts, SI = serum iron, SOD = superoxide dismutase, TIBC = total iron binding capacity, TS = transferrin saturation.

Table 3

Comparison of growth parameters and selected laboratory parameters at corrected age of 3 months.

	Control (n=30)	IS group (n=31)	IS+EPO (n=30)	P	P*
Body weight, g	6825±283	6582±431	6634±312	0.843	0.985
Body length, cm	61.3±3.5	60.1±4.2	60.1±3.6	0.734	0.921
Head circumference, cm	28.5±1.8	29.1±2.4	28.6±2.1	0.785	0.967
RBC, ×10^9	3.8±0.4	4.0±0.3	3.7±0.3	0.134	0.083
Hb, g/L	114.3±21.3	123.7±31.6	125.1±21.2	0.021*	0.034*
MCV, fl	74.1±3.5	78.3±4.7	79.1±5.2	0.017*	0.012*

Hb = hemoglobin, IS = iron-supplemented group, IS+EPO = iron-supplemented combined EPO group, MCV = mean cell volume, P = iron-supplemented group versus control group, P*, iron-supplemented combined EPO versus control group, RBC = red blood cell counts, .

The percentages of RET in the iron-supplemented and iron-supplemented with EPO groups was significantly higher than those in the control group. Robin et al, demonstrated that preterm infants responded to weekly EPO by increasing the percentages of RET and maintaining hematocrit.[11] There were no significant differences in the other indicators of anemia including RBC, Hb, hematorit, and MCV between the 3 groups. Surico et al[12] showed that after 6 days of intravenous iron sucrose therapy in preterm infants, hemoglobin significantly improved. A study on neonatal dairy calves showed parenteral supply of iron can improve mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and iron level.[13] Two studies had shown that intravenous iron therapy can reduce the number of blood transfusions, and the amount of EPO needed.[14,15] There were no differences in the indicators of anemia in the iron-supplemented and iron-supplemented with EPO groups, as compared with the control group. Infants in the control group were significantly more likely to have abnormal Hb and MCV levels at a corrected age of 3 months. This observation could have resulted from use of lower dosages of iron than the dose of 200 µg/kg per day, which is the recommended amount.[7] Another reason could be the short duration of iron intervention and the early application of EPO.

The indicators of iron storage include SI, SF, TIBC, and TS. TIBC in the iron-supplemented and iron-supplemented with EPO groups was significantly lower compared to the control group. There were no significant differences in the other indicators of iron storage, including SI, SF, and TS, among the 3 groups. Previous studies have shown that 120 µg/kg/d of intravenous iron can maintain positive iron balance in preterm infants[4]; however, in the present study, the duration of intervention was short. Serum ferritin is the body's most sensitive indicator of iron deficiency. Pollak and co-workers[5] demonstrated significantly increased serum ferritin levels after 11 days of intravenous iron and EPO in preterm neonates with anemia compared with the control group.

MDA, SOD, and glutathione peroxidase (GSH-Px) in blood or urine are the main indicators of oxidative stress.[16] Pollak et al[5] found no difference in oxidative stress in the intravenous iron treated preterm infants compared with the control group, and iron sucrose caused only a transient increase of MDA in 4 patients. Our study results showed that the indicators of oxidative stress (MDA and SOD) were not significantly changed ($P > 0.05$), indicating 200 µg/kg of iron per day in preterm PN did not increase oxidative injury or decrease antioxidative capacity. Whether intravenous iron is directly involved in oxidative stress in preterm infants needs to be explored.

In summary, preterm infants at risk of iron deficiency should be identified and treated with iron supplementation. Early paren-

teral iron supplementations with EPO were beneficial for the preterm infants. The present study results agree with European Society of Parenteral and Enteral Nutrition (ESPEN) recommendation that early intravenous iron added to PN, and EPO given for 2 weeks could significantly improve TIBC and reticulocyte counts, and improve the Hb and MCV levels at 3 months of age. The potential effect of Epo as a neuroprotective agent for preterm brain needs a longer follow-up period. The effects of longer duration and larger dose of intravenous iron supplementation and the potential beneficial effects of iron on long-term neurocognitive and psychomotor development warrant future study.

Acknowledgments

References

[1] Ohls RK, Ehrenkranz RA, Wright LL, et al. Effects of early erythropoietin therapy on the transfusion requirements of preterm infants below 1250 grams birth weight: a multicenter, randomized, controlled trial. Pediatrics 2001;108:934–42.

[2] Mukhopadhyay K, Yadav RK, Kishore SS, et al. Iron status at birth and at 4 weeks in preterm-SGA infants in comparison with preterm and term-AGA infants. J Matern Fetal Neonatal Med 2012;25:1474–8.

[3] Siddappa AM, Rao R, Long JD, et al. The assessment of newborn iron stores at birth: a review of the literature and standards for ferritin concentrations. Neonatology 2007;92:73–82.

[4] Friel JK, Andrews WL, Hall MS, et al. Intravenous iron administration to very-low-birth-weight newborns receiving total and partial parenteral nutrition. J Parenter Enteral Nutr 1995;19:114–8.

[5] Pollak A, Hayde M, Hayn M, et al. Effect of intravenous iron supplementation on erythropoiesis in erythropoietin-treated premature infants. Pediatrics 2001;107:78–85.

[6] Carnielli VP, Da Riol R, Montini G. Iron supplementation enhances response to high doses of recombinant human erythropoietin in preterm infants. Arch Dis Child Fetal Neonatal Ed 1998;79:F44–48.

[7] Koletzko B, Goulet O, Hunt J, et al. 1. Guidelines on Paediatric Parenteral Nutrition of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Society for Clinical Nutrition and Metabolism (ESPEN), Supported by the European Society of Paediatric Research (ESPR). J Pediatr Gastroenterol Nutr 2005;41(suppl 2):S1–87.

[8] Mayhew SL, Quick MW. Compatibility of iron dextran with neonatal parenteral nutrient solutions. Am J Health Syst Pharm 1997;54:570–1.

[9] Lozoff B, Georgieff MK. Iron deficiency and brain development. Semin Pediatr Neurol 2006;13:158–65.

[10] Chertow GM, Mason PD, Vaage-Nilsen O, et al. Update on adverse drug events associated with parenteral iron. Nephrol Dial Transplant 2006;21:378–82.

[11] Robin KO, Mashid R, Hannah MP, et al. A randomized, masked study of weekly erythropoietin dosing in preterm infants. J Pediatr 2012;160: 790–5.

[12] Surico G, Muggeo P, Muggeo V, et al. Parenteral iron supplementation for the treatment of iron deficiency anemia in children. Ann Hematol 2002;81:154–7.

[13] Coyne DW, Adkinson NF, Nissenson AR, et al. Sodium ferric gluconate complex in hemodialysis patients. II. Adverse reactions in iron dextran-sensitive and dextran-tolerant patients. Kidney Int 2003;63:217–24.

[14] Corwin HL, Gettinger A, Pearl RG, et al. Efficacy of recombinant human erythropoietin in critically ill patients: a randomized controlled trial. JAMA 2002;288:2827–35.

[15] Mohri M, Poorsina S, Sedaghat R. Effects of parenteral supply of iron on RBC parameters, performance, and health in neonatal dairy calves. Biol Trace Elem Res 2010;136:33–9.

[16] Braekke K, Bechensteen AG, Halvorsen BL, et al. Oxidative stress markers and antioxidant status after oral iron supplementation to very low birth weight infants. J Pediatr 2007;151:23–8.