A Randomized, Masked, Placebo-Controlled Study of Darbepoetin Alfa in Preterm Infants

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KEY WORDS

anemia, transfusions, prematurity, erythropoietin

ABBREVIATIONS

ARC—absolute reticulocyte count

Darbe—darbepoetin alfa

ELBW—extremely low birth weight

Epo—erythropoietin

ESA—erythropoiesis stimulating agents

ICH—intracranial hemorrhage

Ig—immunoglobulin

NEC—necrotizing enterocolitis

PRBC—packed red blood cells

ROP—retinopathy of prematurity

Dr Ohls conceptualized and designed the study, performed analyses, and drafted the initial manuscript; Dr Christensen conceptualized and designed the study, was the principal investigator at the Ogden site, and reviewed and revised the manuscript; Dr Kamath-Rayne served as co-principal investigator and supervised data collection at the Colorado site, and critically reviewed and revised the manuscript; Dr Rosenberg was the principal investigator and supervised data collection at the Colorado site, and critically reviewed the manuscript; Dr Wiedmeier was the principal investigator and supervised data collection at the Salt Lake City site, and reviewed the manuscript; Ms Roohi and Ms Lacy coordinated the study and performed data collection at the New Mexico site, and critically reviewed and revised the manuscript; Ms Lambert coordinated the study and performed data collection at the Ogden site, and critically reviewed the manuscript; Ms Burnett coordinated the study and performed data collection at the Salt Lake City site, and critically reviewed the manuscript; Ms Pruckler coordinated the study and performed data collection at the Colorado site, and critically reviewed the manuscript; Dr Schrader performed the statistical analyses, and critically reviewed the manuscript; Dr Lowe performed statistical analyses, and critically reviewed and revised the manuscript; and all authors approved the final manuscript as submitted.

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WHAT'S KNOWN ON THIS SUBJECT: Preterm infants in the NICU receive the greatest number of transfusions of any patient population. The administration of the long-acting erythropoiesis stimulating agent (ESA) darbepoetin to reduce or eliminate transfusions in preterm infants has not been evaluated.

WHAT THIS STUDY ADDS: Infants receiving ESAs received half the number of transfusions and were exposed to approximately half the donors compared with the placebo group. More than half of the ESA recipients (59% darbepoetin recipients, 52% erythropoietin recipients) remained untransfused during their hospitalization.

abstract

BACKGROUND: A novel erythropoiesis stimulating agent (ESA), darbepoetin alfa (Darbe), increases hematocrit in anemic adults when administered every 1 to 3 weeks. Weekly Darbe dosing has not been evaluated in preterm infants. We hypothesized that infants would respond to Darbe by decreasing transfusion needs compared with placebo, with less-frequent dosing than erythropoietin (Epo).

METHODS: Preterm infants 500 to 1250 g birth weight and ≤ 48 hours of age were randomized to Darbe (10 $\mu\text{g/kg}$, 1 time per week subcutaneously), Epo (400 U/kg, 3 times per week subcutaneously) or placebo (sham dosing) through 35 weeks' gestation. All received supplemental iron, folate, and vitamin E, and were transfused according to protocol. Transfusions (primary outcome), complete blood counts, absolute reticulocyte counts (ARCs), phlebotomy losses, and adverse events were recorded.

RESULTS: A total of 102 infants (946 ± 196 g, 27.7 ± 1.8 weeks' gestation, 51 ± 25 hours of age at first dose) were enrolled. Infants in the Darbe and Epo groups received significantly fewer transfusions ($P = .015$) and were exposed to fewer donors ($P = .044$) than the placebo group (Darbe: 1.2 ± 2.4 transfusions and 0.7 ± 1.2 donors per infant; Epo: 1.2 ± 1.6 transfusions and 0.8 ± 1.0 donors per infant; placebo: 2.4 ± 2.9 transfusions and 1.2 ± 1.3 donors per infant). Hematocrit and ARC were higher in the Darbe and Epo groups compared with placebo ($P = .001$, Darbe and Epo versus placebo for both hematocrit and ARCs). Morbidities were similar among groups, including the incidence of retinopathy of prematurity.

CONCLUSIONS: Infants receiving Darbe or Epo received fewer transfusions and fewer donor exposures, and fewer injections were given to Darbe recipients. Darbepoetin and Epo successfully serve as adjuncts to transfusions in maintaining red cell mass in preterm infants. *Pediatrics* 2013;132:e119–e127

Erythropoiesis stimulating agents (ESAs) have been used clinically for more than 20 years to stimulate red blood cell production. Erythropoietin (Epo) successfully stimulates erythropoiesis and decreases transfusion requirements in adults and children with anemia attributable to end-stage renal disease or cancer. In preterm infants, ESAs have shown varied success in decreasing the total number and volume of transfusions.^{1,2} Darbepoetin alfa (Darbe) is a biologically modified long-acting ESA.³ The increased half-life of Darbe allows for dosing every 1 to 4 weeks in adults with anemia attributable to end-stage renal disease or cancer. Single-dose pharmacokinetics in preterm infants revealed a similar prolonged half-life^{4,5}; however, weekly dosing of Darbe has not been evaluated.

Transfusion guidelines have been adapted by many US neonatal units; yet, few studies have evaluated outcomes of restrictive transfusion guidelines,^{6–8} and transfusion studies have not been performed at high-altitude centers (>4800 feet above sea level) where oxygen use (a neonatal transfusion trigger in previous studies^{6,7}) is generally increased in NICU populations. After publication of the Iowa transfusion study,⁶ many centers considered using more liberal transfusion guidelines. However, long-term neurodevelopmental outcomes among those randomized to be liberally transfused were considerably worse.^{9,10} Recent studies suggest an association between preterm infant morbidities, such as necrotizing enterocolitis (NEC) and significant intracranial hemorrhage (ICH), with transfusions^{11–13}; therefore, using ESAs to avoid these possible risks might prove clinically important. We designed a study to assess whether Darbe would decrease transfusions in preterm infants, using a restrictive transfusion protocol applied at 4 high-altitude centers (the University of New

Mexico in Albuquerque, NM; McKay Dee Hospital in Ogden, UT; LDS Hospital/Intermountain Medical Center in Murray, UT; and the University of Colorado in Denver, CO). Although not designed as an equivalence trial, we hypothesized that similar to Epo, the administration of Darbe to preterm infants would result in increased reticulocyte counts and decreased transfusions compared with placebo. If successful, Darbe might be more beneficial to preterm infants in that fewer injections would be required to achieve the same benefit.

METHODS

Subjects

Infants of 500 to 1250 g birth weight, ≤48 hours of age, and who were expected to survive the first days of life (as determined by the attending neonatologist) were eligible. Infants transfused before enrollment were not excluded. Infants with trisomies, significant congenital anomalies (including known neurologic anomalies), hypertension, seizures, thromboses, hemolytic disease, or receiving Epo clinically were ineligible for study. The study was approved by the institutional review boards at the Universities of New Mexico and Colorado, and Intermountain Health Care. Parental consent was obtained. An investigational new drug application was approved by the Food and Drug Administration (IND 100138). The study was registered on ClinicalTrials.gov (NCT 00334737).

Randomization was stratified by center using a computer-generated permuted block method. Multiples were randomized to the same treatment group. All caregivers and investigators (except research pharmacists and coordinators) were masked to treatment assignment.

Dosing of Study Drug

Infants were randomized in masked fashion to 1 of 3 groups: Epo, 400 U/kg,

given subcutaneously 3 times a week (Monday, Wednesday, and Friday); Darbe, 10 µg/kg, given subcutaneously once a week,^{14,15} with sham dosing 2 other times per week; or placebo, consisting of 3 sham doses per week. The study drug was brought to the bedside in a closed container, and injections were shielded behind screens and out of earshot from caregivers and parents. An adhesive bandage covered the true and sham injection sites. Dosing continued until 35 completed weeks' gestation, discharge, transfer to another hospital, or death. Doses of Darbe and Epo were initially based on study entry weight and adjusted weekly. Study drug concentrations were chosen to give equivalent volumes (0.1 mL/kg body weight) of Darbe or Epo.

Criteria for Withholding or Stopping the Study Drug

Criteria for withholding the study drug included absolute neutrophil count <500/µL; hematocrit >50% (not due to transfusion) associated with an absolute reticulocyte count (ARC) ≥200 000 cells/µL; thrombocytopenia (<50 000/µL) or thrombocytosis (>700 000/µL); elevated creatinine (>2.7 mg/dL); a systemic adverse reaction to injection (hypotension, respiratory compromise, or systemic rash); or hypertension, defined as mean blood pressure >2 SD above gestational age average. The study drug was restarted when these conditions resolved. Treatment was stopped if clinical seizures occurred, if thromboses were diagnosed, or if hypertension or neutropenia recurred.

Supplements

All infants (regardless of treatment arm) received supplemental iron, folate (50 µg per day oral), and vitamin E (15 IU per day oral).¹⁶ Iron dextran, 3 mg/kg once a week was added to

parenteral nutrition while infants were receiving <60 mL/kg per day enteral feedings. Oral iron 3 mg/kg per day was started when feedings were $\geq$ 60 mL/kg per day, and increased to 6 mg/kg per day when feedings reached 120 mL/kg per day. Serum ferritin concentrations were used to adjust iron dosing. For infants in whom ferritin concentrations were >400 ng/mL, the parenteral or enteral dose of iron was decreased by 50%; for infants in whom ferritin concentrations were <50 ng/mL, the parenteral or enteral dose was doubled.

Laboratory Evaluations

Complete blood counts and ARC were obtained at day 1 (before study drug) and every 2 weeks during the study period. Treating clinicians were unaware of study laboratory results unless values met criteria for holding/stopping the study drug or met transfusion criteria. Ferritin concentrations were evaluated at 14 and 42 days of study. All infants (regardless of treatment arm) were evaluated for anti-ESA antibodies.

Data Collection

Blood pressure was recorded daily. The number and volume of transfusions, donor exposures, phlebotomy losses (determined by laboratory-specified blood volumes and bedside recording), and adverse events were recorded from birth through 36 weeks' gestation, transfer, or death. The number of ventilator days, length of hospital stay, and incidence of the following were recorded: bronchopulmonary dysplasia (oxygen administration at 36 weeks corrected gestational age¹⁷), retinopathy of prematurity (ROP; all stages¹⁸), ICH (including periventricular leukomalacia¹⁹), patent ductus arteriosus and treatment (surgery or indomethacin), NEC,²⁰ late-onset sepsis (a positive blood or cerebral spinal fluid culture obtained in the presence of compatible signs of

septicemia after 72 hours of age, or culture-negative clinical infection after 72 hours of age for which the infant received antibiotics for $\geq$ 5 days), and any incidence of neutropenia or thrombocytopenia. A data safety monitoring board reviewed adverse and serious adverse events every 6 months.

Transfusion Guidelines

Infants were transfused based on clinical or research laboratory results according to a standardized, restrictive transfusion protocol used clinically at the University of New Mexico (Table 1). Each infant was assigned a matched, leuko-reduced, citrate-phosphate-dextrose adenine anticoagulant-preserved donor unit, made available in a sterile docking device capable of 50-mL aliquots of packed red blood cells (PRBCs) with $\geq$ 4 transfusions per unit, and a shelf life of 28 days. PRBCs were irradiated just before administration over 2 to 4 hours.

Sample Size Estimate

The primary outcome was number of transfusions. Secondary outcomes included donor exposure, ARC, hematocrit, hospital stay, and morbidities associated with preterm birth, specifically ROP. A total of 27 infants in each group were required to identify a difference of 2 transfusions between

Darbe and placebo, using an α of 0.05 and 80% power. Two-year follow-up was planned; to account for deaths (15%) and loss to follow-up (10%), 34 infants per group were enrolled.

Statistical Analysis

The number of transfusions, donors, and transfusion violations were compared using Poisson regression. For univariate variables, differences among groups were compared by using 2-tailed Fisher's exact tests for categorical variables and 1-way analysis of variance or Kruskal-Wallis test (depending on distributional characteristics) for continuous variables. For repeated measures data, groups were compared by using linear mixed models (random coefficient regression).

RESULTS

A total of 905 infants were screened for the study. Of those, 513 were eligible. Reasons for nonrandomization included parent refusal (49%), consent not requested (8%), or enrollment in other studies (43%). A total of 102 infants were enrolled in the study between July 2006 and May 2010. Three subjects (1 who had the study drug mistakenly held at the start of the study and subsequently never received any study drug; 1 who was found to be

TABLE 1 Transfusion Protocol

Hct/Hgb	Respiratory Support and/or Symptoms	PRBC Volume
$\leq 30/\leq 10$	Moderate/significant ventilation (MAP >8 cm H ₂ O and FiO ₂ >0.4)	15–20 mL/kg
$\leq 25/\leq 8$	Minimal respiratory support (any mechanical ventilation with FiO ₂ $\leq$ 0.4, OR CPAP >6 cm H ₂ O and FiO ₂ $\geq$ 0.4)	20 mL/kg
$\leq 20/\leq 7$	Supplemental oxygen or CPAP with an FiO ₂ $\leq$ 0.4, and at least 1 of the following: <ul style="list-style-type: none"> • ≥ 24 h of tachycardia (heart rate >180) or tachypnea (RR >60); • a doubling of the oxygen requirement from the previous 48 h • lactate ≥ 2.5 mEq/L or an acute metabolic acidosis (pH <7.20); • weight gain <10 g/kg/d over the previous 4 d while receiving ≥ 120 kcal/kg/d; • undergoing surgery within 24 h 	20 mL/kg
$\leq 18/\leq 6$	Asymptomatic and an ARC <100 000 cells/ μ L	20 mL/kg

CPAP, continuous positive airway pressure; Hct, hematocrit; Hgb, hemoglobin; MAP, mean airway pressure; RR, respiratory rate. Transfusions could be administered in 2 10-mL/kg aliquots, as determined by attending clinician.

ineligible based on congenital neurologic anomaly on head ultrasound noted before receiving study drug; and 1 who died of a pulmonary hemorrhage before receiving study drug) were excluded from analysis. One infant had the study drug stopped at 34 weeks' corrected gestation at the request of parents. All infants who received at least 1 dose of study drug were included in analysis.

Maternal and infant characteristics are shown in Table 2. By chance, a greater number of female infants were enrolled in the Darbe group ($P = .039$), and more mothers were diagnosed with abruption in the placebo group ($P = .024$). There were no other significant differences among the 3 groups in maternal or neonatal characteristics. Prestudy hematocrit, transfusions, and phlebotomy losses were similar among groups.

Infants in the Darbe and Epo groups (both separately and combined) required significantly fewer transfusions ($P = .015$, combined comparison) and were exposed to significantly fewer donors during the study ($P = .044$, combined comparison) than those in the placebo group (Table 3). Fifty-nine percent of Darbe, 52% of Epo, and 38% of the placebo group did not receive any transfusions during their hospitalization. Transfusion protocol violations occurred at similar rates for transfused subjects, either because the hematocrit exceeded the value specified by the transfusion guidelines or because less than the prescribed transfusion volume was administered (Table 3). There were no significant differences in phlebotomy losses among groups during the study (Table 3).

Changes in ARC are shown in Fig 1A. Infants treated with Darbe or Epo had significantly higher ARC than infants in the placebo group ($P = .001$). Similarly, hematocrits were significantly greater in the ESA groups compared

TABLE 2 Baseline Characteristics

	Darbe, <i>n</i> = 33	Epo, <i>n</i> = 33	Placebo, <i>n</i> = 33	<i>P</i> =
Maternal information				
Antenatal steroids, % (<i>n</i>)	94 (31)	88 (29)	85 (28)	.614
Chorio, % (<i>n</i>)	18 (6)	9 (3)	12 (4)	.820
Antibiotics, % (<i>n</i>)	61 (20)	58 (19)	67 (22)	.813
Preeclampsia, % (<i>n</i>)	48 (16)	36 (12)	36 (12)	.543
Abruption, % (<i>n</i>)	0	9 (3)	18 (6)	.039
Previa, % (<i>n</i>)	3 (1)	3 (1)	6 (2)	1.000
Diabetes, % (<i>n</i>)	9 (3)	15 (5)	12 (4)	.926
C/S, % (<i>n</i>)	76 (25)	73 (24)	79 (26)	.956
Black, % (<i>n</i>)	9 (3)	6 (2)	3 (1)	.869
White, % (<i>n</i>)	39 (13)	55 (18)	36 (12)	.301
Hispanic, % (<i>n</i>)	42 (14)	30 (10)	52 (17)	.239
Native American, % (<i>n</i>)	3 (1)	9 (3)	9 (3)	.694
Pacific Islander, % (<i>n</i>)	6 (2)	0	0	.327
Neonatal information				
Gestation, wk	27.9 ± 1.8 28.0 [26.4, 29.0]	27.8 ± 1.9 28.0 [26.3, 29.0]	27.3 ± 1.8 27.6 [26.0, 28.6]	.528
Birth weight, g	941 ± 164 940 [855, 1090]	957 ± 212 942 [781, 1150]	933 ± 221 995 [740, 1141]	.816
Head circumference, cm	25.7 ± 2.9 25.5 [24.0, 26.5]	25.5 ± 2.1 25.5 [24.5, 26.5]	25.1 ± 2.8 25.0 [23.0, 26.5]	.655
Length, cm	33.4 ± 6.3 35.3 [33.0, 36.0]	35.5 ± 3.5 36.0 [34.0, 37.5]	34.7 ± 3.6 36.0 [33.0, 37.5]	.192
Female, % (<i>n</i>)	64 (21)	33 (11)	39 (13)	.039
SGA, % (<i>n</i>)	21 (7)	18 (6)	12 (4)	.368
Singleton % (<i>n</i>)	76 (25)	82 (27)	70 (23)	.564
Twin sets, <i>n</i>	3	2	4	.559
Inborn, % (<i>n</i>)	94 (31)	88 (29)	91 (30)	1.00
Fetal distress, % (<i>n</i>)	27 (9)	44 (14)	48 (16)	.188
1-min Apgar	5.0 ± 2.3 5 [3, 7]	4.7 ± 2.6 5 [2, 7]	4.8 ± 2.4 4.5 [3, 7]	.913
5-min Apgar	7.4 ± 1.9 8 [7, 9]	7.3 ± 1.8 8 [7, 8]	7.3 ± 1.4 8 [6.5, 8]	.742
Hematocrit, %	44.5 ± 7.4 46.2 [41, 50]	44.9 ± 7.3 45.7 [39.9, 49.5]	44.5 ± 5.4 45 [43.0, 47.7]	.870
MCV, fL	116 ± 9 116 [112, 123]	117 ± 8 117.5 [112, 124]	117 ± 7 116 [111, 118]	.835
WBC, × 10 ⁶ /μL	10.1 ± 8.8 80.0 [5.6, 11.2]	9.3 ± 6.5 6.9 [5.3, 11.5]	7.2 ± 3.3 6.4 [4.4, 9.3]	.182
Absolute neutrophil count, × 10 ³ /μL	4.3 ± 6.9 3.0 [1.7, 4.3]	4.2 ± 5.1 2.5 [1.4, 5.1]	2.9 ± 2.8 1.9 [1.1, 3.7]	.272
Platelets, × 10 ³ /μL	203 ± 71 190 [146, 235]	210 ± 61 217 [166, 254]	203 ± 77 201 [147, 239]	.724
Transfused before study, % (<i>n</i>)	18 (6)	15 (5)	18 (6)	1.00
PRBC volume, mL/kg	13.2 ± 5.2 15.2 [14.6, 15.5]	14.2 ± 2.3 14.8 [14.8, 15.1]	27.2 ± 14.5 30.5 [10.0, 35.9]	.413
Prestudy phlebotomy loss, mL/kg	5.0 ± 4.5 3.9 [1.6, 7.1]	5.5 ± 3.5 5.5 [3, 7.8]	6.7 ± 5.1 5.4 [3.5, 9.3]	.189
Age at first dose, h	46 ± 27 47 [21, 61]	54 ± 25 59 [34, 73]	62 ± 65 48 [36, 65]	.414

C/S, cesarean section; fL, femtoliter; MCV, mean corpuscular volume (fL); SGA, small for gestational age; WBC, white blood cells. *P* values represent comparison among 3 groups. Under Neonatal information, values in the top line of each section are mean ± SD, and values in the second line of each section are median (first and third quartile).

with placebo ($P = .001$; Fig 1B). There were no differences in absolute neutrophil count (Fig 1C) or platelet counts (Fig 1D) among groups during the study.

Ferritin concentrations were higher in the placebo group at days 14 and 42 of the study (Table 4). More infants in the ESA groups had their iron dose increased on day 14 and day 42 compared

TABLE 3 Transfusions and Phlebotomy Losses

	Darbe, <i>n</i> = 33	Epo, <i>n</i> = 33	Placebo, <i>n</i> = 33	<i>P</i> ₁ =	<i>P</i> ₂ =
Transfusions/subject	1.2 ± 2.4 0 [0, 1]	1.2 ± 1.6 0 [0, 2]	2.4 ± 2.9 1 [0, 4]	.015	.949
Donors/subject	0.7 ± 1.2 0 [0, 1]	0.8 ± 1.0 0 [0, 2]	1.2 ± 1.3 1 [0, 2]	.044	.558
Volume, mL/kg	30 ± 58 0 [0, 29.2]	23 ± 33 0 [0, 36.6]	51 ± 65 20.0 [0, 87.9]	.041	.600
Volume, mL/kg, transfused subjects only	75.6 ± 73.2 45 [20.0, 102.4]	48.0 ± 32.7 39.8 [21.5, 65.7]	80.7 ± 65.3 83.3 [20.0, 97.8]	.36	.61
Subjects not transfused, <i>n</i> (%)	20 (59%)	17 (52%)	12 (38%)	.088	.620
Transfusion protocol violations/transfused subject, total	0.8 ± 1.7 0 [0, 1]	1.1 ± 1.1 1 [0, 1]	0.9 ± 1.4 0 [0, 1]	.805	.661
Due to high hematocrit	0.7 ± 1.7 0 [0, 1]	0.8 ± 1.1 0.5 [0, 1]	0.5 ± 1.3 0 [0, 0]	.435	.895
Due to low transfusion volume	0.2 ± 0.4 0 [0, 0]	0.3 ± 0.8 0 [0, 0]	0.4 ± 0.6 0 [0, 1]	.300	.381
Total phlebotomy losses, mL/kg	32.3 [20.3–42.5]	29.9 [20.4–42.5]	24.9 [20.4–53.0]	.732	.802
Pre study	43.7 ± 45.3 3.6 [1.6–7.2]	42.5 ± 31.0 5.8 [3.4–8.9]	55.9 ± 53.9 6.4 [3.7–9.0]	.137	.267
During study	5.6 ± 6.5 32.3 [20.3–42.5]	6.1 ± 4.4 29.9 [20.4–42.5]	7.9 ± 7.5 24.9 [20.4–53.0]	.974	.801
Post study	37.7 ± 43.1 0 [0–0]	35.5 ± 30.7 0.2 [0–1.3]	46.9 ± 49.5 0.3 [0–1.0]	.191	.015
	0.5 ± 1.1	0.9 ± 2.0	1.1 ± 2.1		

Values represent mean ± SD and median [first quartile, third quartile]. Phlebotomy losses are represented by 95% confidence intervals. *P*₁, Darbe plus Epo (combined) versus placebo; *P*₂, Darbe versus Epo

FIGURE 1

Hematologic indices during the study curves were generated for ARC (A), hematocrit (B), absolute neutrophil count (C), and platelet count (D) using nonparametric Lowess (locally weighted scatterplot smoothing) regression smoothers calculated for each treatment arm separately. Data were fit with linear mixed models where each subject was allowed a separate quadratic trajectory (random coefficient regression), with interaction allowed between time and treatment arm, and *P* values reported are for the fixed effects contrasts comparing groups. A, Changes in ARC. Infants treated with Darbe (dotted line) or Epo (hatched line) had significantly higher ARCs than infants in the placebo group (solid line; *P* = .001). Similarly, hematocrits were significantly greater in the ESA groups compared with placebo (*P* = .001; B). There were no differences in absolute neutrophil counts (C) or platelet counts (D) among groups during the study.

with the placebo group. More infants in the placebo group had their iron dose decreased on day 42 because of ferritin >400. The average number of parenteral iron doses per infant was similar, as was average age at which oral iron was started. No adverse events were attributed to administration of parenteral or enteral iron.

Mortality and all preterm morbidities evaluated were similar among groups (Table 5). Specifically, the incidence of all stages of ROP was no different among groups. The incidence of ICH grade ≥3 was not statistically different among groups. Side effects (hypertension, thromboses, seizures, neutropenia) were minimal and similar among treatment groups (Table 5). There were no significant differences in mean blood pressures among groups.

None of the ESA recipients tested had evidence of antibody formation (D. Mytych, PhD, Amgen Scientific Director, personal communication, 2013). One infant in the placebo group had measurable anti-Epo antibody (2.5 μg/mL) and anti-Darbe antibody (8 μg/mL) concentrations. More detailed antibody isotype analysis detected anti-ESA immunoglobulin (Ig)M antibodies at day 56. No anti-ESA IgG was detected. This infant did not receive any blood products; nor did the infant receive Darbe, Epo, or other medications that could result in antibody formation. Two additional infants (1 placebo, 1 on Epo) had evidence of weak anti-Epo antibody formation that was not clinically relevant (<0.25 μg/mL).

DISCUSSION

The number of transfusions preterm infants receive has gradually decreased in the past decade. Although PRBC transfusions are generally considered safe, there is a small but measurable risk of infection, transfusion reaction, or transfusion-associated morbidities, such

TABLE 4 Iron Dosing and Ferritin Concentrations

	Darbe, <i>n</i> = 33	Epo, <i>n</i> = 33	Control, <i>n</i> = 33	<i>P</i> =
Ferritin d 14	121 [63, 184]	104 [58, 197]	204 [114, 348]	.003
Ferritin d 42	50 [27, 77]	61 [33, 99]	127 [67, 216]	.002
No. of intravenous iron doses	1.7 ± 1.3	1.6 ± 1.5	1.9 ± 2.3	.317
Oral iron started, days of age	15.5 ± 8.5	12.9 ± 8.4	14.5 ± 7.7	.205
Ferritin <50 d 14	7	5	2	.024
Ferritin <50 d 42	12	13	2	<.001
Ferritin >400 d 14	1	1	5	.118
Ferritin >400 d 42	3	0	5	.045

Ferritin concentrations reported as median [first quartile, third quartile]; *P* values represent comparisons among groups.

TABLE 5 Clinical Outcomes

	Darbe, <i>n</i> = 32	Epo, <i>n</i> = 32	Placebo, <i>n</i> = 30
CLD, <i>n</i>	22	21	20
Days on ventilation	19 ± 52 3 [1, 18]	13 ± 20 4 [1, 15]	16 ± 20 7 [2, 26]
No ROP, <i>n</i>	22	22	21
ROP ≤stage 2, <i>n</i>	10	9	8
ROP >stage 2-regressed, <i>n</i>	0	1	2
ROP >stage 2-required intervention, <i>n</i>	2	1	2
ICH ≥grade 3, <i>n</i>	3	3	7
NEC >stage 2, <i>n</i>	2	1	2
Patent ductus arteriosus, <i>n</i>	16	16	15
Medically treated, <i>n</i>	10	10	5
Surgically treated, <i>n</i>	3	2	7
Positive culture, <i>n</i>	6	5	5
Hypertension, <i>n</i>	2	0	1
Seizures, <i>n</i>	3	1	0
Thromboses, <i>n</i>	0	0	1
Neutropenia, <i>n</i>	0	0	0
Mortality, <i>n</i>	2	1	3
Hospital stay, d	76 ± 46 69 [51, 87]	72 ± 26 73 [55, 89]	74 ± 33 63 [52, 88]
Weight at 36 wk, g	2046 ± 346 2060 [1730, 2332]	2059 ± 417 2126 [1779, 2258]	2025 ± 507 1980 [1845, 2369]
Head circumference at 36 wk, cm	31.2 ± 2.7 30.7 [29.5, 32.5]	31.1 ± 1.6 31.0 [30.0, 31.9]	31.6 ± 2.1 31.5 [30.5, 32.1]

Values represent mean ± SD and median [first quartile, third quartile]. All differences among groups in clinical outcomes were not significant (all *P* values >0.2).

as transfusion-related lung injury.²¹ In addition, with new evidence from recent studies that demonstrate potential side effects of red cell transfusions, such as extension of ICH and transfusion-associated NEC,^{11–13, 22} the need to study the safety and efficacy of ESAs, in combination with restrictive transfusion guidelines, urgently increases.

This is the first prospective, randomized, masked, multicenter study of early Darbe administered to preterm infants, and the first study of ESA administration to preterm infants that resulted in a significant decrease in both donor exposure and transfusion number.

ESA-treated infants were exposed to approximately half the donors and received half the number of transfusions compared with the placebo group. In addition, although not the primary outcome of the study, 59% of the Darbe recipients and 52% of the Epo recipients did not receive any transfusions during their hospitalization. Although previous studies outside the United States have achieved similar levels of success,²³ this is the first US study to report a >50% transfusion-free population of very low birth weight infants. Moreover, although the number of transfusions has decreased

over the past 20 years, extremely low birth weight (ELBW) infants are still receiving 3 to 5 PRBC transfusions per hospitalization, and are being exposed to 2 to 3 donors.^{24,25}

The success of this study compared with previous studies may be in part because of differences in study design. We compared early Darbe administration and the usual erythropoietic dose of Epo with placebo to determine if ESAs could decrease or prevent transfusions. Study medications were generally initiated before 60 hours of life, 12 to 24 hours earlier than previous studies.¹⁶ The earlier administration of ESAs might also benefit longer-term developmental outcomes. The absence of antibody formation was specifically tested in all available infant samples. Finally, we applied stringent transfusion guidelines at sites that were above 4800 feet elevation. Despite being at higher elevation, infants appeared to tolerate the restrictive guidelines, although long-term developmental outcomes require further study.

Instituting strict transfusion guidelines can have a significant impact on the number of transfusions infants receive. This is most beneficial to preterm infants in a practical sense, because it can be implemented without additional cost. A previous study reported \$780 074 savings 1 year after implementation of transfusion guidelines.²⁶ Moreover, the risks of transfusions continue to be evaluated. The 2006 Cochrane analysis of “early” Epo usage in the NICU included 23 studies involving 2074 preterm infants.²⁷ Summary statistics indicated a modest reduction in early transfusions. Similarly, summary statistics of the effect of the “late” use of Epo²⁸ indicated a significant but small reduction in transfusions. In both analyses the importance of 1 or 2 fewer transfusions was questioned.^{27,28} However, this benefit might have

greater clinical significance in light of recent associations between early transfusions and severe ICH,¹² late transfusions and the development of NEC,^{11,22} and potentially damaging concentrations of heavy metals, such as lead and mercury.²⁹ It is possible that even 1 to 2 fewer transfusions at key periods during a NICU course could be beneficial.

Previous studies evaluating specific transfusion strategies reported conflicting results. Bell and colleagues⁶ reported an increase in serious ICH and PVL in those infants randomized to a restrictive transfusion strategy. Kirpalani and colleagues⁷ randomized 451 infants to low- or high-threshold hemoglobin strategies and reported no difference in neurologic morbidities in the low-hemoglobin threshold group; however, analyses at 2 years of age revealed increased mild cognitive impairment in the low-hemoglobin threshold group.⁸ Conversely, improved cognitive and MRI outcomes were reported in the restrictively transfused group of the Iowa study,^{9,10} underscoring the need for long-term evaluation and more comprehensive study.

Side effects were minimal and not different between ESA-treated and placebo groups. In addition, similar to adult studies (particularly Darbe studies), none of the ESA-treated infants tested developed antibodies. The presence of polyreactive IgM (which cross-reacts with multiple antigens) is not unexpected because of an immature immune response. Anti-ESA IgM reactivity to glycosylated therapeutic proteins has been previously demonstrated to ESAs in other patient populations.³⁰ At present, there are no published reports of neonates receiving more than single doses of Darbe, and further study is required in a larger population of ELBW infants to identify side effects specific to

neonates. This study was not powered to identify differences in the low incidence of any side effects, specifically ROP, a preterm morbidity that has been linked to early use of Epo.²⁷ Similar to our previous randomized trials of Epo administration to preterm infants, we saw no difference in the incidence of any stage of ROP.^{16,31,32} Close ophthalmologic evaluation of all ESA-treated infants remains a priority in treatment studies. To determine a similar difference reported in the study by Romagnoli et al³³ (where ROP was a significant side effect), a total of 450 infants would be required. However, because that study³³ began Epo in the second week of life, it is difficult to compare with our current study.

Recent animal studies have identified neuroprotective properties of both Darbe and Epo.^{15,33–41} In addition to stimulating erythropoiesis, ESAs are protective in the developing brain, making it possibly beneficial for very premature infants who are at risk for intraventricular hemorrhage, hypoxic-ischemic injury, and developmental delay.³⁷ The neuroprotective mechanisms of ESAs include decreased neuronal apoptosis, decreased inflammation, increased neurogenesis, promotion of oligodendrocyte differentiation and maturation, and improved white matter survival.^{35–41} Recent clinical studies suggest a strong potential for neuroprotection.^{42–45} Our group previously reported an improved cognitive outcome in former ELBW infants with serum Epo concentrations ≥ 500 mU/mL.⁴² Brown and colleagues⁴³ reported improved cognitive outcomes in preterm infants exposed to higher cumulative doses of Epo, and European investigators reported improved developmental outcomes at 8 to 12 years in former Epo-treated preterm infants, especially those with significant brain

injury during initial hospitalization.⁴⁴ McAdams et al randomized ELBW infants to 3 doses of 500 to 2500 U/kg Epo and found improved neurodevelopmental outcomes compared with concurrent controls.⁴⁵ Neurodevelopmental follow-up of study infants is ongoing.

We conclude that Darbe and Epo are effective in decreasing donors and transfusions in preterm infants without increasing morbidities. With increasing evidence of possible risks of transfusions, it was significant that more than 50% of the treated infants remained untransfused. Importantly, Darbe provides this effect with weekly dosing. Our study found no differences in preterm infant morbidities, and in particular, no differences in ROP between groups; however, conclusions regarding overall safety require larger randomized studies.

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REFERENCES

- Bishara N, Ohls RK. Current controversies in the management of the anemia of prematurity. *Semin Perinatol*. 2009;33(1):29–34
- Maier RF, Obladen M, Müller-Hansen I, et al; European Multicenter Erythropoietin Beta Study Group. Early treatment with erythropoietin beta ameliorates anemia and reduces transfusion requirements in infants with birth weights below 1000 g. *J Pediatr*. 2002;141(1):8–15
- Egrie JC, Browne JK. Development and characterization of novel erythropoiesis stimulating protein (NESP). *Br J Cancer*. 2001;84(suppl 1):3–10
- Warwood TL, Ohls RK, Wiedmeier SE, et al. Single-dose darbepoetin administration to anemic preterm neonates. *J Perinatol*. 2005;25(11):725–730
- Warwood TL, Ohls RK, Lambert DK, et al. Intravenous administration of darbepoetin to NICU patients. *J Perinatol*. 2006;26(5):296–300
- Bell EF, Strauss RG, Widness JA, et al. Randomized trial of liberal versus restrictive guidelines for red blood cell transfusion in preterm infants. *Pediatrics*. 2005;115(6):1685–1691
- Kirpalani H, Whyte RK, Andersen C, et al. The Premature Infants in Need of Transfusion (PINT) study: a randomized, controlled trial of a restrictive (low) versus liberal (high) transfusion threshold for extremely low birth weight infants. *J Pediatr*. 2006;149(3):301–307
- Whyte RK, Kirpalani H, Asztalos EV, et al; PINTOS Study Group. Neurodevelopmental outcome of extremely low birth weight infants randomly assigned to restrictive or liberal hemoglobin thresholds for blood transfusion. *Pediatrics*. 2009;123(1):207–213
- McCoy TE, Conrad AL, Richman LC, Lindgren SD, Nopoulos PC, Bell EF. Neurocognitive profiles of preterm infants randomly assigned to lower or higher hematocrit thresholds for transfusion. *Child Neuropsychol*. 2011;17(4):347–367
- Nopoulos PC, Conrad AL, Bell EF, et al. Long-term outcome of brain structure in premature infants: effects of liberal vs restricted red blood cell transfusions. *Arch Pediatr Adolesc Med*. 2011;165(5):443–450
- Christensen RD. Association between red blood cell transfusions and necrotizing enterocolitis. *J Pediatr*. 2011;158(3):349–350
- Baer VL, Lambert DK, Henry E, Snow GL, Christensen RD. Red blood cell transfusion of preterm neonates with a Grade 1 intraventricular hemorrhage is associated with extension to a Grade 3 or 4 hemorrhage. *Transfusion*. 2011;51(9):1933–1939
- Christensen RD, Lambert DK, Baer VL, et al. Postponing or eliminating red blood cell transfusions of very low birth weight neonates by obtaining all baseline laboratory blood tests from otherwise discarded fetal blood in the placenta. *Transfusion*. 2011;51(2):253–258
- Roohi M, Peceny MC, Ohls RKA. Randomized, masked, dose response study of darbepoetin administered to preterm infants. *Pediatr Res*. 2007;61:14
- Belayev L, Khoutorova L, Zhao W, et al. Neuroprotective effect of darbepoetin alfa, a novel recombinant erythropoietic protein, in focal cerebral ischemia in rats. *Stroke*. 2005;36(5):1071–1076
- 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(4):934–942
- Ehrenkranz RA, Walsh MC, Vohr BR, et al; National Institutes of Child Health and Human Development Neonatal Research Network. Validation of the National Institutes of Health consensus definition of bronchopulmonary dysplasia. *Pediatrics*. 2005;116(6):1353–1360
- The Committee for the Classification of Retinopathy of Prematurity. An international classification of retinopathy of prematurity. *Arch Ophthalmol*. 1984;102(8):1130–1134
- Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. *J Pediatr*. 1978;92(4):529–534
- Bell MJ, Ternberg JL, Feigin RD, et al. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. *Ann Surg*. 1978;187(1):1–7
- Sanchez R, Toy P. Transfusion related acute lung injury: a pediatric perspective. *Pediatr Blood Cancer*. 2005;45(3):248–255
- Mohamed A, Shah PS. Transfusion associated necrotizing enterocolitis: a meta-analysis of observational data. *Pediatrics*. 2012;129(3):529–540
- Haiden N, Schwindt J, Cardona F, et al. Effects of a combined therapy of erythropoietin, iron, folate, and vitamin B12 on the transfusion requirements of extremely low birth weight infants. *Pediatrics*. 2006;118(5):2004–2013
- Carroll PD, Widness JA. Nonpharmacological, blood conservation techniques for preventing neonatal anemia—effective and promising strategies for reducing transfusion. *Semin Perinatol*. 2012;36(4):232–243
- Widness JA, Madan A, Grindeanu LA, Zimmerman MB, Wong DK, Stevenson DK. Reduction in red blood cell transfusions among preterm infants: results of a randomized trial with an in-line blood gas and chemistry monitor. *Pediatrics*. 2005;115(5):1299–1306
- Baer VL, Henry E, Lambert DK, et al. Implementing a program to improve compliance with neonatal intensive care unit transfusion guidelines was accompanied by a reduction in transfusion rate: a pre-post analysis within a multihospital health care system. *Transfusion*. 2011;51(2):264–269
- Ohlsson A, Aher SM. Early erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. *Cochrane Database Syst Rev*. 2006;(3):CD004863
- Aher S, Ohlsson A. Late erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. *Cochrane Database Syst Rev*. 2006;(3):CD004868
- Bearer CF, Linsalata N, Yomtovian R, Walsh M, Singer L. Blood transfusions: a hidden source of lead exposure. *Lancet*. 2003;362(9380):332
- Barger TE, Wrona D, Goletz TJ, Mytych DT. A detailed examination of the antibody prevalence and characteristics of anti-ESA antibodies. *Nephrol Dial Transplant*. 2012;27(10):3892–3899
- Ohls RK, Roohi M, Peceny HM, Schrader R, Brier R. A randomized, masked study of weekly erythropoietin dosing in preterm infants. *J Pediatr*. 2012;160(5):790–795.e1
- Ohls RK, Harcum J, Schibler KR, Christensen RD. The effect of erythropoietin on the transfusion requirements of preterm infants weighing 750 grams or less: a randomized, double-blind, placebo-controlled study. *J Pediatr*. 1997;131(5):661–665
- Romagnoli C, Zecca E, Gallini F, Girlando P, Zuppa AA. Do recombinant human erythropoietin and iron supplementation increase the risk of retinopathy of prematurity? *Eur J Pediatr*. 2000;159(8):627–628
- McPherson RJ, Juul SE. Recent trends in erythropoietin-mediated neuroprotection. *Int J Dev Neurosci*. 2008;26(1):103–111

35. Digicaylioglu M, Lipton SA. Erythropoietin-mediated neuroprotection involves cross-talk between Jak2 and NF-kappaB signalling cascades. *Nature*. 2001;412(6847):641–647
36. Kumral A, Baskin H, Gokmen N, et al. Selective inhibition of nitric oxide in hypoxic-ischemic brain model in newborn rats: is it an explanation for the protective role of erythropoietin? *Biol Neonate*. 2004;85(1):51–54
37. Chattopadhyay A, Choudhury TD, Bandyopadhyay D, Datta AG. Protective effect of erythropoietin on the oxidative damage of erythrocyte membrane by hydroxyl radical. *Biochem Pharmacol*. 2000;59(4):419–425
38. Akisu M, Tuzun S, Arslanoglu S, Yalaz M, Kultursay N. Effect of recombinant human erythropoietin administration on lipid peroxidation and antioxidant enzyme(s) activities in preterm infants. *Acta Med Okayama*. 2001;55(6):357–362
39. Genc S, Akhisaroglu M, Kuralay F, Genc K. Erythropoietin restores glutathione peroxidase activity in 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine-induced neurotoxicity in C57BL mice and stimulates murine astroglial glutathione peroxidase production in vitro. *Neurosci Lett*. 2002;321(1-2):73–76
40. Vairano M, Dello Russo C, Pozzoli G, et al. Erythropoietin exerts anti-apoptotic effects on rat microglial cells in vitro. *Eur J Neurosci*. 2002;16(4):584–592
41. Sugawa M, Sakurai Y, Ishikawa-Ieda Y, Suzuki H, Asou H. Effects of erythropoietin on glial cell development; oligodendrocyte maturation and astrocyte proliferation. *Neurosci Res*. 2002;44(4):391–403
42. Beirer R, Peceny MC, Hartenberger CH, Ohls RK. Erythropoietin concentrations and neurodevelopmental outcome in preterm infants. *Pediatrics*. 2006;118(3). Available at: www.pediatrics.org/cgi/content/full/118/3/e635
43. Brown MS, Eichorst D, Lala-Black B, Gonzalez R. Higher cumulative doses of erythropoietin and developmental outcomes in preterm infants. *Pediatrics*. 2009;124(4). Available at: www.pediatrics.org/cgi/content/full/124/4/e681
44. Neubauer AP, Voss W, Wachtendorf M, Jungmann T. Erythropoietin improves neurodevelopmental outcome of extremely preterm infants. *Ann Neurol*. 2010;67(5):657–666
45. McAdams RM, McPherson RJ, Mayock DE, Juul SE. Outcomes of extremely low birth weight infants given early high-dose erythropoietin. *J Perinatol*. 2013;33(3):226–230

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