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Darbepoetin as a Neuroprotective Agent in Mild Neonatal Encephalopathy: A Randomized, Placebo Controlled, Feasibility Trial

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Abstract

Objective: To assess the feasibility and safety of one dose of Darbepoetin alpha (Darbe) administered to neonates ≥34 weeks with mild neonatal encephalopathy (NE)

Methods: Randomized, masked, placebo-controlled study including neonates ≥34 weeks gestation with mild NE. Neonates were randomized to receive one dose of Darbe (10 μg/kg IV) or placebo. Clinical and laboratory maternal and newborn data were collected. The Bayley Scales of Infant and Toddler Development, third edition (Bayley-III) and a standardized neurological examination at 8–12 months corrected age was assessed.

Results: There were no differences in baseline characteristics of the 21 infants randomized (9 Darbe, 12 placebo). Adverse events were not reported at any time. Bayley-III scores were average in both Darbe and placebo groups.

Conclusion: This study demonstrates that a randomized, masked, placebo-controlled trial is safe and feasible. A large, randomized trial is warranted to assess the effect of Darbe in this population.

Keywords

mild hypoxic-ischemic encephalopathy; neonatal encephalopathy; erythropoiesis stimulating agents; neuroprotection

Introduction

Twenty-five to 42% of infants with neonatal encephalopathy (NE), likely due to hypoxic-ischemic encephalopathy, are classified as having mild NE (1–4). Infants with mild NE have not been included in previous trials of therapeutic interventions for infants with NE because they were previously thought to have neurodevelopmental outcomes similar to those without

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evidence of NE (5,6). In the therapeutic hypothermia era, reviews have found that 20 to 40% of newborns with mild NE (varying definitions) have abnormal short and long-term outcomes (1,3,7–9). A recent prospective, multicenter study on infants with mild NE (The PRIME study), found 40% had a The Bayley Scales of Infant and Toddler Development, third edition (Bayley-III) scores <85 in either cognition, motor, or language and 16% had a disability at 18–22 months of age; adding further concern to the neurodevelopmental outcomes of infants with mild NE (9). In clinical practice, providers are becoming increasingly concerned about neuroprotection for infants with mild NE, as there is a trend towards providing therapeutic hypothermia to these babies without clinical evidence of benefit (2,10). Infants with mild NE initially appear well with few overt signs of neurological injury (5).

An ideal neuroprotective agent for infants with mild NE would provide neuroprotection with minimal adverse effects for these well-appearing newborns. Erythropoiesis stimulating agents (ESAs), specifically Darbepoetin (Darbe) due to its extended half-life, could be an ideal agent to provide neuroprotection to this at-risk, but seemingly well population. ESAs have been used in neonates for over 30 years with few side effects noted. In recent years, ESAs have been studied extensively in preclinical trials of neuroprotection driven by the finding that the erythropoietin (Epo) receptor is expressed in the developing human brain and can activate cellular mechanisms that provide both neuroprotection (anti-apoptotic, anti-oxidant, and anti-inflammatory properties); as well as neuroregeneration (cellular maturation, neurovascular remodeling, revascularization, neurogenesis, oligodendrogenesis) (11–17). Both Epo and Darbe administered peripherally can cross the blood-brain barrier by way of extracellular pathways in amounts that can provide neuroprotection (18). Darbe appears to be safe in neonates with evidence of perinatal asphyxia who have moderate or severe NE, but further study is needed in infants with mild NE (19). The current study aimed to assess the feasibility and safety of administering a single dose of Darbe as monotherapy for infants with mild NE.

Methods

Study design and population

We conducted a multicenter, randomized, masked, placebo-controlled feasibility study from October 2017 to December 2019 (NCT03071861) in 4 hospitals (University of New Mexico Hospital, Albuquerque, New Mexico; University of Utah Hospital, Salt Lake City, Utah; Intermountain Medical Center, Salt Lake City, Utah, and Primary Children’s Hospital, Salt Lake City, Utah). The Institutional Review Board of each center approved the study, and written informed consent was obtained from parents. An investigational new drug application was approved by the Food and Drug Administration (IND 132207). This study was supported by an inter-institutional pilot project award through the National Center for Advancing Translational Science of the National Institute of Health which provided 12 months of funding to both the University of New Mexico and the University of Utah. As a feasibility study, our sample size was determined by the number of patients recruited over 12 months at each site (New Mexico site October 2017 to October 2018, Utah site March 2018 to March 2019) with follow up performed 8–12 months after enrollment. Newborns were

eligible for enrollment in the first 24 hours of age if they were ≥34 weeks' gestational age, admitted to the neonatal intensive care unit, and had severe perinatal acidosis or received delivery room resuscitation as defined by the National Institute of Child Health and Human Development, Neonatal Research Network (20). Exclusion criteria included moderate/severe encephalopathy on modified Sarnat examination at <6 hours of age, major congenital and/or chromosomal abnormalities, prenatal diagnosis of brain abnormality or hydrocephalus, severe growth restriction (weight <3%), central venous hematocrit >65%, platelet count >600,000/μL, neutrophil count <500/μL, hypothermia therapy instituted at ≤6 hours of age, or a completely normal neurological examination.

Data were collected on the modified Sarnat exam findings (normal, mild, moderate, and severe) as previously outlined in the PRIME study (4). Mild NE was defined as one or two moderate or severe findings on the modified Sarnat (level of consciousness, spontaneous activity, posture, tone, primitive reflexes, and autonomic nervous system) in any of the six categories (20). All exams were performed by an attending neonatologist who underwent certification with a "gold standard examiner" to perform the examination.

Study procedures and data collection

Maternal demographics (age, education, race, ethnicity) were collected at study entry. Neonatal vital signs, admission physical exam, initial modified Sarnat exam (with findings of mild NE), and a modified Sarnat exam at discharge, transfer, or 96 hours of age (whichever occurred first) were documented. The following neonatal data were collected if clinically performed: MRI, hemoglobin/hematocrit, CBC with differential, and liver function chemistries.

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) were masked to treatment assignment. Infants were randomized to receive a single intravenous (IV) dose of Darbe, 10 μg/kg, or placebo (equivalent volume of normal saline) within 24 hours of birth. The study drug was brought to the bedside (as close to birth as possible after consent and randomization) in a closed container and administered via slow IV push.

Infants were screened for adverse events during their first 7 days of life or at hospital discharge (whichever occurred first). Adverse events included alterations in blood pressure, secondary infections, neutropenia, thrombotic/vascular events, hematologic events (thrombocytopenia, polycythemia), and hepatic/renal function that were outside of the normal range for the study population. Predetermined hospital outcomes were survival, gavage feeds at discharge, length of stay, and seizures.

Neurodevelopmental outcomes

Evaluation of neurodevelopmental outcome consisted of the third edition of the Bayley-III and a detailed standardized neurological examination performed by trained examiners at 8–12 months of age, adjusted for prematurity. The Bayley-III was performed by one trained examiner at each site, masked to the treatment assignment. The Bayley-III cognitive, language, and motor scales (mean = 100 [standard deviation (SD) 15]) were included.

Information on growth, hearing, and vision were obtained from history and medical records. Families completed a questionnaire at the 8–12 month visit regarding general health, hospitalizations, surgeries, and need for developmental services.

Statistics

Demographics, clinical and hospital outcomes of interest were summarized using mean and standard deviation (SD) or median and interquartile range (IQR) for continuous variables; for categorical variables, counts and percentages were reported. Variables were summarized by treatment group. The two groups were compared using the Wilcoxon rank-sum test for continuous variables and Fisher's exact test for categorical variables. Univariable and multivariable linear regression models were used to evaluate the association between risk factors and Bayley-III scores. Risk factors were treatment (Darbe/placebo), age at testing, total Sarnat score, the subject's cord gas or first hour of age pH, base deficit, and center (NM/UT). Regression coefficients or odds ratio and their 95% confidence interval (CI) were reported. Statistical significance was assessed at the 0.05 level. Statistical analyses were implemented using R v. 3.6.0 (R Core Team, 2019).

Results

Seventy-eight newborns with NE were screened from October 2017 to March 2019. Twenty-nine (37%) met eligibility criteria, and all parents were approached for consent (Figure 1). Twenty-one (72% consent rate) were randomized to receive Darbe (n = 9) or normal saline (n = 12). All infants received the treatment to which they were randomized. The treatment groups were similar with respect to baseline maternal and infant characteristics (Table 1). One set of twins was randomized to the same treatment (Darbe). One infant was less than 36 weeks gestation (35^{6}) and was randomized to placebo group. The total Sarnat score (21) was not significantly different between the groups (mean (SD) Darbe 4.0 (1.2), placebo 4.2 (1.9)). The study drug dose was administered at a median of 15.8 hours of age (IQR:12.2, 22.7); infants received Darbe at a median of 13.8 hours of age (IQR:12.2, 24.9). There were two infants in the Darbe group who received the study drug beyond 24 hours (25 and 30 hours). Hyperthermia (>38°C) was noted on admission after resuscitation in four infants (3 Darbe,1 placebo). Aside from admission temperature, hyperthermia was otherwise not present over the first 3 days of age. Hypothermia (36.1–36.4°C) was noted in four infants upon admission (2 Darbe, 2 placebo). Three infants received delayed cord clamping (1 Darbe, 2 placebo).

Hospital Outcomes

Hospital outcomes are listed in Table 2. Length of stay was significantly shorter in the Darbe group (median (IQR) 5.0 (4.0, 5.0) vs 8.5 (7.0, 16), p = 0.01). There were no differences in temperature between the two groups after the study drug was administered. There was no hyperthermia noted after the initial admission temperature; two Darbe recipients had mild hypothermia (36.4°C) on day 2 and day 3. Enteral feeds were started at a median of 1.8 days of age (Darbe 1.4 vs placebo 2.0, p= 0.13). One infant in the placebo group required gavage feeding at discharge. Infants in the placebo group took longer to reach full oral feeds (median 3 days for Darbe group and 6 days for placebo group; p= 0.01).

There were no adverse events reported. No patients in either group developed polycythemia, hypertension, secondary infections, neutropenia, thrombotic or vascular events, or hepatic or renal dysfunction after the study drug was administered. The mean, highest hematocrit recorded did not differ between the Darbe and placebo groups (51.0 vs 51.9, p= 0.91). At 8–12 month follow up none of the families reported infant health problems such as, hypertension, anemia, or growth problems.

Neurodevelopmental Outcome

Twenty (95%) infants were successfully evaluated at a mean age of 10.6±1.7 months (Table 3). Age at follow-up did not differ between the groups. At follow-up, the Bayley-III cognitive (p= 0.48), language (p=0.76), and motor (p=0.62) scores were not significantly different between the treatment and placebo groups. One infant (placebo) had abnormal tone and movement on neurological examination. Five infants were noted to have a mild abnormality on neurological examination (preference to use one hand more than the other, asymmetric parachute reflex, or preference to roll to one side; 3 Darbe, 2 placebo). Five infants (3 Darbe, 2 placebo) were receiving special developmental services at the time of follow up.

Discussion

This is the first multicenter, masked, placebo-controlled trial for infants with mild NE. We found the administration of Darbe to be safe and feasible in these infants. Our results are encouraging in that they suggest that mild NE treated with Darbe may have shorter hospital stays. Cognitive Bayley-III scores trended higher (although not statistically significant) in Darbe recipients, suggesting Darbe may provide neuroprotection for infants with mild NE.

Infants with mild NE have not been included in previous trials on therapeutic interventions for infants with NE. They are a unique population in that they are seemingly well at the time of birth and often don’t require extensive medical interventions. However, recent data suggest they are still at risk for adverse neurodevelopmental outcomes (1,9,22).

Many centers have adopted providing therapeutic hypothermia for infants with mild NE with limited evidence to support the practice and risk of harm (2,10). In a piglet model, there is some evidence that cooling a piglet without hypoxic injury results in increased apoptosis (23,24). The small observational studies reporting on hypothermia therapy for infants with mild NE do not suggest there is a benefit over normothermic conditions. There are notable side effects associated with therapeutic hypothermia, such as increased length of hospital stay, acute kidney injury, coagulopathy, and opioid and benzodiazepine exposure (8,25–27). These side effects are acceptable and mitigated in infants with moderate to severe NE as the risk of death or severe neurodevelopmental impairment (NDI) is significantly higher than infants with mild NE.

We therefore proposed to explore the use of Darbe for neuroprotection in this seemingly well but at-risk population. Darbe administration could be safer and require fewer medical resources than therapeutic hypothermia. Infants can receive Darbe treatment and resume normal newborn care with minimal prolongation of their hospital stay, making this therapy

potentially more economic than therapeutic hypothermia. Moreover, early Darbe treatment may facilitate parental-infant bonding as this treatment does not prevent infants from being held by their parents and/or breastfeeding (28,29).

In assessing feasibility of a larger trial, it was important to know the feasibility of obtaining consent and administering the study drug within a specific time frame; specifically, in infants with mild NE as they often do not appear overtly ill. The study drug was given as soon as possible after birth when consent was obtained. Over 50% of our deliveries were emergent and parents often needed time to recover before approaching for informed consent. Although our consent rate was high, we noted many parents required some time (hours) to consider study. In our study the study drug was administered at a median of 15.8 hours of age (IQR:12.2, 22.7) suggesting a larger trial is feasible. There were two infants in the Darbe group who received the study drug beyond 24 hours (25 and 30 hours). Unlike therapeutic hypothermia, ESAs appear to be less time sensitive and some preclinical studies suggest they can provide neuroprotection days after an injury allowing for a longer treatment window as compared to therapeutic hypothermia (30).

Our results suggest that Darbe may decrease length of stay for infants with mild NE. Chalak and colleagues noted the length of stay was significantly shorter in infants without NDI versus those with NDI (median 4 vs. 9.5 days; $p \leq 0.01$) (9). Retrospective studies on infants with mild NE who receive therapeutic hypothermia report a median length of stays of 8–9 days (range: 8–12) (8,25). Our results are encouraging as infants in the Darbe group were noted to have a shorter length of stay, while infants in the placebo group had length of stays similar to cooled and non-cooled infants with mild NE. However, these results should be interpreted with caution due to the small sample size.

In the present study, 14 infants had a length of stay >5 days. We reviewed each of these charts to assess for confounding factors leading to a longer length of stay. There were two infants in the placebo group who likely had prolonged length of stays unrelated to their mild NE (persistent pulmonary hypertension and neonatal opioid withdrawal). Removing those two from the analysis gives a median length of stay of 5 days (IQR: 4,5) for the Darbe group and 7 days (IQR: 6.75,12.25) for the placebo group ($p= 0.08$). Slow oral feeding was the predominant cause for length of stay >5 days (57%), which could be related to mild NE. Infants with NE who received therapeutic hypothermia experienced delayed establishment of feeding due to poor latching, inadequate sucking, and dysphagia (31–33). Infants in the placebo group took longer to reach full oral feeds. Retrospective reviews of infants with mild NE reported time to full oral feeds of 4–6 days similar to our placebo group (1,8).

No adverse events were identified in this study. In Walsh and colleagues' observational study on infants with mild NE who received therapeutic hypothermia, 17% required blood products for thrombocytopenia, coagulopathy, or anemia (8). Transfusions have not been reported in other studies on infants with mild NE who receive standard supportive care. NE is associated with thrombocytopenia, prolongation of coagulation studies, and increased nucleated red blood cell count (34). In infants with moderate to severe NE, therapeutic hypothermia delays the production of enzymes involved in the coagulation cascade; however, an increase in hemorrhagic events has not been reported (35). Assessment for

coagulopathy was not mandated in our study and clinicians could order laboratory studies as they deemed necessary. There were relatively few laboratory draws over the first week of age; none of the infants had coagulopathy parameters evaluated. Thrombocytopenia was identified in 25% of infants but none required transfusion. Additionally, there were no adverse events (hypertension, anemia, or growth problems) noted at the 8–12 month follow up visit when a medical history was taken to assess for safety.

We were encouraged by the high rate of follow up (95%) at 8–12 months of age. In assessing the feasibility of a large randomized controlled trial there was concern that families may be less interested in follow up assessments, as children with mild NE may have less overt signs of developmental delay. Unlike studies for moderate and severe NE, death and moderate to severe disability cannot be the outcome measure as these are uncommon in mild NE. A large trial will need to be powered for a difference in Bayley-III cognitive scores or disability. This study provides guidance as to what the potential magnitude of effect could be for a larger trial.

Scores from the Bayley-III exam, performed at 8–12 months, were not significantly different between groups. However, cognitive and language scores were 5 points higher in the Darbe group (median 100, IQR: 95–115). In untreated infants with mild NE, Chalak and colleagues reported median cognitive Bayley-III scores of 95 (IQR: 90–106) and Finder and colleagues reported a mean Bayley-III cognitive score of 97.6 ±11.9 (9,22). In the present study's placebo group the Bayley-III cognitive scores (median 95.0, IQR: 95.0, 102.5) were similar to both Chalak and Finder's studies. Rao and colleagues published mean Bayley-III cognitive scores of 93 ±12.3 in a retrospective study on 16 infants with mild NE treated with therapeutic hypothermia, consistent with standard normothermia care outcomes reported in our study and others (9,22).

In the present study, only one infant was not meeting his/her motor milestones and had a neurological examination that was concerning (marked hypotonia at 11 months of corrected age). This baby's gestational age was 35 weeks, participated in the placebo group, and was not referred to early intervention at the time of discharge; additionally, this infant was not receiving early intervention services before the study follow up examination. Five infants (two in placebo and three in Darbe group) were noted to have a mild abnormality (hand preference, asymmetric parachute reflex, or preference to roll to only one side).

Both the University of Utah and the University of New Mexico participate in the Neonatal Research Network. As such, all examiners have been certified and receive annual training on performing a modified Sarnat exam. The Bayley-III exams were performed by two certified examiners. The follow-up neurological examinations were performed by three examiners each of which performed a certifying exam with the primary investigator (TLD) who also performed 75% of all follow-up neurological examinations in this study.

A limitation of this study is that it was not powered to detect a difference in outcomes between treatment groups. In addition, the 10-month follow-up may not be adequate to assess long-term neurodevelopmental outcomes. While a single dose of Darbe is feasible in this population we have an ongoing study to assess its pharmacokinetics. This study was

designed to be pragmatic, therefore lab work and neuroimaging were not mandated. Although MRI and EEG/aEEG can be early predictors of outcome, we chose to focus on neurodevelopmental assessment. Our findings will need to be confirmed with a larger study with an appropriate sample size and later follow-up.

Conclusions

This study demonstrates that a randomized, masked, placebo-controlled trial is safe and feasible with a high consent rate (72%) and high follow-up rate (95%). A single dose of Darbe (10 μg/kg) given intravenously before 24 hours of age may be associated with decreased hospital length of stay and improved neurodevelopmental outcome. A large randomized trial is planned to confirm these findings and determine if Darbe will improve neurodevelopmental outcomes for infants with mild NE.

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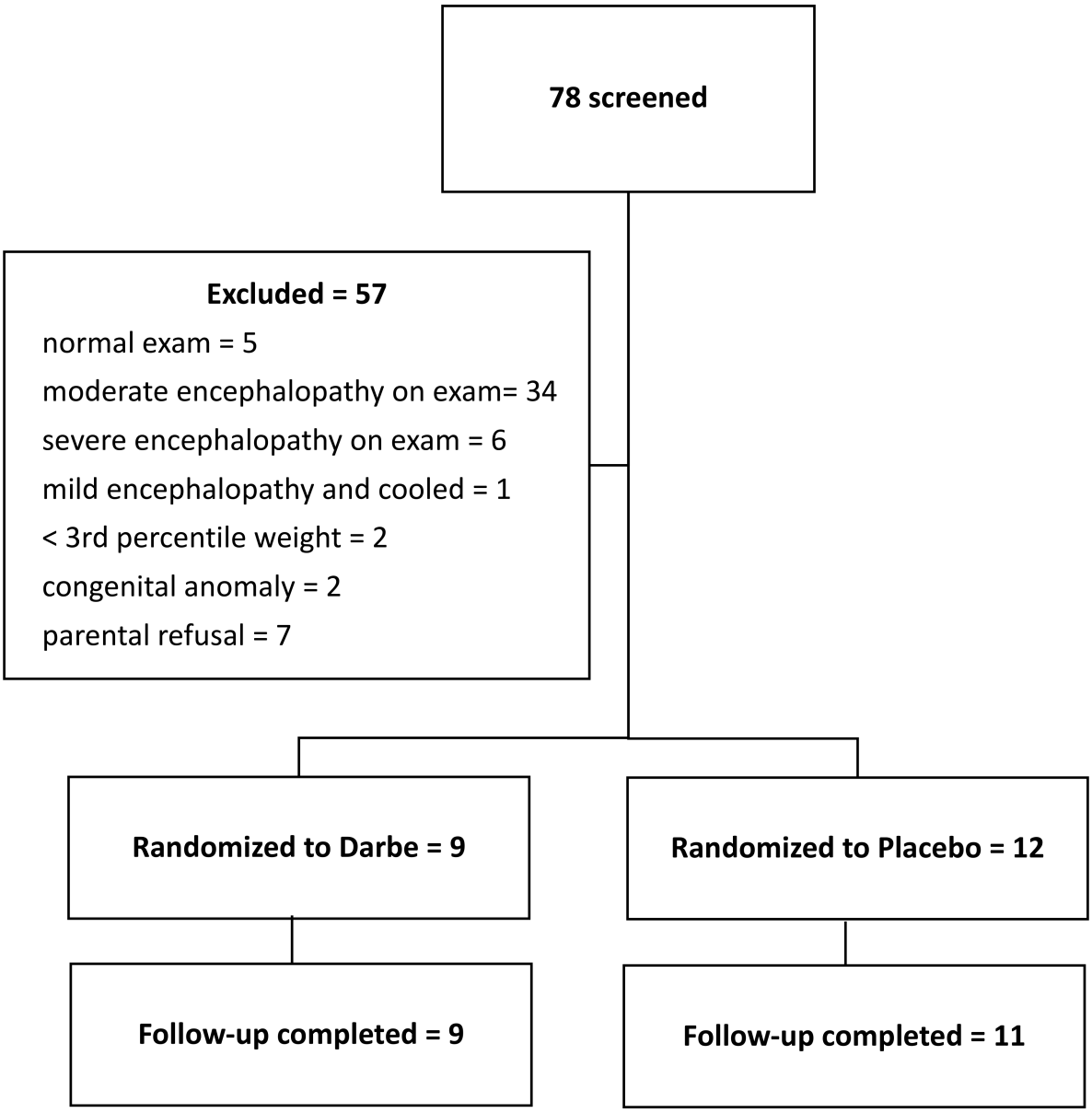

Figure 1.
CONSORT Flow Diagram. Numbers of infants who were screened for eligibility randomly assigned to receive Darbepoetin or placebo, and follow to 8–12 months of age.

Table 1:

Maternal and perinatal characteristics

Variable	Placebo (n=12)	Darbe (n=9)
Maternal age (years): mean (SD)	27.3 (4.2)	31.8 (9.9)
Maternal hypertension: number (%)	3 (18.2)	2 (22.2)
Gestational diabetes: number (%)	2 (16.6)	2(22.2)
Abnormal fetal heart rate pattern: number (%)	11 (91.7)	9 (100)
Meconium prior to delivery: number (%)	7 (58.3)	5 (55.6)
Cord accident: number (%)	4 (33.3)	0 (0)
Placental abruption, previa, accreta: number (%)	2 (16.7)	0 (0)
Maternal pyrexia >/= 37.6 C: number (%)	4 (33.3)	2 (22.2)
Emergent cesarean section: number (%)	6 (50.0)	4 (44.4)
Delayed cord clamping/milking: number (%)	2 (16.7)	1 (11.1)
Male: number (%)	6 (50.0)	6 (66.7)
Gestational age (weeks): mean (SD)	39.3 (1.9)	39.5 (1.5)
Cord blood pH: mean (SD)	7.1 (0.1)	7.0 (0.0)
Cord blood base deficit: mean (SD)	11.8 (4.3)	15.7 (3.3)
1st patient pH: mean (SD)	7.1 (0.1)	7.2 (0.2)
1st patient base deficit: mean (SD)	11.9 (3.4)	13.9 (7.7)
Thrombocytopenia (<150): number (%)	4 (33.3)	1 (11.1)
1 min APGAR: median (IQR)	2.5 (1, 3)	2.0 (2.0, 4.5)
5 min APGAR: median (IQR)	4 (3, 6)	6 (4.5, 7)
Need for extensive resuscitation (CPR, epinephrine): number (%)	0	1 (11.1)

All p values were >0.05

Table 2:

Hospital Outcomes

Variable	Placebo (n=12)	Darbe (n=9)
Time from birth to dose given (hr): median (IQR)	16.8 (14.8, 20.6)	13.8 (12.2, 24.9)
Hematocrit >65: number (%)	1(8.3)	0
Hypoglycemia <50 mg/dL: number (%)	2(16.7)	2 (22.2)
Days on oxygen: median (IQR)	4.0 (1.0, 7.8)	1.0 (1.0, 3.0)
Number of laboratory draws in 1st week: median (IQR)	5 (3.3, 6.8)	4 (3.0, 5.5)
Day of life enteral feedings started: median (IQR)	2.0 (1.8, 2.0)	1.0 (1.0, 2.0)
Days to full feeding (off IV Fluid and gavage): ** median (IQR)	6 (4, 13)Δ	3 (1.5, 4.5)
Receiving breastmilk at discharge: number (%)	7 (58.3)	7(77.8)
Multivitamin with iron at discharge: number (%)	8 (66.7)	4 (44.4)
Discharge with gavage tube feedings: number (%)	1(8.3)	0
Discharge with home therapy: number (%)	4 (33.3)	1 (11.1)
Abnormal hearing screen: number (%)	0	1(11.1)
Length of stay: median (IQR) *	8.5 (7.0, 16.0)	5.0 (4.0, 5.0)

* $p<0.05$

** $p<0.01$

Δ n=11 (excluded the one infant who was discharged on home gavage feeding)

Table 3

Neurodevelopmental Outcomes

Variable	Placebo (n=11)	Darbe (n=9)
Age at testing (months): mean (SD)	10.4 (1.7)	10.9 (1.7)
Abnormal neurological exam: number (%)	1 (9.1)	0
Mild neurological abnormality: number (%)	2 (18.2)	3 (33.3)
Receiving Early Intervention at follow up: number (%)	2(18.2)	3(33.3)
Cognitive Composite: median (IQR)	95.0 (95.0, 102.5)	100.0 (95.0, 115.0)
Language Composite: median (IQR)	89.0 (87.5,95.5)	94.0 (89.0, 97.0)
Receptive language scaled score: median (IQR)	7.0 (7.0, 9.5)	9.0 (5.0, 9.0)
Expressive language scaled score: median (IQR)	8.0 (8.0, 10.5)	9.0 (8.0, 10.0)
Motor Composite: median (IQR)	98.5 (88.8, 106.0)	94.0 (88.0, 103.0)
Fine motor scaled score: median (IQR)	10.0 (8.5, 11.0)	9.0 (8.0, 10.0)
Gross motor scaled score: median (IQR)	8.5 (7.0, 10.8)	10.0 (8.0, 11.0)
Cognitive Composite <85: number (%)	0	0
Language Composite <85: number (%)	1(9.1)	1(11.1)
Motor Composite <85$^{\Delta}$ number (%)	1(10.0)	1(11.1)
Cognitive Composite <70: number (%)	0	0
Language Composite <70: number (%)	0	0
Motor Composite <70$^{\Delta}$: number (%)	1(10.0)	1(11.1)

$^{\Delta}$**n=19,** 1 infant in the placebo group did not complete the motor portion of the examination.

All p values >0.05