Recombinant Human Erythropoietin Improves Neurological Outcomes in Very Preterm Infants

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Objective: To evaluate the efficacy and safety of repeated low-dose human recombinant erythropoietin (rhEPO) in the improvement of neurological outcomes in very preterm infants.
Methods: A total of 800 infants of ≤32-week gestational age who had been in an intensive care unit within 72 hours after birth were included in the trial between January 2009 and June 2013. Preterm infants were randomly assigned to receive rhEPO (500IU/kg; n = 366) or placebo (n = 377) intravenously within 72 hours after birth and then once every other day for 2 weeks. The primary outcome was death or moderate to severe neurological disability assessed at 18 months of corrected age.
Results: Death and moderate/severe neurological disability occurred in 91 of 338 very preterm infants (26.9%) in the placebo group and in 43 of 330 very preterm infants (13.0%) in the rhEPO treatment group (relative risk [RR] = 0.40, 95% confidence interval [CI] = 0.27–0.59, $p < 0.001$) at 18 months of corrected age. The rate of moderate/severe neurological disability in the rhEPO group (22 of 309, 7.1%) was significantly lower compared to the placebo group (57 of 304, 18.8%; RR = 0.32, 95% CI = 0.19–0.55, $p < 0.001$), and no excess adverse events were observed.
Interpretation: Repeated low-dose rhEPO treatment reduced the risk of long-term neurological disability in very preterm infants with no obvious adverse effects.

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Preterm and very preterm births have increased globally, and the survival rate of very preterm infants has increased with the progress of prenatal and neonatal intensive care. [1] However, very preterm infants have a high risk of severe short-term complications such as intracranial hemorrhage (ICH), periventricular leukomalacia (PVL), retinopathy of prematurity (ROP), and long-term neurodevelopmental disorders such as cerebral palsy, intellectual disabilities, and hearing and vision impairment,[2,3] and prematurity is still a leading cause of neonatal death. Although active perinatal care has improved neurodevelopmental outcomes in extremely preterm infants, the most extremely preterm infants continue to have high rates of neurological disabilities.[3,4] These neurological disabilities are still a significant burden on families and society, and the current therapy for preterm brain injury is mainly supportive and seeks to maintain physiological parameters. There is, therefore, a pressing need for interventions to prevent injury or enhance injury repair and to improve long-term neurological outcome.[5]

Erythropoietin (EPO) was first recognized for its hematopoietic properties; recombinant human EPO (rhEPO) has been used to treat a number of anemic states, including early and late anemia of prematurity, and it has been found to be safe and to reduce the need for blood transfusions.[6] EPO produced in the central

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nervous system[7] is upregulated after insult and plays a role in neuroprotection.[8–11] Experimental studies have reported that rhEPO possesses neuroprotective properties in different neonatal brain injury animal models,[12,13] and clinical studies have shown that rhEPO treatment reduces brain injury and the incidence of neurological disabilities in infants.[8,14–17] In addition, improved neurodevelopmental outcomes have been observed in preterm infants with anemia after rhEPO treatment.[17–20] The neuroprotective effect of rhEPO was suggested to be through acting against apoptosis, inflammation, and neurotoxicity and by acting as an antioxidant in protecting white matter from injury and in promoting neural regeneration, injury repair, and normal development.[5]

It has been shown that serum rhEPO concentrations are positively correlated with the degree of maturation during mental development in preterm infants,[21] and high-dose rhEPO (2,500U/kg) is well tolerated by preterm infants, causing no excess morbidity or mortality.[14,20] However, it is still a significant concern to use high-dose rhEPO in very preterm infants because this might increase the potential risk of ROP.[19,22,23] Based on previously observed neurodevelopmental improvement in very preterm infants who received low-dose rhEPO treatment for anemia prevention—and our own previous study, where repeated low-dose rhEPO (300–500U/kg) treatment of hypoxic–ischemic encephalopathy in term infants improved long-term neurological outcomes—we tested the hypothesis that early repeated low-dose rhEPO (500U/kg) treatment started within 72 hours after birth and then once every other day for 2 weeks will reduce early severe complications and improve long-term neurological outcomes in very preterm infants. This study provides important evidence that early repeated low-dose rhEPO treatment improves long-term neurological outcomes in very preterm infants and without obvious adverse effects.

Subjects and Methods

Patient Population

Between January 2009 and June 2013, a total of 800 preterm infants admitted to the neonatal intensive care unit (NICU) of the Third Affiliated Hospital of Zhengzhou University and Zhengzhou Children's Hospital within 72 hours after birth and with gestational age $\leq$ 32 weeks were deemed eligible for the study. The sample size was determined based on the assumption that 30% of the selected preterm infants would develop adverse neurodevelopmental outcomes in the control group at 18 months of corrected age.[24] If the relative risk (RR) were to be reduced by 40% with rhEPO treatment, then 363 patients would need to be recruited for each group for a significance level of 5% with 95% power and 10% loss to follow-up. Infants with genetic metabolic diseases, congenital abnormalities, polycythemia, severe infection (infection-induced multiple organ failure), unstable vital signs (multiple organ failure such as respiration or circulation failure), or grade III/IV intracranial hemorrhage before randomization were excluded from the study. Written informed consent was obtained from the parents, and the infants were assigned randomly to rhEPO treatment or placebo. This study was approved by the Life Science Ethics Committee of Zhengzhou University and Henan Medical Academy in accordance with the Helsinki Declaration and was registered at ClinicalTrials.gov (NCT02036073).

Study Design and Treatment Procedures

For infants who fulfilled the inclusion criteria, their mothers' complete obstetric histories were obtained, including chorioamnionitis, maternal hypertension, premature rupture of membrane, and mode of delivery. A simple randomization plan was established in the Department of Medical Documentation of each center before the start of the trial. Each patient was randomly assigned to rhEPO or placebo in a 1:1 allocation using a computer-based random-number generator. The resulting group assignment for each consecutive patient was concealed in sealed envelopes before the patients' inclusion. In the rhEPO treatment group, patients were given rhEPO at 500U/kg intravenously every other day for 2 weeks (a cumulative dose of 3,500U/kg over the course of 7 separate intravenous injections regardless of gestational age), starting with the first dose within 72 hours after birth. Patients in the placebo group received an equivalent volume of saline with the same treatment procedure as the rhEPO group. The supportive care, including keeping warm, parenteral nutrition, feeding, and other treatments according to different clinical symptoms were identical between the two treatment groups. Blood transfusion criteria followed strict clinical criteria.[25]

Discontinuation was defined as infants who were lost to follow-up, who did not complete the treatment, or who suffered serious side effects. Criteria for withholding or stopping the study drug included major venous thrombosis, polycythemia (hematocrit $>$ 60% or hematocrit increase $\geq$ 15% not due to transfusion), hypertension (systemic blood pressure $>$95mmHg at 0–7 days of age, $>$ 100mmHg at 8–14 days, or $>$105mmHg at over 2 weeks), and unexpected death (not caused by defined disorders acquired in the neonatal period due to prematurity).

Data Collection

Blood pressure and body weight of the very preterm infants were recorded daily for the first 2 weeks of life. In addition, weekly complete blood cell counts and the number and volume of blood transfusions during hospitalization were recorded. Routine blood tests were obtained before and after rhEPO treatment. Renal and liver function tests were obtained weekly. If infants had blood transfusions, blood samples were collected at least 3 days after that. Head ultrasound was performed on all patients within 3 days after birth and then weekly until discharge (at least 4 sessions of ultrasonography were performed,

which depended on the gestational age), and cerebral magnetic resonance imaging (MRI) was performed at 40 weeks of corrected age to determine the presence and extent of brain injury, hemorrhage, or thrombosis.[26] Data regarding complications of prematurity, including ICH, PVL, bronchopulmonary dysplasia (BPD), necrotizing enterocolitis (NEC), sepsis, and ROP, were recorded.

ICH was classified as grade I to IV according to Papile et al.[27] PVL, including cystic PVL and diffuse PVL, was diagnosed by ultrasound and MRI.[28,29] BPD was determined by an oxygen reduction test at 36 weeks of postmenstrual age, and the severity was further judged using criteria adapted from the National Institute of Child Health and Human Development.[30] NEC was determined by symptoms and x-ray examination and classified according to Berman and Moss.[31] ROP screening was performed in accordance with Chinese guidelines with both eyes examined by an experienced ophthalmologist. The severity of ROP was graded according to the international classification of ROP.[32]

Outcome Measures

All surviving infants were regularly evaluated every 3 months through a standardized nervous system examination, and a final examination at 18 months of corrected age was performed to evaluate motor functions and mental developmental index (MDI) using the Bayley Scales of Infant Development (2nd edition). Hearing status was determined from parental reports and supplemented with auditory brainstem response measurements. Deafness was defined as a hearing disability that required amplification.[33] Blindness was defined as a corrected visual acuity of $<20/200$.[33] Moderate or severe disabilities were defined as survival with at least 1 of the following complications: cerebral palsy, MDI < 70, deafness, or blindness.

In this study, the investigators enrolled the participants. The investigators performing the short-term and long-term outcome assessments and the parents were blinded to the patients' group allocation, although the doctors and nurses responsible for treatment were not blinded according to the rules of medical procedure in China. The final evaluation at 18 months of corrected age was performed by doctors from the Child Growth and Development Department, who were blinded to the treatment protocol and not allowed to have access to the treatment history of the infants.

Statistical Analysis

All data were analyzed using SPSS 19.0 software (IBM, Armonk, NY). Quantitative data are expressed as mean $\pm$ standard deviation. The gestational age, birth weight, blood cell counts, alanine aminotransferase level, serum creatinine level, and numbers of blood transfusions between the two groups were compared using Student t test. The differences between the two groups for sex ratio, obstetric history, asphyxia, and surfactant administration were compared using the chi-square test. Neonatal complications and outcomes at 18 months of corrected age between the two groups were compared using the univariate logistic regression test. Interaction analysis in subgroups was performed with Mantel–Haenszel tests. Risk factors of adverse neurological outcome were determined using multivariate logistic regression analysis. The level of statistical significance was set at 2-sided $\alpha = 0.05$.

Results

Baseline Characteristics and Follow-up

A total of 800 very preterm infants of ≤ 32 weeks of gestational age were admitted to the NICU during the observation period (Fig 1). There were no significant differences in baseline characteristics between the two treatment groups (Table 1).

Neurological Outcomes at 18 Months of Corrected Age

For analysis of long-term outcomes, there were 613 of 668 surviving very preterm infants who completed the follow-up at 18 months of corrected age, including 304 in the placebo group and 309 in the rhEPO group (see Fig). Regarding the primary outcomes, death or moderate/severe neurological disability occurred in 91 of the 338 very preterm infants (26.9%) in the placebo group and in 43 of the 330 very preterm infants (13.0%) in the rhEPO treatment group (RR = 0.40, 95% confidence interval [CI] = 0.27–0.59, $p < 0.001$; Table 2) at 18 months of corrected age. There was no significant difference in the mortality rate between the two groups ($p = 0.077$).

The total number of patients with moderate or severe neurological disability at 18 months of corrected age was 57 (57 of 304, 18.8%) infants from the placebo group and 22 (22 of 309, 7.1%) infants from the rhEPO treatment group (RR = 0.32, 95% CI = 0.19–0.55, $p < 0.001$; see Table 2). The median MDI value was significantly lower in the placebo group (90.00, range = 63–106) compared to the rhEPO group (100.00, range = 68–117; $p < 0.001$). The median psychomotor development index value was significantly lower in the placebo group (90.00, range = 62–109) compared to the rhEPO group (99.00, range = 66–112; $p < 0.001$). The incidence of MDI < 70 was 16.1% in the infants from the placebo group (84.1 $\pm$ 12.1, median = 90.0, range = 63.0–106.0) versus 6.1% in the infants from the rhEPO treatment group (95.2 $\pm$ 12.5, median = 100, range = 68–117; RR = 0.33, 95% CI = 0.19–0.58, $p < 0.001$). The incidence of deafness was significantly lower in infants from the rhEPO group compared to infants in the placebo group (0.3% vs 2.6%, respectively; RR = 0.12, 95% CI =0.02–0.99, $p = 0.019$). The incidences of cerebral palsy ($p = 0.065$) and blindness ($p = 0.662$) were similar between infants from the rhEPO treatment and the placebo group (see Table 2).

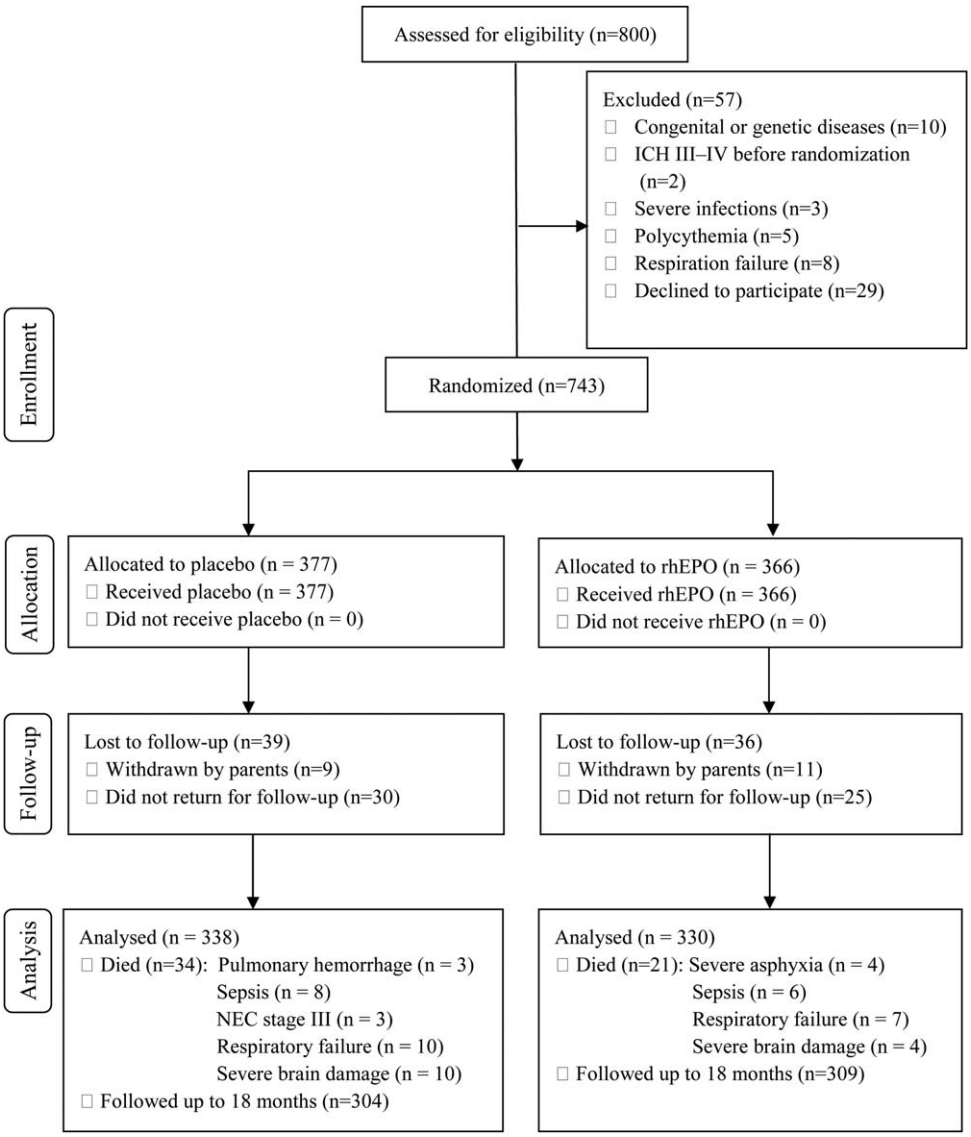

FIGURE 1: Study flow. Schematic flowchart shows the numbers of infants who were screened for eligibility, randomly assigned to recombinant human erythropoietin (rhEPO) or placebo groups, and followed up to 18 months of corrected age. Lost to follow-up means that contact with the family was lost during the follow-up period. ICH = intracranial hemorrhage; NEC = necrotizing enterocolitis.

Subgroup analyses were performed based on gestational age (<28 weeks, 28–$29^{6/7}$ weeks, 30–32 weeks), birth weight (<1,000g, 1,000–1,499g, ≥1,500g), and sex. Moderate or severe disability, including MDI < 70 at 18 months of corrected age, was associated with gestational age and birth weight ($p < 0.01$ and $p < 0.05$, respectively; Table 3). rhEPO treatment significantly reduced the incidence of moderate or severe disability, including MDI < 70 in the gestational age 28–$29^{6/7}$ weeks and 30–32 weeks groups, in the birth weight 1,000–1,499g group, and in boys ($p < 0.01$; see Table 3). There was no significant interaction between rhEPO and any subgroup in preterm infants with disability at 18 months of corrected age (Table 4).

Multivariate Analysis for Adverse Neurological Outcome

Logistic regression analysis of risk factors for moderate/severe neurological disability at 18 months of corrected age was performed using 16 items, including gestation, birth weight, sex, severe asphyxia, ventilation > 7 days, severe anemia, PVL, ICH III–IV, sepsis, BPD, NEC, cholestasis syndrome, respiratory distress syndrome, EPO treatment, pneumonia, and persistent hypoglycemia. We found that PVL, ICH III–IV, ventilation > 7 days, and gestation age of 28–$29^{6/7}$ weeks were risk factors for poor neurological outcome. EPO treatment was a protective factor against adverse neurological outcome (Table 5).

TABLE 1. Baseline Characteristics

Characteristic	Placebo, n = 377	rhEPO, n = 366	*p*
Gestational age, wk	30.40 ± 1.46	30.39 ± 1.38	0.906
Birth weight, g	1,396 ± 239	1,372 ± 209	0.153
Male, No. (%)	261 (69.2)	251 (68.6)	0.848
Female, No. (%)	116 (30.8)	115 (31.4)	0.848
Premature rupture of membrane, No. (%)	63 (16.7)	79 (21.6)	0.081
Maternal hypertension, No. (%)	53 (14.1)	62 (16.9)	0.255
Twins/multiple births, No. (%)	46 (12.2)	32 (8.7)	0.115
Caesarean section, No. (%)	205 (54.3)	198 (54.1)	0.972
1-minute Apgar < 3′, No. (%)	9 (2.4)	8 (2.2)	0.990
5-minute Apgar < 7′, No. (%)	6 (1.6)	5 (1.4)	0.811
Surfactant administration, No. (%)	229 (60.7)	227 (62.0)	0.700
Mechanical ventilation > 7 days, No. (%)	28 (7.4)	19 (5.2)	0.224

Probability value is for placebo group versus recombinant human erythropoietin (rhEPO) group using Student *t* test or chi-square test. $p < 0.05$ was considered statistically significant.

Neonatal Complications

The incidence of PVL and grade III–IV ICH in the infants from the rhEPO treatment group was significantly lower compared to the infants from the placebo treatment group (RR = 0.52, 95% CI = 0.35–0.79, $p = 0.002$ and RR = 0.38, 95% CI = 0.23–0.62, $p < 0.001$, respectively; Table 6). Similarly, the occurrence of NEC and sepsis was significantly lower in the rhEPO-treated infants than in the placebo treatment group (RR = 0.44, 95% CI = 0.27–0.73, $p = 0.001$ and RR = 0.65, 95% CI = 0.46–0.92, $p = 0.015$, respectively). Further analysis showed the rate of NEC stage I (RR = 0.39, 95% CI = 0.22–0.68, $p = 0.001$), but not stage II (RR = 0.44, 95% CI = 0.13–1.44, $p = 0.165$) or III (RR = 1.52, 95% CI = 0.25–9.13, $p = 0.685$), was significantly decreased in the rhEPO group. The occurrence of ROP did not show significant differences between the two groups of infants ($p = 0.196$), and there were no

TABLE 2. Outcomes at 18 Months of Corrected Age

Outcome	Placebo	rhEPO	Relative Risk [95% CI]	*p*
Primary outcomes				
Death, No. (%)	34/338 (10.1)	21/330 (6.4)	0.60 [0.34–1.06]	0.077
Disability, No. (%)	57/304 (18.8)	22/309 (7.1)	0.32 [0.19–0.55]	<0.001[a]
Death + disability, No. (%)	91/338 (26.9)	43/330 (13.0)	0.40 [0.27–0.59]	<0.001[a]
Secondary outcomes				
Cerebral palsy, No. (%)	16/304 (5.3)	7/309 (2.3)	0.43 [0.17–1.06]	0.065
MDI < 70, No. (%)	49/304 (16.1)	19/309 (6.1)	0.33 [0.19–0.58]	<0.001[a]
Deafness, No. (%)	8/304 (2.6)	1/309 (0.3)	0.12 [0.02–0.99]	0.019[a]
Blindness, No. (%)	3/304 (1.0)	2/309 (0.6)	0.67 [0.11–4.04]	0.662

Probability value is for placebo group versus rhEPO group using univariate logistic regression. $p < 0.05$ was considered statistically significant. Disability indicates the incidence of moderate or severe disability, which is defined as survival with at least 1 of the following complications: cerebral palsy, MDI < 70, deafness, or blindness.
[a]Statistically significant.
CI = confidence interval; MDI = mental developmental index; rhEPO = recombinant human erythropoietin.

TABLE 3. Outcomes at 18 Months of Corrected Age in Subgroups

Characteristic	CP, No. (%)	MDI < 70, No. (%)	Blindness, No. (%)	Deafness, No. (%)	Disability, No. (%)
Gestational age					
<28 weeks					
Total, n = 25	5 (20)	10 (40)	4 (16)	0	12 (48)
Placebo, n = 13	3 (23)	6 (46)	2 (15.4)	0	6 (46.2)
rhEPO, n = 12	2 (16.7)	4 (33.3)	2 (16.7)	0	6 (50)
28–$29^{6/7}$ weeks					
Total, n = 172	10 (5.8)	20 (11.6)	1 (0.6)	4 (2.3)	26 (15.1)
Placebo, n = 77	7 (9.1)	15 (19.5)	1 (1.3)	3 (3.9)	20 (26)
rhEPO, n = 95	3 (3.2)	5 (5.3)[a]	0	1 (1.1)	6 (6.3)[a]
30–32 weeks					
Total, n = 416	8 (1.9)[b]	38 (9.1)[b]	0	5 (1.2)	41 (9.9)[b]
Placebo, n = 214	6 (2.8)	28 (13.1)	0	5 (2.3)	31 (14.5)
rhEPO, n = 202	2 (1)	10 (5)[a]	0	0	10 (5)[a]
Birth weight					
<1,000g					
Total, n = 27	2 (7.4)	6 (22.2)	2 (7.4)	0	7 (25.9)
Placebo, n = 14	2 (14.3)	5 (35.7)	1 (7.1)	0	5 (35.7)
rhEPO, n = 13	0	1 (7.7)	1 (7.7)	0	2 (15.4)
1,000–1,499g					
Total, n = 363	17 (4.7)	49 (13.5)	3 (0.8)	9 (2.5)	59 (16.3)
Placebo, n = 172	11 (6.4)	34 (19.8)	2 (1.2)	8 (4.7)	42 (24.4)
rhEPO, n = 191	6 (3.1)	15 (7.9)[a]	1 (0.5)	1 (0.5)[c]	17 (8.9)[a]
≥1,500g					
Total, n = 213	4 (1.9)	13 (6.1)[d]	0	0	13 (6.1)[b]
Placebo, n = 118	3 (2.5)	10 (8.5)	0	0	10 (8.5)
rhEPO, n = 105	1 (1)	3 (2.9)	0	0	3 (2.9)
Sex					
Boys					
Total, n = 428	16 (3.7)	49 (11.4)	4 (0.9)	7 (1.6)	60 (14.0)
Placebo, n = 216	10 (4.6)	36 (16.7)	2 (0.9)	7 (3.2)	44 (20.3)
rhEPO, n = 212	6 (2.8)	13 (6.1)[a]	2 (0.9)	1 (0.5)[c]	16 (7.5)[a]
Girls					
Total, n = 185	7 (3.8)	19 (10.2)	1 (0.5)	1 (0.5)	19 (10.3)
Placebo, n = 88	6 (6.8)	13 (14.8)	1 (1.1)	1 (1.1)	13 (14.8)
rhEPO, n = 97	1 (1.0)	6 (6.2)	0	0	6 (6.2)

$p < 0.05$ was considered statistically significant.
[a] $p < 0.01$, placebo group versus rhEPO group using Fisher exact test in each stratum.
[b] $p < 0.01$, comparing poor outcome with gestational age, birth weight, and sex using Fisher exact test.
[c] $p < 0.05$, placebo group versus rhEPO group using Fisher exact test in each stratum.
[d] $p < 0.05$, comparing poor outcome with gestational age, birth weight, and sex using Fisher exact test.
CP = cerebral palsy; rhEPO = recombinant human erythropoietin.

TABLE 4. Subgroup Interaction Analyses in Preterm Infants with Disability at 18 Months of Corrected Age

Subgroup	rhEPO, n = 22, No. (%)	Placebo, n = 57, No. (%)	Relative Risk [95% CI]	Interaction *p*
Sex				
Boys	16/212 (7.5)	44/216 (20.4)	0.31 [0.17–0.57]	
Girls	6/97 (6.2)	13/88 (14.8)	0.37 [0.14–1.03]	
Total			0.32 [0.19–0.55]	0.76
Gestational age				
<28 weeks	6/12 (50)	6/13 (46.2)	1.17 [0.24–5.62]	
28–$29^{6/7}$ weeks	6/95 (6.3)	20/77 (26)	0.19 [0.07–0.51]	
30–32 weeks	10/202 (5)	31/214 (14.5)	0.30 [0.14–0.63]	
Total			0.32 [0.19–0.55]	0.15
Birth weight				
<1,000g	2/13 (15.4)	5/14 (35.7)	0.33 [0.05–2.11]	
1,000–1,499g	17/191 (8.9)	42/172 (24.4)	0.30 [0.17–0.56]	
≥1,500g	3/105 (2.9)	10/118 (8.5)	0.29 [0.08–1.10]	
Total			0.32 [0.19–0.54]	0.99

Probability value is for interaction analysis in subgroups using the Mantel–Haenszel test. $p < 0.05$ was considered statistically significant.
CI = confidence interval; rhEPO = recombinant human erythropoietin.

significant differences in the occurrence of BPD between the two groups of infants ($p = 0.168$; see Table 6).

Safety Analysis

The red blood cell counts ($p = 0.567$), hemoglobin levels ($p = 0.849$), hematocrit ($p = 0.725$), and blood platelet counts ($p = 0.309$) were similar between the two groups of infants before rhEPO treatment (Table 7). At 2 weeks after treatment, there were significantly higher red blood cell counts ($p = 0.001$), hemoglobin levels ($p < 0.001$), and hematocrit ($p = 0.004$) in the rhEPO-treated infants compared to the infants who received placebo. The total

TABLE 5. Logistic Regression Analysis of Risk Factors for Adverse Neurological Outcome

Factor	B	SE	Wald	*df*	*p*	Exp (B)	95% CI Exp (B) Lower	95% CI Exp (B) Upper
Ventilation > 7 days	3.079	1.126	7.481	1	0.006[a]	21.728	2.393	197.286
PVL	2.205	0.746	8.729	1	0.003[a]	9.073	2.101	39.184
ICH, III–IV	3.990	1.331	8.993	1	0.003[a]	54.062	3.984	733.596
rhEPO	−1.385	0.606	5.219	1	0.022[a]	0.250	0.076	0.821
Gestational age								
<28 weeks			6.137	2	0.046[a]			
28–$29^{6/7}$ weeks	2.334	0.943	6.119	1	0.013[a]	10.315	1.624	65.529
Constant	−3.564	1.181	9.110	1	0.003[a]	0.028		

CI = confidence interval; ICH = intracranial hemorrhage; PVL = periventricular leukomalacia; rhEPO = recombinant human erythropoietin; SE = standard error; Wald = Wald test.
[a]Statistically significant.

TABLE 6. Neonatal Complications and rhEPO Treatment

Complication	Placebo, n = 377, No. (%)	rhEPO, n = 366, No. (%)	Relative Risk [95% CI]	*p*
ICH, III-IV	60 (15.9)	24 (6.6)	0.38 [0.23–0.62]	<0.001
PVL	74 (19.6)	41 (11.2)	0.52 [0.35–0.79]	0.002
ROP	97 (25.7)	79 (21.6)	0.80 [0.57–1.12]	0.196
NEC	54 (14.3)	25 (6.8)	0.44 [0.27–0.73]	0.001
BPD	51 (13.5)	37 (10.1)	0.73 [0.46–1.14]	0.168
Sepsis	99 (26.3)	71 (19.4)	0.65 [0.46–0.92]	0.015

Probability value is for the placebo group versus rhEPO group using univariate logistic regression. $p < 0.05$ was considered statistically significant.
BPD = bronchopulmonary dysplasia; CI = confidence interval; ICH = intracranial hemorrhage; NEC = necrotizing enterocolitis; PVL = periventricular leukomalacia; rhEPO = recombinant human erythropoietin; ROP = retinopathy of prematurity.

number of blood transfusions was significantly decreased in the rhEPO-treated infants compared to the infants in the placebo group ($p < 0.001$). There were no significant differences regarding the blood platelet count, alanine aminotransferase levels, or serum creatinine levels between the infants in the two treatment groups (see Table 7). No other obvious side effects such as thrombosis, hypertension, polycythemia, or polyplastocytosis were observed in the rhEPO treatment group or the placebo group.

Discussion

Very preterm infants are at high risk for brain injury and subsequent neurodevelopmental disabilities such as cerebral palsy and neurocognitive deficits, and there are no

TABLE 7. Blood Analysis before and after Treatment during Hospitalization

Blood Analysis	Placebo, n = 377	rhEPO, n = 366	*p*
Before treatment			
RBC, 10^{12}/l	3.9 ± 0.8	3.8 ± 0.6	0.567
Hemoglobin, g/l	139 ± 29	140 ± 28	0.849
Hematocrit, %	42.3 ± 9.1	42.8 ± 8.7	0.725
Platelet, 10^9/l	236 ± 113	247 ± 110	0.309
ALT	7.6 ± 5.3	7.8 ± 3.5	0.299
SCR	62.3 ± 33.1	53.5 ± 22.3	0.195
After treatment			
RBC, 10^{12}/l	2.9 ± 0.6	3.2 ± 0.4	0.001
Hemoglobin, g/l	98 ± 18	109 ± 15	<0.001
Hematocrit, %	28.6 ± 6.3	33.3 ± 4.6	0.004
Platelet, 10^9/l	267 ± 126	270 ± 111	0.823
ALT	11.5 ± 5.1	9.8 ± 8.2	0.428
SCR	41.5 ± 10.5	37.2 ± 14.8	0.522
Transfusions	2.2 ± 1.7	1.1 ± 1.2	<0.001

Probability value is for the erythropoietin group versus the control group using Student *t* test. $p < 0.05$ means a significant difference.
ALT = alanine aminotransferase; RBC = red blood cell; rhEPO = recombinant human erythropoietin; SCR = serum creatinine.

widely accepted preventive or therapeutic strategies for preterm brain injury and neurological sequelae. Recent experimental[34,35] and clinical[17,36–38] studies indicate that rhEPO is a promising candidate for very preterm infants with brain injury and that it improves neurodevelopment and reduces the risk of neurodevelopmental disability. However, the safe dose range, timing, and duration of rhEPO administration still require careful exploration and consideration. In this study, we found that repeated administration of low-dose rhEPO (500U/kg) for 2 weeks significantly reduced the incidence of brain injuries, including ICH and PVL. This suggests that rhEPO treatment confers short-term neuroprotection in very preterm infants. In addition, the incidence of neurological disabilities was significantly reduced after rhEPO treatment. This rhEPO treatment protocol did not increase the incidence of ROP, and no adverse side effects were observed. Overall, our study demonstrated that repeated low-dose rhEPO affords both short-term and long-term neuroprotection and suggests that rhEPO is a promising preventive and therapeutic option for very preterm infants. The subgroup analysis showed that rhEPO improved long-term outcomes for very preterm infants of different gestational ages, birth weights, and sex. In this study, we also observed that the incidence of neurological disabilities was associated with gestational age and birth weight. The earlier the preterm birth, or the lower the birth body weight, the higher the chances of suffering from neurological disability, and this is in agreement with previous findings.[39]

The current study has some limitations. First, the number of subjects lost to follow-up was relatively high. This was probably related to the high cost of hospitalization and lack of health insurance or poor prognosis. The migration of some families might be another important reason, because many young people frequently change their work in China. Second, although the rate of enrollment was highly dependent on the parents' understanding and trust in the intervention, the number of the very preterm infants in the gestational age < 28 weeks subgroup and the birth weight $< 1{,}000$g subgroup was low, and this might be why we did not observe any difference between rhEPO and vehicle-treated very preterm infants in these subgroups. In addition, we would likely need more infants in the birth weight $\geq 1{,}500$g subgroup to see a significant difference because the incidence of neurological disabilities decreases with increasing birth weight. Third, there were more boys than girls in this study, and such an imbalance in sex ratio has been reported in other studies in Chinese populations.[40] Male preterm infants have a higher risk of abnormal neurological outcome than female preterm infants,[41] and more boys in the current study might have influenced the incidence of neurological disability and the effects of rhEPO treatment. Fourth, a subsequent follow-up for 5 years[42] or until the children start school[43] might further help determine whether early administration of rhEPO to very preterm infants improves neurodevelopmental outcomes.rhEPO was originally used at a low dose to prevent anemia in preterm infants, and it is now widely accepted as a routine application for all preterm infants as a substitute for blood transfusion, although the dose, duration, and route of rhEPO administration has been modified and is different from its original usage.[23,25,44] Animal experiments showed that high-dose rhEPO (10,000–30,000U/kg) decreases both short-term and long-term brain injury, but it must be given at the time of, or very soon after, brain injury.[12,45] This high-dose rhEPO in rodents was equivalent to that in preterm infants given at a high dose of 1,000 to 2,500U/kg.[20] Based on those results from neonatal rodent brain injury models, clinical studies showed that high-dose rhEPO (2,500–3,000U/kg) administered after birth was well tolerated in preterm and term infants[14,20,33,37,42] and conferred neuroprotection.[16,36] In a previous study using low-dose rhEPO to prevent anemia in preterm infants, it was observed that preterm infants with rhEPO treatment also had significant neurodevelopmental improvement.[38] Another previous study using low-dose rhEPO (250–400U/kg, 3 times a week for 6 weeks) to prevent anemia in premature infants of birth weight $< 1{,}500$g found that the neurodevelopmental outcome improvement was related to the total cumulative dose of rhEPO,[46] suggesting that repeated low-dose rhEPO treatment could be beneficial. Repeated administration of as low as 500U/kg of rhEPO for 2 weeks in the present study proved to be neuroprotective.

With the increasing number of premature infants, rhEPO is increasingly being used in neonatal medicine as a substitute for blood transfusions in preterm infants. The side effects of rhEPO in very preterm infants have drawn much concern, especially because rhEPO has been reported to promote angiogenesis and ROP.[47] However, studies in the current literature show conflicting results with regard to ROP being associated with the use of rhEPO in preterm infants.[17,20,22,23,48] In a recent review of 27 studies with a total of 2,209 preterm infants, early administration of rhEPO reduced the risk associated with increased blood transfusion, but there was a tendency, although not significant, for an increase in the risk of ROP. The rates for mortality and morbidities, including ICH and NEC, were not significantly changed by early rhEPO treatment.[23] Other recent studies showed that early high-dose rhEPO (2,500–3,000U/kg) administered

once in very preterm infants did not increase the incidence or severity of ROP.[20,37] In the current study, low-dose rhEPO (500U/kg) treatment decreased the risk of NEC and sepsis and did not increase the risk of ROP or BPD. The mechanisms behind the reduced incidence of NEC and sepsis are not fully understood; however, rhEPO protects epithelial barrier function and protects intestine from excessive autophagy and apoptosis, and this, as well as rhEPO's immunomodulation actions, might provide beneficial effects in preventing NEC and sepsis.[49,50] Furthermore, there were no adverse effects such as thrombosis, hypertension, polycythemia, or polyplastocytosis observed, which is in line with previous reports,[20,37] suggesting that the current protocol of repeated low-dose rhEPO treatment in this study is protective and safe.

In summary, this study provides important evidence for a safe and beneficial effect of rhEPO on improving the short-term and long-term outcomes in very preterm infants who received repeated low doses of rhEPO. No signs of adverse effects of this early rhEPO treatment in very preterm infants were identified. We speculate, therefore, that rhEPO treatment could be an important preventive and therapeutic option for very preterm infants with brain injury. Future research on the neuroprotective effect of rhEPO will focus on very preterm infants of gestational age < 28 weeks by increasing the sample size and prolonging the treatment time up to 32 weeks collective age. A subsequent follow-up to 5 years will be performed in the future to determine whether early administration of rhEPO to very preterm infants improves neurodevelopmental outcomes.

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Author Contributions

J.S. and H.S. contributed equally to this work. Concept and study design: C.Z., X.W., H.S., and J.S. Data acquisition and analysis: all authors. Drafting the manuscript and the figures: J.S., C.Z., H.S., and X.W.

Potential Conflicts of Interest

Nothing to report.

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