Safety of Early High-Dose Recombinant Erythropoietin for Neuroprotection in Very Preterm Infants

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Objective To investigate the safety and short term outcome of high dose recombinant human erythropoietin (rhEpo) given shortly after birth and subsequently over the first 2 days for neuroprotection to very preterm infants.
Study design Randomized, double masked phase II trial. Preterm infants (gestational age 26 0/7-31 6/7 weeks) were given rhEpo (n_t = 229; 3000 U/kg body weight) or NaCl 0.9% (n_c = 214) intravenously at 3, 12-18, and 36-42 hours after birth.
Results There were no relevant differences between the groups for short-term outcomes such as mortality, retinopathy of prematurity, intraventricular hemorrhage, sepsis, necrotizing enterocolitis, and bronchopulmonary dysplasia. At day 7-10, we found significantly higher hematocrit values, reticulocyte, and white blood cell counts, and a lower platelet count in the rhEpo group.
Conclusions Early high-dose rhEpo administration to very premature infants is safe and causes no excess in mortality or major adverse events. *(J Pediatr 2015;167:52-7)*
Trial registration ClinicalTrials.gov: NCT00413946.

See editorial, p 10

Premature birth, intraventricular hemorrhage (IVH), and hypoxic ischemic encephalopathy remain major risk factors for delayed psychomotor development, cognitive deficits, and cerebral palsy in newborn infants.[1-7] The most critical period centers around birth when oxygenation, especially of the brain, may be impaired because of insufficient placento-fetal perfusion, or after birth because of respiratory and circulatory disorders. Additional factors such as inflammation, excitotoxicity, or oxidative stress can alter brain development, which remains particularly vulnerable during the phase of oligodendrocyte development.[8-10]

Although therapeutic hypothermia improves long-term neurodevelopmental outcome in term neonates with hypoxic-ischemic encephalopathy,[11] there is still urgent need for alternative or additional treatment options for proven and safe neuroprotection in infants born prematurely. Although there are some drugs which show promise for ante- and postnatal neuroprotection in preclinical studies, their specific interactions in developing organ systems, their dosing, and their timing are still insufficiently known.

Recombinant human erythropoietin (rhEpo), the primary regulator of erythropoiesis, has been evaluated for postnatal neuroprotection in newborn infants.[12] Several reasons explain the high potential of rhEpo for neuroprotective treatment: (1) the endogenous erythropoietin (Epo)/Epo receptor system is essential for proper development of the central nervous system as shown in mutant mice with conditional ablation of one of these genes[13]; (2) the Epo/Epo receptor system in the brain is stimulated in response to hypoxic-ischemic injury and subsequently induces receptor-mediated, cell-specific effects of early and late tissue healing[14-16]; (3) in vitro and in vivo experiments demonstrate the anti-inflammatory, anti-excitotoxic, anti-oxidant, and anti-apoptotic effects of Epo on neurons and oligodendroglia and characterize the molecular mechanisms of these processes[17,18]; (4) rhEpo promotes neurogenesis and angiogenesis, which are essential for repair processes after brain injury and the development of compensatory mechanisms for proper neurodevelopment[17,19-22]; and (5) retrospectively performed analysis of clinical data from preterm infants treated with

DOL	Day of life
ELBW	Extremely low birth weight
Epo	Erythropoietin
GA	Gestational age
IVH	Intraventricular hemorrhage
MDI	Mental developmental index
PMA	Postmenstrual age
PVL	Periventricular leukomalacia
rhEpo	Recombinant human erythropoietin
ROP	Retinopathy of prematurity
WBC	White blood cell

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rhEpo for the anemia of prematurity demonstrated a robust improvement of neurodevelopmental outcome.[23-26]

Several interventional clinical trials on the neuroprotective effect of rhEpo or of its highly glycosylated derivate darbepoetin have been completed in neonates, including infants at high risk for long-term neurodevelopmental disorders because of extreme prematurity,[27-30] (near-) term neonates with asphyxia (with or without therapeutic hypothermia),[31-33] neonates with stroke,[34] neonates undergoing cardiac surgery,[35] infants suffering from cerebral palsy,[36] and in adults with arterial ischemic stroke, subarachnoidal hemorrhage, chronic schizophrenia, progressive multiple sclerosis, or cardiac surgery.[37,38] Some clinical trials initiated for neuroprotection in very premature neonates were either placed on hold by the Food and Drug Administration because of safety concerns and later restarted with or without modified doses and dosing schedules, or never started recruitment for various reasons (NCT00451698, NCT00491413, and NCT00589953).

The primary objective of our phase II trial was to investigate the neuroprotective effect of rhEpo in very premature infants with the goal to improve their long-term neurodevelopmental outcome. The primary hypothesis of this trial is that high-dose rhEpo improves the Bayley mental developmental index (MDI) of infants born very preterm at 24 months of age compared with placebo. Herein, we report the short-term safety of early high-dose rhEpo administered immediately after birth and subsequently over the first 2 days of life to very preterm infants.

Methods

This phase II clinical trial was a randomized, double-masked, placebo-controlled multicenter trial (ClinicalTrials.gov: NCT00413946). The full study protocol has been reported.[27] Briefly, very preterm infants born between 26 $^{0}/_{7}$ and 31 $^{6}/_{7}$ weeks of gestation were eligible for enrollment. The exclusion criteria were genetically defined syndromes, congenital malformations adversely affecting neurodevelopment, or severe IVH ≥III° before randomization. The patients were randomized within the first 3 hours after birth. The study medication (rhEpo or NaCl 0.9%) was randomly assigned to each patient number in advance, using a computer-based random number generator. Epoetin beta (3000 U rhEpo/kg body weight at birth, equal to 1 mL solution/kg birth weight; Roche, Basel Switzerland), or an equivalent volume of saline placebo was given intravenously at <3, 12-18, and 36-42 hours after birth over a period of 10 minutes.[27] **Figure 1** (available at www.jpeds.com) gives the standardized evaluations performed during the hospital stay. Intracranial hemorrhage was graded according to Papile,[39] and white matter disease according to de Vries (periventricular echodensity at 7 days and periventricular leukomalacia [PVL] at 36 postmenstrual weeks).[40] Peripheral blood cell counts were performed on day 1 and between day of life (DOL) 7 and 10 using an automated counter. The severity of retinopathy of prematurity (ROP) was graded according to the international classification of ROP.[41] The gain in weight, length, and head circumference was documented prior to discharge.

Serious adverse events and adverse events were continuously reported to the safety monitoring board. Coordination and data management were provided by the Swiss Neonatal Network, and by a study nurse (B.K.). An external safety and data monitoring board performed regular reviews of the patient data. Approval to conduct this study was from the Ethical Committee of the University Children's Hospital Zurich, by the Ethical Committee of the Canton Zurich, and by Swissmedics Berne. Written informed consent was from the parents. Health care providers, parents, outcome assessors, and external statisticians were unaware of treatment allocation.

Statistical Analyses

A minimal sample size for each group of 176 infants was calculated for a difference of 0.3 SD of the Bayley MDI, assuming a 2-sided alpha 0.05, and power (1-beta) 0.8.[27] We assumed a 20% loss to follow-up and deaths. Frequencies of traits were compared by OR and 95% CIs for the OR. Continuous measurements were summarized by means, differences of means, and 95% CI for the differences of means (IBM SPSS Statistics for Macintosh, v 20.0; IBM Corporation, Armonk, New York).

Results

Between September 2005 and December 2012, 1359 very premature infants were eligible. Among them, 161 infants did not meet all inclusion criteria (including 2 infants with preexisting IVH ≥grade 3), 176 parents refused, and 572 parents were not asked because of emergency situations or language problems precluding informed consent, or because of a high unlikeliness of compliance with long-term follow-up exams. Thus, 450 infants were randomized. Seven of them (1 infant in the Epo group; 6 infants in the placebo group) were excluded from the analysis because the infants did not get the full medication dose as allocated, ending up with 229 infants in the Epo group and 214 infants allocated to the placebo group (**Figure 2**; available at www.jpeds.com). The rhEpo and placebo groups were almost equal for gestational age (GA), weight, and head circumference at birth, sex, pregnancy-related complications, antenatal steroids for lung maturation, mode of delivery, and umbilical artery pH (**Table I**). Only the Apgar score at 5 minutes was significantly higher in the Epo group (7.6 vs 7.3; $P = .037$). Both groups were also comparable with regard to mechanical ventilation and days on nasal continuous positive airway pressure or on supplemental oxygen.

There were 196 (85.6%) survivors without any severe IVH (≥grade 3), PVL, and ROP (≥grade 3, plus disease) in the rhEpo group, and 183 (85.5%) in the placebo group (**Table II**). No significant differences were found in the mortality rate with 12 infants (5.2%) in the rhEpo group and 12 infants (5.6%) in the control group. Furthermore, there were no differences with regard to IVH, ventricular dilation, cystic and noncystic PVL, ROP, sepsis,