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High-Dose Erythropoietin for Asphyxia and Encephalopathy (HEAL): A Randomized Controlled Trial: Background, Aims, and Study Protocol

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Abstract

Background—Hypoxic ischemic encephalopathy (HIE) remains an important cause of neonatal death and frequently leads to significant long-term disability in survivors. Therapeutic hypothermia, while beneficial, still leaves many treated infants with lifelong disabilities. Adjunctive therapies are needed, and erythropoietin (Epo) has the potential to provide additional neuroprotection.

Objectives—To review the current incidence, mechanism of injury and sequelae of HIE, and to describe a new phase III randomized placebo-controlled trial of Epo neuroprotection in term and near-term infants with moderate to severe HIE treated with therapeutic hypothermia.

Methods—This article presents an overview of HIE, neuroprotective functions of Epo, and the design of a double-blind, placebo-controlled, multicenter trial of high-dose Epo administration, enrolling 500 neonates ≥ 36 weeks of gestation with moderate or severe HIE diagnosed by clinical criteria.

Results and Conclusions—Epo has robust neuroprotective effects in preclinical studies, and phase I/II trials suggest that multiple high doses of Epo may provide neuroprotection against brain injury in term infants. The High Dose Erythropoietin for Asphyxia and Encephalopathy (HEAL) Trial will evaluate whether high dose Epo reduces the combined outcome of death or

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neurodevelopmental disability when given in conjunction with hypothermia to newborns with moderate/severe HIE.

Keywords

hypoxia-ischemia; neonatal encephalopathy; therapeutic hypothermia; neuroprotection

Introduction

Perinatal asphyxia, a lack of oxygen and perfusion to the brain and other vital organs, globally contributed to an estimated 11% of deaths among children under 5 years of age, and 22% of deaths in the first month of life in 2013 [1]. Risk of perinatal asphyxia or hypoxic ischemic encephalopathy (HIE) varies widely by country, ranging from 2–4 per 1000 live births in high-income countries up to 26 per 1000 live births in low/middle-income countries [2]. Therapeutic hypothermia (TH) is currently the only proven treatment for HIE, and is standard of care in high-income countries [3]. While TH is clearly beneficial (number needed to treat is 8) [3], neonates with moderate/severe HIE treated with TH still experienced unacceptably high complications: mortality 28%, range 24–38; cognitive impairment 24%, range 21–25; cerebral palsy (CP) 22%, range 13–28; epilepsy 19%, range 15–24; cortical visual impairment 6%, range 1–10, with combined death or moderate/severe disability 48%, range 44–53) [3]. Adjunctive therapies to further improve outcomes are desperately needed. In high-income countries, experimental therapies must be tested in conjunction with the standard of care.

Epo has neuroprotective and neuroregenerative effects, which are the net effects of the anti-inflammatory, anti-excitotoxic, anti-oxidant, and anti-apoptotic effects on neurons and oligodendrocytes. Furthermore, Epo promotes neurogenesis, oligodendrogenesis, and angiogenesis, which are essential for normal neurodevelopment and injury repair. These beneficial effects of Epo have been well documented in experimental models of neonatal brain injury [4,5], with emerging clinical data also showing benefit [6–8]. The safety profile of high dose Epo in neonates is also reassuring [9]. Epo pharmacokinetics in HIE and hypothermia have been well defined [10,11]. Given the compelling preclinical data, the suggestive findings from human trials, favorable safety and pharmacokinetic data, and the unacceptable rate of adverse long-term neurologic outcomes in HIE, we are conducting a randomized, placebo-controlled, multi-center, phase III clinical trial in 500 term/near-term infants with moderate/severe HIE to determine whether multiple doses of high-dose Epo reduce the rate of death or neurodevelopmental disability, and reduce severity of brain injury on neonatal MRI and MRS (“High dose Epo for Asphyxia and Encephalopathy” - HEAL Trial, NCT-02811263)

Pathophysiology of HIE

Lack of both oxygen (hypoxia) and perfusion (ischemia) must be present for a significant period to result in clinically significant HIE. Large animal models suggest occlusion of umbilical cord blood flow for 18 to 25 minutes is sufficient to cause moderate/severe brain injury [12]. The initial insult results in primary energy failure with impairment of

mitochondrial respiration, anaerobic metabolism, lactic acidosis, failure of cell membrane pumps leading to an influx of sodium and calcium, cell swelling and death [13]. A cascade of inflammatory events follows with an increased release of pro-apoptotic proteins and subsequent apoptotic cell death. Injury progression continues with ongoing inflammation, impaired neurogenesis, and alteration in synaptogenesis and axonal growth. The mechanism of cell death changes from early necrosis, to later apoptosis with a continuum of phenotypes emerging over time (the apoptosis-necrosis continuum) [14]. The final phase is regeneration and repair. Key targets for neuroprotection might include early anti-oxidant therapy, interruption of acute inflammation and apoptotic cell death, and finally, stimulation of repair mechanisms.

Epo Neuroprotection

Epo is unique as a potential neuroprotective agent in that it has acute effects (anti-inflammatory, anti-excitotoxic, anti-oxidant, and anti-apoptotic) and regenerative effects (neurogenesis, angiogenesis, and oligodendrogenesis), essential for repair of injury and normal neurodevelopment [4,5]. Epo functions by binding to its homodimeric cell surface receptor (Epo- R). Epo-Rs are expressed by a variety of cell types in brain, including neuronal progenitor cells, subsets of mature neurons, astrocytes, oligodendrocytes, microglia, and vascular endothelial cells [4,5]. Epo and Epo-R expression in the brain is high during fetal development but declines rapidly after birth. During hypoxia-ischemia, Epo-R expression in neurons, astrocytes, and microglia is upregulated [15]. If the insult is of sufficient duration, increased Epo expression follows, mediated via hypoxia-inducible factor-1α. In the absence of Epo-Epo-R binding, cells are predisposed to apoptosis, while in the presence of Epo, cells are preserved [15]. The established functions of Epo create an important rationale for providing exogenous Epo administration to infants with HIE, given that brain injury can occur after brief but catastrophic insults such as placental abruption or cord accidents, and such events are insufficient to stimulate an endogenous increase in Epo synthesis.

In animal models and stroke studies, Epo has been shown to reduce neuronal loss, learning impairment, and CP following brain injury. These effects are dose-dependent, with multiple doses being more effective than a single dose [16,17]. Even when initiated as late as one week after injury in neonatal rodents, there is evidence of improved behavioral outcomes, enhanced neurogenesis, increased axonal sprouting, and reduced white matter injury [17].

Clinical Studies of Epo neuroprotection for HIE in the setting of therapeutic hypothermia

In a phase I open label dose escalation study of Epo plus hypothermia in an HIE population, we evaluated the pharmacokinetics and safety of 5 IV doses of Epo administered between 1–9 days of age (NCT#00719407) [11]. Of the doses tested (250, 500, 1000, 2500 U/kg/dose), 1000 U/kg/dose IV produced target plasma Epo levels that conferred optimal neuroprotection in animal studies [16,18]. Neurodevelopmental outcomes were available for 22 of 24 infants [7] at 22 months (range 8–34). There were no deaths. Moderate to severe

disability occurred in only 1 child (4.5%), despite 8 (36%) having moderate to severe brain injury on neonatal MRI [7].

Based on the pharmacokinetic data obtained in our phase I study, we randomized 50 newborns undergoing hypothermia for moderate/severe HIE to 5 doses of IV Epo (1000 U/kg/dose) or placebo (NCT# 01913340). Infants received study drug on days 1, 2, 3, 5 and 7 of age. The 24 infants who received Epo exhibited less brain injury on MRI, and better 12-month motor outcomes on the Alberta Infant Motor Scale than the 26 infants who received placebo [19]. These phase I and II data demonstrating safety and potential efficacy led us to design the phase III randomized controlled trial described herein.

STUDY DESIGN

The High-dose Erythropoietin for Asphyxia and Encephalopathy (HEAL) Trial is a multicenter, randomized, double-masked, placebo-controlled trial.

HYPOTHESIS—Multiple doses of Epo (1000 U/kg/dose) given to cooled infants with moderate/severe HIE will reduce the primary outcome of death or neurodevelopmental impairment at 24 months from 49% to 33%. We further hypothesize that neonatal Epo will be safe, will decrease brain injury severity on neonatal brain MRI and MRS, and will decrease serial inflammatory cytokines and biomarkers of brain injury. We expect these findings will change clinical practice.

POPULATION—The HEAL Trial will enroll 500 newborns ≥ 36 weeks gestational age with moderate/severe HIE determined by neurologic symptoms, Apgar score, acidosis level, and need for resuscitation. Parental consent, randomization and initial treatment occur within 24 hours of birth. Inclusion and exclusion criteria are described in Table 1. Enrollment is irrespective of gender, religion, race and ethnicity.

INTERVENTION—Randomization is stratified by site and by severity of encephalopathy (Table 2). Following randomization, study drug (Epoetin alpha 1000 U/kg/dose or equal volume normal saline placebo) will be given via IV on study days 1, 2, 3, 4, and 7. Study members are blinded to treatment group with the exception of site pharmacists and the study statistician.

NEUROIMAGING—A standardized 3T brain MRI and MRS will be performed after rewarming, between 96–144 hours of age. MRI and MRS data acquisition were harmonized across different MRI makes and models, using a standardized HEAL MRI and MRS protocol through an iterative quality control process. The brain MRI studies will be read centrally by two independent readers blinded to treatment arm. Severity and location of brain injury will be determined using the validated Washington University MRI scoring system specifically designed to evaluate extent of HIE injury [19]. Quantitative MRS data will be processed using LC Model.

PLASMA AND URINE BIOMARKERS—Each subject will have 3 blood samples (prior to study drug, and on study days 2 and 4), and 2 urine samples (prior to study drug, and after

rewarming). Figure 1 summarizes timing of treatment administration, collection of study specimens, and ascertainment of clinical outcomes.

OUTCOME—After hospital discharge, standardized parental telephone interviews at 4, 8, 12, 18 and 24 months will collect interval medical and developmental history. The Warner Initial Developmental Evaluation A (WIDEA) parental questionnaire, which assesses development in 4 domains (self-care, mobility, communication and social cognition), will be administered at 12, 18 and 24-months [20].

The primary composite outcome of death or mild/moderate/severe neurodevelopmental impairment (NDI) will be assessed at 24-months of age. Impairment is defined as of any of the following: Gross Motor Function Classification System (GMFCS) level ≥ 1, or GMFCS =0 or 0.5 and CP (any type), or Bayley Scales of Infant and Toddler Development 3rd Edition (Bayley III) Cognitive Score <90.

CP presence and type will be determined by a validated standardized neurologic examination [21]. All neurologic and Bayley III examiners will be centrally trained and certified. Since death is a competing outcome with neurodevelopmental impairment, it is critical to include it in the primary outcome measure.

We will assess the effect of Epo on secondary outcomes at 24 months of age: a) presence of CP, b) severity of motor impairment based on GMFCS score, c) Bayley III cognitive and language scores, d) epilepsy (i.e., ≥ 2 afebrile, unprovoked seizures), and e) behavioral abnormalities (i.e., attention problems or aggressive behavior) based on the Child Behavior Checklist externalizing score.

To evaluate whether Epo shifts the overall severity of impairment, we will perform a secondary analysis of the effect of Epo on a 4-level outcome: 1) normal, 2) mild motor and/or cognitive impairment, 3) moderate/severe motor and/or cognitive impairment, and 4) death. Severity of motor impairment will be determined by type of CP and GMFCS level. Severity of cognitive impairment will be determined by the Bayley III Cognitive Score.

SAFETY—Complications of HIE and potentially of study drug are captured through standardized Case Report Forms (CRFs). Pre-specified Serious Adverse Events (SAEs) are documented and adjudicated by an independent medical monitor masked to treatment allocation (Table 3).

All CRF data are remotely reviewed for data quality and completeness. On-site monitoring visits ensure protocol and regulatory compliance. An NIH-led Data Safety Monitoring Board (DSMB) meets regularly to oversee study conduct and safety. At pre-specified interim enrollment time points of n=125, 250, and 375 subjects, the DSMB will formally compare mortality and rates of SAEs across the two treatment groups using appropriate small sample methods such as Fisher's exact test. The DSMB will monitor mortality as a primary safety endpoint; overall significance level will be controlled using O'Brien-Fleming boundaries [22]. An interim analysis of efficacy/futility will not be conducted, as at the time the primary measure of treatment efficacy can be assessed, the majority of patients will have already been randomized.

SAMPLE SIZE—Using data from three large sites in the phase II study [19] we estimated the following outcomes: death-14%; moderate/severe impairment-18%; mild impairment-17%; normal-51%. Therefore, we anticipate the rate of death or NDI, the primary outcome, to be 49% in placebo-treated controls. The primary outcome will be assessed between 22–26 months of age. We consider data from a long-term follow-up phase I study [11] in which 24 cooled infants were given multiple doses of Epo ranging from 250U/kg to 2500U/kg [7]. At 22–26 months of age, n=22 subjects were followed. 0/22 subjects died, 1/22 had moderate-severe NDI, and 6/22 had mild NDI (overall primary outcome rate of 7/22 =31.8%; exact confidence interval = 14%–55%) for our planned intervention group). Based on animal data [23], phase I data [11], and projections from phase II data [19], we expect primary outcome rates from 31–35% among Epo-treated subjects with a protective relative risk of 0.65 to 0.71. An enrolled sample of 500 subjects, and assuming an intervention rate of 33%, yields greater than 90% power to detect a difference from a control rate of 49% (relative risk = 0.67). We assume a 90% follow-up rate with n=225/250 subjects evaluated in each arm.

STATISTICAL ANALYSES—Analyses will be based on a modified Intention to Treat (mITT) approach. All randomized neonates receiving at least one study drug dose will be analyzed. Neonates withdrawn after randomization but before the first dose will be excluded from the mITT. The primary analysis will test equality of the primary outcome (death or NDI) rate across the two groups using a likelihood ratio test based on logistic regression, with stratification by site and HIE severity. For the ordered categorical secondary long-term outcome (death; moderate/severe impairment; mild impairment; normal), we will use a generalization of the Wilcoxon test that controls for site and HIE severity.

We will select a random subset of 200 subjects (100 treated and 100 controls, including both moderate and severe HIE) to measure circulating biomarkers of inflammation and brain injury. Time-specific comparisons of the mean biomarker will be measured across treatment groups using appropriate regression methods while controlling for site and HIE severity. In addition, we will conduct longitudinal analysis using linear mixed models [24] that permit an omnibus test across all three blood sample measurement times, and allow inference on differential rates of change across treatment groups. For the quantitative MRI-based injury score (Washington University MRI scoring system), we will use a stratified t-test to provide inference regarding the mean response across the treatment groups adjusting for site and HIE severity.

Limitations

The majority of HEAL subjects are transferred from referring institutions, challenging the time-sensitive consent process. Successful enrollments require timely coordination amongst transport, neonatology, and neurology teams at referring and receiving hospitals. Furthermore, the Sarnat exam (on which randomization is based) must be performed consistently across sites, and within a 1–6 hours of life. To facilitate consistent Sarnat exams, we created an online training and examiner certification process. Harmonization of MR studies across platforms is also challenging, and the timing of the MR exams cannot always be done within the ideal window due to clinical complications.

Conclusions/Discussion

This research has the potential to reduce suffering from life-long neurologic disabilities, and to significantly reduce the societal costs of caring for survivors with neurodevelopmental impairment. In 2012 currency, the lifetime cost of caring for an individual with CP is estimated at 1.15 million dollars [25]. Using a conservative estimate of 20% CP rates in infants with HIE treated with hypothermia and a conservative HIE incidence of 2 per 1000, each year infants born with HIE introduce an economic burden that will total $1.7 billion in lifetime costs due to CP alone. Similar calculations using CDC cost data [25], and rates of disability derived from hypothermia studies suggest that each year, HIE produces additional lifetime costs of $1.6 billion for intellectual disability. While assessments at 2 years of age are critical, ultimately, longer-term follow-up will be necessary to determine whether combined Epo and hypothermia treatment results in sustained improved long-term outcomes such as school readiness, IQ, executive function, and motor function. If no benefits of Epo are found, the HEAL Trial will nevertheless generate important information about clinical and laboratory biomarkers of neurodevelopmental disabilities related to newborn encephalopathy, and the data will inform the next generation of neonatal neuroprotection trials.

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Appendix

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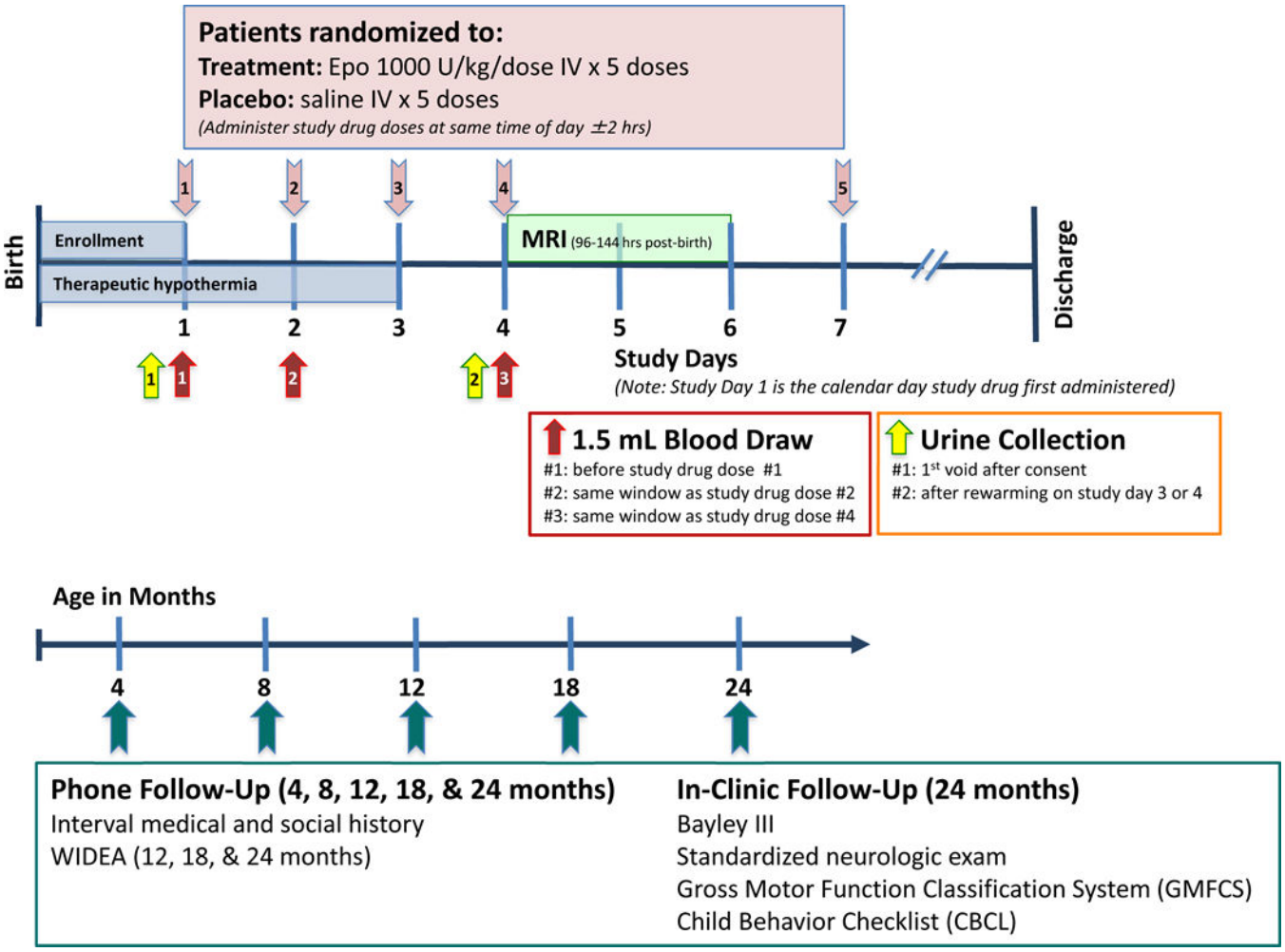

Figure 1.
Study Protocol Summary

Table 1

Inclusion and Exclusion Criteria

Inclusion Criteria	**1**	≥ 36 weeks gestational age
	2	Active or passive whole-body cooling started prior to 6 hours of age
	3	Perinatal depression based on at least one of the following:
		a. Apgar score <5 at 10 minutes
		b. Need for resuscitation (i.e., chest compressions, mechanical ventilation or CPAP) at 10 minutes of age
		c. pH <7.00 in a cord (arterial or venous) gas, or in an infant gas (arterial or venous) obtained at <60 minutes of age
		d. Base deficit ≥15 mmol/L in a cord (arterial or venous) gas, or in an infant gas (arterial or venous) obtained at <60 minutes of age
	4	Moderate or severe encephalopathy, based on presence of at least 3 of 6 Sarnat criteria, present between 1–6 hours after birth. Table 2 shows the modified Sarnat scoring system developed for the HEAL Trial.
Exclusion Criteria	**1**	Study drug unlikely to be administered within 26 hours of birth
	2	Infant has living twin (or higher order multiple) who is also being cooled.
	3	Birth weight < 1800 g (i.e., intrauterine growth restriction)
	4	Genetic or congenital condition that affects neurodevelopment or requires multiple surgeries (e.g., congenital viral infection, hydrops, complex congenital heart disease, severe dysmorphic features, etc.)
	5	Head circumference <30 cm
	6	Redirection of care is being considered due to moribund condition
	7	Patient anticipated to be unavailable for evaluation at age 2
	8	Polycythemia (hematocrit > 65.0%)
	9	Parents/legal guardians with diminished capacity or autonomy
	10	Infant is participating or intends to participate in another interventional study during the birth hospitalization (note: does not include observational studies)
	11	Sentinel event and encephalopathy occurred only after birth
	12	Unable to consent in primary language of parent(s)

Table 2

Modified HEAL Sarnat Scoring System*

Category	Mild Abnormality	Moderate Abnormality	Severe Abnormality	Unable to assess
1. Level of consciousness	Hyperalert or irritable	Lethargic or poorly responsive	Minimal or no responsiveness	
2. Spontaneous activity	Slightly decreased	Decreased	Absent	
3. Posture	Mild distal flexion	Distal flexion, complete extension	Decerebrate	
4. Tone	Hypertonic	Hypotonic	Flaccid	
5. Primitive reflexes a. Suck	Normal	Weak or bite	Absent	Unable to assess
b. Moro	Low threshold to elicit	Weak or incomplete	Absent	Unable to assess
6. Autonomic a. Pupils	Equal and reactive	Constricted	Dilated and either fixed or sluggishly reactive; asymmetric	Unable to assess
b. Respiration	Normal	Periodic breathing	Intubated and ventilated	

Note: To be eligible for the HEAL study, 3 of the 6 categories must be either moderate or severe. Level of consciousness may be the deciding factor to assign HIE stage in the event of a tie.

*The **ideal qualifying Sarnat exam** should be the WORST exam that is performed PRIOR TO receiving sedating medications, and that is performed between 1–6 hours of age.

Table 3

Pre-Specified Serious Adverse Events (SAEs)

Serious Adverse Events
- Systemic hypertension requiring anti-hypertensive therapy - Polycythemia - Disseminated intravascular coagulation (clinical bleeding/oozing requiring transfusion of blood product) - Major venous or arterial thrombosis not related to a central line - Pulmonary hypertension treated with inhaled nitric oxide or extracorporeal life support - Intracranial hemorrhage visualized on head ultrasound or MRI T1 or T2 sequences - Cardiopulmonary arrest not secondary to ETT obstruction or other mechanical issue - Death