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Original Article

Erythropoietin and Hypothermia for Hypoxic-Ischemic Encephalopathy



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ABSTRACT

BACKGROUND: Erythropoietin is neuroprotective in animal models of neonatal hypoxic-ischemic encephalopathy. We previously reported a phase I safety and pharmacokinetic study of erythropoietin in neonates. This article presents the neurodevelopmental follow-up of infants who were enrolled in the phase I clinical trial. **METHODS:** We enrolled 24 newborns with hypoxic-ischemic encephalopathy in a dose-escalation study. Patients received up to six doses of erythropoietin in addition to hypothermia. All infants underwent neonatal brain magnetic resonance imaging (MRI) reviewed by a single neuroradiologist. Moderate-to-severe neurodevelopmental disability was defined as cerebral palsy with Gross Motor Function Classification System levels III-V or cognitive impairment based on Bayley Scales of Infant Development II mental developmental index or Bayley III cognitive composite score. **RESULTS:** Outcomes were available for 22 of 24 infants, at mean age 22 months (range, 8-34 months). There were no deaths. Eight (36%) had moderate-to-severe brain injury on neonatal MRI. Moderate-to-severe disability occurred in one child (4.5%), in the setting of moderate-to-severe basal ganglia and/or thalamic injury. Seven infants with moderate-to-severe watershed injury exhibited the following outcomes: normal (three), mild language delay (two), mild hemiplegic cerebral palsy (one), and epilepsy (one). All 11 patients with a normal brain MRI had a normal outcome. **CONCLUSIONS:** This study is the first to describe neurodevelopmental outcomes in infants who received high doses of erythropoietin and hypothermia during the neonatal period. The findings suggest that future studies are warranted to assess the efficacy of this new potential neuroprotective therapy.

Keywords: neonatal encephalopathy, hypoxic-ischemic encephalopathy, neuroprotection, neurodevelopmental outcomes, erythropoietin

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Introduction

Perinatal hypoxic-ischemic encephalopathy (HIE) is an important cause of neonatal encephalopathy and occurs in one to three per 1000 term births,^{1,2} affecting up to 12,000 infants each year in the United States. Therapies remain limited. Hypothermia initiated within 6 hours of birth provides modest improvements in outcome.³⁻⁷ Yet despite this therapy, over 40% of infants with moderate-to-severe

HIE die or suffer moderate-to-severe disabilities including cerebral palsy, intellectual impairment, and epilepsy. New neuroprotective therapies are needed to further reduce the unacceptably high risk of adverse outcomes after HIE.

The hematopoietic cytokine erythropoietin (Epo) has neuroprotective and neuroregenerative effects in the brain.^{8–13} High doses of Epo administered to neonatal rodents after hypoxic-ischemic brain injury result in improved histologic and functional outcomes and enhanced neurogenesis and repair.^{14–20} In a nonhuman primate model of HIE in which hypothermia alone did not significantly improve outcomes, the combined treatment of Epo and hypothermia resulted in a significantly lower rate of death or moderate-to-severe cerebral palsy than did treatment with saline alone (0% versus 43%, $P < 0.05$).²¹ Compared with animals treated with saline, those that received both Epo and hypothermia also demonstrated improved long-term motor and cognitive responses, enhanced cerebellar growth, and improved fractional anisotropy on early diffusion tensor imaging.²¹

Two clinical trials reported that human infants with HIE who received five to seven doses of Epo during the first week of age, in the absence of hypothermia, experienced improved neurological outcomes.^{22,23} After hypothermia became the standard of care in the treatment of HIE, we evaluated the safety and pharmacokinetics of combined Epo and hypothermia therapy in a phase I trial and found that multiple doses of Epo ranging from 250 to 2500 U/kg IV appeared safe in the neonatal period.²⁴ However, longer term outcome data have yet to be reported in cooled infants who received high-dose Epo as a neonate. Therefore, we present the neurodevelopmental outcomes of infants with HIE who received high doses of Epo and hypothermia therapy during the first week of age.

Study design

We previously reported a phase I safety and pharmacokinetic study of Epo in neonates.²⁴ The current article presents the neurodevelopmental follow-up of infants who were enrolled in the phase I clinical trial. In an open-label dose-escalation study,²⁴ 24 newborns ≥ 37 weeks of gestational age undergoing hypothermia for HIE received one of the following four Epo doses IV: 250 ($n = 3$), 500 ($n = 6$), 1000 ($n = 7$), and 2500 U/kg per dose ($n = 8$). We studied these doses to determine which would achieve target plasma Epo levels based on available data from animal studies. We administered up to six doses of Epo every 48 hours, starting by 24 hours of age. Each patient met inclusion and exclusion criteria for encephalopathy and perinatal depression as previously described.^{24,25} All patients also underwent standard 72 hours of hypothermia therapy using either whole body ($n = 21$) or head ($n = 3$) cooling. All patients received a brain magnetic resonance imaging (MRI) at the completion of hypothermia therapy as part of routine clinical care. A study neuroradiologist (A.J.B.) who was blinded to patient outcomes interpreted the MRI studies using a previously validated scoring system.²⁶ The MRI was classified as normal, abnormal with a predominant watershed pattern of injury, or abnormal with predominant basal ganglia and/or thalamic injury. Severity of injury was dichotomized as being either moderate and/or severe or

mild and/or normal as previously described.²⁷ The study received institutional review board's approval at each of five participating hospitals.

After hospital discharge, patients were evaluated in the high-risk infant follow-up programs of each of the five study sites, as part of routine clinical care. During these visits, patients were evaluated for neurodevelopmental abnormalities: cerebral palsy, tone abnormalities, motor delay, language delay, and presence of seizures. The Bayley Scales of Infant Development (Bayley) II or III was performed in 16 patients at median age 24.4 months (range, 13–34 months). The eight patients who did not receive Bayley testing were either enrolled at a site where Bayley testing is not performed routinely ($n = 5$) or did not receive Bayley testing as part of their follow-up assessment ($n = 3$). We defined moderate-to-severe disability as either a clinical diagnosis of cerebral palsy with Gross Motor Function Classification System (GMFCS) III–V or moderate-to-severe cognitive delay based on Bayley II MDI of < 70 or Bayley III cognitive composite score of < 80 . Mild impairment was defined as cognitive or language delays requiring referral to early intervention services, epilepsy, or abnormal neurological examination without a diagnosis of cerebral palsy or functional impairment.

Results

Twenty-four of 26 infants consented to the study. Hypotonia, lethargy, and poor suck were the most common signs of encephalopathy (Table 1). Fifteen infants (63%) had a 10-minute Apgar score of ≤ 5 , and mean arterial or venous cord pH was 6.87 (S.D. = 0.14). Almost half of infants (45.8%) were delivered via emergent cesarean section. A sentinel event occurred in seven patients (29%), including placental abruption (four), uterine rupture (two), and prolapsed cord (one). Over half ($n = 13$) had either clinical ($n = 9$) or electrographic ($n = 7$) seizures during the hospital stay. Average length of hospitalization was 13.5 ± 7.2 days (range, 6–36 days).

Patients received a mean of $4.8 (\pm 1.2)$ Epo doses (range, 2–6 doses). Patients who did not receive all six doses of study drug were either discharged to home before the last dose (10), lost IV access (four), or had a protocol violation (one). All doses of Epo were tolerated well with no apparent adverse effects. There were no neonatal deaths, and the frequency of systemic complications was not statistically different from that reported in historical controls who received hypothermia alone.^{24,25}

MRI findings

Brain MRI performed at a median age of 6 days (range, 4–13 days) revealed no abnormalities in 13 of 24 patients (54%). Of the 11 who had MRI evidence of brain injury, nine had injury predominantly in the watershed distribution, one had basal ganglia predominant injury, and one had a focal arterial infarction. Moderate-to-severe brain injury was present in eight infants (seven watershed and one basal ganglia and/or thalamus), whereas mild injury was observed in three infants (two watershed and one focal arterial infarction).

TABLE 1.

Baseline Characteristics of 24 Infants With Neonatal Encephalopathy Who Received Hypothermia and High-Dose Erythropoietin Therapy

Characteristic	n*	%
Birth weight (g)	3255 (571)	
Gestational age (wk)	39 (1.8)	
Head circumference (cm)	34.4 (1.8)	
Female	12	50.0
Encephalopathic		
Altered consciousness	24	100.0
Hypotonia	18	75.0
Lethargy	17	70.8
Poor suck	14	58.3
Stupor or coma	8	33.3
Reflex abnormality	8	33.3
Clinical seizures	8	33.3
Hyperalert	4	16.7
Perinatal depression	24	100.0
5-min Apgar (n = 24)		
0-3	14	58.3
4-6	7	29.2
7-10	3	12.5
10-min Apgar (n = 20)		
0-3	5	25.0
4-6	11	55.0
7-10	4	20.0
Resuscitation > 10 min	21	87.5
Chest compressions	6	25.0
Cord gas pH (n = 14)	6.87 (0.14)	
Blood gas within 60 min of birth (n = 20)	6.92 (0.16)	
Delivery mode		
Emergent cesarean section	11	45.8
Spontaneous vaginal	8	33.3
Vacuum or forceps delivery	4	16.7
Elective cesarean section	1	4.2

* All data are mean (S.D.) or number of patients and percentage. n = 24 unless otherwise indicated.

Follow-up data

Two patients were lost to follow-up before reaching 6 months of age. Both infants had a normal MRI, and normal final examination (i.e., at 1 and 3 months), but were dropped from follow-up analyses given the uncertainty of their long-term outcomes. Among the remaining 22 (92%) infants, the mean age at final evaluation was 22 months (S.D. = 7.4; range, 8–34 months). The majority (20 of 22) were evaluated at 12 months or more.

There were no deaths during available follow-up. Of the 22 patients with more than 6 months of follow-up, only one (4.5%) had a moderate-to-severe motor or cognitive disability. This child had quadriplegic cerebral palsy at 20 months of age, a GMFCS level of III, gastrostomy tube feedings, and severe language delay. Six of 22 (27%) had a mild neurodevelopmental abnormality: language delay requiring speech therapy referral (three), hemiplegic cerebral palsy with GMFCS level of I (one), increased tone on neurological examination with normal function (one), and epilepsy with normal development and examination (one).

Brain MRI in relation to outcome

Of eight infants with moderate-to-severe brain MRI abnormalities, one developed moderate-to-severe disability

(i.e., quadriplegic cerebral palsy) in the setting of bilateral basal ganglia and thalamic injury. The other seven infants with moderate-to-severe watershed distribution injury (Table 2) were either normal (three) or had mild abnormalities on follow-up: mild language delay (two), mild hemiplegic cerebral palsy (one), and epilepsy with normal examination and development (one). Mild MRI brain injury was present in three infants: two had watershed injury and one had a small focal arterial infarction. Neurodevelopment was normal in all three infants, although the patient with arterial infarction had mildly increased tone on neurological examination. Brain MRI was normal in 11 patients; all 11 had a normal neurodevelopmental outcome.

Six of nine infants with watershed distribution brain injury underwent Bayley testing at median age 26 months (range, 13–31 months). All six had normal cognitive and/or language and motor scores, in spite of the presence of moderate-to-severe (four) or mild (two) watershed injury.

Discussion

In this small, open-label, phase I trial of combined Epo and hypothermia therapy for HIE, we report no deaths and a relatively low rate (4.5%) of moderate-to-severe neurodevelopmental disability at median age 22 months. This study lacked controls and was not designed to test efficacy. However, our data provide evidence extending beyond the neonatal period that high-dose Epo given in conjunction with hypothermia does not worsen outcomes when given to newborns with HIE.

This study is subject to a number of limitations. Because our inclusion criteria were not identical to the criteria used in hypothermia trials, we are unable to compare our outcomes to those published in HIE trials to determine efficacy. The study lacked controls, had an inconsistent length and quality of follow-up, used varying MRI protocols across sites, and lacked blinding because all patients received Epo. Furthermore, our previously reported pharmacokinetic study suggested that an Epo dose of 1000 U/kg is optimal to achieve neuroprotective plasma Epo levels in cooled infants.²⁴ Our patients received four different dosing regimens, and only seven of 24 received the optimal Epo doses of 1000 U/kg.

Therapeutic hypothermia has clearly improved the outlook of infants with moderate-to-severe HIE. Yet there remains a pressing need for additional neuroprotective therapies that will further reduce the unacceptably high rate of long-term adverse outcomes. Epo has neuroprotective effects that have been demonstrated in numerous preclinical studies.^{11,15,17,20,21,28} Although the mechanisms by which Epo exerts neuroprotection are not fully understood, benefits include acute effects such as reduced neuronal apoptosis,^{29–31} inflammation,^{32–34} oxidative injury,^{35,36} and glutamate toxicity,^{37,38} as well as long-term effects such as enhanced neurogenesis and repair.^{19,20,39,40} These benefits may complement the more immediate neuroprotective effects of hypothermia.

Although our study was not designed to determine the efficacy of Epo therapy, it is worth noting that our rate of adverse outcomes was no worse than what has been reported in patients treated with hypothermia alone. For instance, the rate of death or moderate-to-severe disability

TABLE 2.

Follow-up Data of Eight Infants Who Received High-Dose Erythropoietin (Epo) and Hypothermia and Who Also Had Neonatal Magnetic Resonance Imaging (MRI) Evidence of Moderate-to-Severe Brain Injury

MRI Pattern of Injury	Neurodevelopmental Impairment	Cerebral Palsy	Language Delay	Epilepsy	Epo (U/kg/dose)	Follow-up (mo)
Basal ganglia	Moderate and/or severe	Yes	Yes	No	2500	26
Watershed	Mild	Yes	No	No	2500	20
Watershed	Mild	No	No	Yes	2500	22
Watershed	Mild	No	Yes	No	500	20
Watershed	Mild	No	Yes	No	2500	25
Watershed	None	No	No	No	1000	30
Watershed	None	No	No	No	500	18
Watershed	None	No	No	No	250	30

in our study (4.5%) was lower than that reported in infants who received hypothermia in large trials (44–51%).^{3-5,7} Only one of eight patients (12.5%) with moderate-to-severe MRI brain injury in our study developed a significant neurodevelopmental disability. In contrast, 70–80% of cooled infants who had moderate-to-severe MRI brain injury in the National Institute of Child Health and Human Development hypothermia trial either died or had moderate-to-severe neurodevelopmental disability.⁴¹ Although our numbers are small, the relatively low rate of adverse outcomes, even in the setting of significant brain injury, suggests that additional studies are warranted to evaluate whether Epo can effectively enhance repair and improve outcomes after HIE.

About half of our patients (54%) had a normal brain MRI. This is consistent with previously reported rates of normal brain MRI in infants with HIE treated with hypothermia (range, 41–54%).⁴¹⁻⁴³ Among our patients with MRI brain abnormalities, only one of 11 (9%) demonstrated basal ganglia predominant injury. In contrast, studies of cooled infants with HIE have reported a higher rate of basal ganglia injury, ranging from 24% to 60%.⁴¹⁻⁴³ Basal ganglia and thalamic injury occurs most commonly in infants who have experienced a sentinel event such as uterine rupture, placental abruption, or cord prolapse.⁴⁴ A sentinel event occurred in only seven of 24 patients (29%) in our study, whereas such an event was reported in 35 of 73 cooled infants (47%) in the NICHD hypothermia trial. Thus, it is likely that the lower rate of sentinel events in our population accounts for the lower rate of basal ganglia injury in our patients. Hypothermia effectively reduces the incidence of basal ganglia injury after HIE.^{35,42,43} Whether high-dose Epo can further reduce the incidence of basal ganglia injury is unknown.

Neonatal brain injury occurring in the watershed areas and sparing the basal ganglia can cause epilepsy and cognitive and motor delays, whereas this pattern of injury less frequently causes death or moderate-to-severe cerebral palsy.³⁶ In our study, several infants with MRI findings of moderate-to-severe watershed white matter injury demonstrated only mild neurodevelopmental abnormalities. Of note, mild abnormalities such as cerebral palsy with GMFCS I-II, and mild motor or cognitive delay, have typically been combined with normal outcomes in published hypothermia trials. However, neuroprotective therapies may in fact reduce adverse outcomes across all severities. Future HIE neuroprotection trials may benefit from a closer examination of several outcome categories to better appreciate potential benefits across the entire spectrum of severity.

The clinical signs and symptoms currently used to diagnose HIE are nonspecific and are hard to distinguish from neonatal encephalopathy because of other conditions such as perinatal arterial ischemic stroke, sinovenous thrombosis, and even epidural hemorrhage.³⁷ It is not surprising that one of our patients with clinically diagnosed HIE was found on MRI to have manifested a perinatal arterial infarction, instead of global hypoxic-ischemic injury, in keeping with rates of arterial ischemic stroke in past studies.³⁸ Interestingly, Epo improves histologic and functional outcomes in animal models of focal arterial infarction.^{17,20} In newborn infants with acute perinatal arterial stroke, high-dose Epo also appears to be safe.⁴⁵ Future studies of Epo for neuroprotection will need to address the heterogeneous timing and pathogenetic mechanisms that contribute to neonatal brain injury.

Several clinical trials of Epo with hypothermia are currently underway. These studies will provide additional information in the coming years regarding the safety and efficacy of this potential therapy for HIE. It is unknown whether Epo therapy provides optimal neuroprotection when given during the first 3 days as an add-on therapy to hypothermia or whether Epo enhances regenerative and repair mechanisms best when given days later. Because Epo has both early and late neuroprotective effects, most clinical trials are testing the administration of multiple doses of Epo, given over a period of 3–7 days after delivery. A multicenter, phase II, double-blinded, randomized controlled trial in the United States will evaluate preliminary efficacy by assessing biomarkers of long-term neurodevelopmental outcome (NCT01913340). Two large phase III randomized controlled trials in France (NCT01732146) and Australia will assess neurodevelopmental outcomes at age 2 years in cooled infants with HIE. Finally, darbepoetin is a long-acting formulation of Epo that has also been evident to be safe when administered with hypothermia to newborns with HIE (NCT01471015).⁴⁶

Conclusions

High-dose Epo does not appear to worsen neurodevelopmental outcomes when administered in conjunction with therapeutic hypothermia for HIE. In addition to the neonatal safety data we previously reported, the neurodevelopmental outcome data presented here provide additional reassurance that this new potential therapy has no adverse long-term consequences and therefore appears

safe. We eagerly await the results of future trials that will provide additional data regarding whether this promising therapy can effectively reduce the rate of long-term neurodevelopmental disability after HIE.

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Comparative experiment is the “sine qua non” of scientific experimental medicine; without it a physician walks at random and becomes the plaything of endless illusions. A physician, who tries a remedy and cures his patients, is inclined to believe that the cure is due to his treatment. But the first thing to ask them is whether they have tried doing nothing. i.e. not treating other patients; for how can they otherwise know whether the remedy or nature cured them?

Claude Bernard
An Introduction to the Study of Experimental Medicine, 1865