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Authors

Wu, Yvonne W
Bauer, Larry A
Ballard, Roberta A
et al.

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Erythropoietin for Neuroprotection in Neonatal Encephalopathy: Safety and Pharmacokinetics

AUTHORS: Yvonne W. Wu, MD, MPH,[a,b] Larry A. Bauer, PharmD,[c] Roberta A. Ballard, MD,[b] Donna M. Ferriero, MD,[a,b] David V. Glidden, PhD,[d] Dennis E. Mayock, MD,[e] Taeun Chang, MD,[f] David J. Durand, MD,[g] Dongli Song, MD, PhD,[h] Sonia L. Bonifacio, MD,[b] Fernando F. Gonzalez, MD,[b] Hannah C. Glass, MD,[a,b] and Sandra E. Juul, MD, PhD[e]

Departments of [a]Neurology, [b]Pediatrics, and [d]Epidemiology and Biostatistics, University of California, San Francisco, California; Departments of [e]Pediatrics and [c]Pharmacy, University of Washington, Seattle, Washington; [f]Department of Neurology, Children's National Medical Center, Washington, DC; [g]Department of Neonatology, Children's Hospital Oakland, Oakland, California; and [h]Department of Neonatology, Santa Clara Valley Medical Center, San Jose, California

KEY WORDS

neonatal encephalopathy, asphyxia, hypoxia-ischemia, neuroprotection

ABBREVIATIONS

aEEG—amplitude-integrated EEG
ANOVA—analysis of variance
AUC—area under the curve
Cmax—maximum concentration
CSF—cerebrospinal fluid
Epo—erythropoietin
HI—hypoxic-ischemic
HIE—hypoxic-ischemic encephalopathy
IP—intraperitoneally
IV—intravenously
MRT—mean residence time
SBP—systemic blood pressure
SC—subcutaneously

All authors have made substantive intellectual contributions to this study. All authors meet the following criteria: (1) they provided substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) they provided substantial contributions to drafting the article or revising it critically for important intellectual content; and (3) they approved the final version to be submitted.

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Address correspondence to Yvonne Wu, MD, MPH, University of California, San Francisco, Child Neurology, 350 Parnassus Ave, Suite 609, San Francisco, CA 94143-0137. E-mail: wuy@neuropeds.ucsf.edu

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WHAT'S KNOWN ON THIS SUBJECT: Infants with hypoxic-ischemic encephalopathy suffer a high rate (>40%) of death or moderate to severe disability, even after therapeutic hypothermia. High-dose erythropoietin (Epo) reduces brain injury and improves neurologic function in animal models of neonatal hypoxic-ischemic brain injury.

WHAT THIS STUDY ADDS: Multiple doses of Epo (up to 2500 U/kg intravenously) given in conjunction with hypothermia are well tolerated in newborns with HIE. Epo doses of 1000 U/kg intravenously in cooled infants produce plasma concentrations that are neuroprotective in animal studies.

abstract

OBJECTIVE: To determine the safety and pharmacokinetics of erythropoietin (Epo) given in conjunction with hypothermia for hypoxic-ischemic encephalopathy (HIE). We hypothesized that high dose Epo would produce plasma concentrations that are neuroprotective in animal studies (ie, maximum concentration = 6000–10 000 U/L; area under the curve = 117 000–140 000 U*h/L).

METHODS: In this multicenter, open-label, dose-escalation, phase I study, we enrolled 24 newborns undergoing hypothermia for HIE. All patients had decreased consciousness and acidosis (pH $<$ 7.00 or base deficit $\geq$ 12), 10-minute Apgar score $\leq$ 5, or ongoing resuscitation at 10 minutes. Patients received 1 of 4 Epo doses intravenously: 250 (N = 3), 500 (N = 6), 1000 (N = 7), or 2500 U/kg per dose (N = 8). We gave up to 6 doses every 48 hours starting at $<$24 hours of age and performed pharmacokinetic and safety analyses.

RESULTS: Patients received mean 4.8 $\pm$ 1.2 Epo doses. Although Epo followed nonlinear pharmacokinetics, excessive accumulation did not occur during multiple dosing. At 500, 1000, and 2500 U/kg Epo, half-life was 7.2, 15.0, and 18.7 hours; maximum concentration was 7046, 13 780, and 33 316 U/L, and total Epo exposure (area under the curve) was 50 306, 131 054, and 328 002 U*h/L, respectively. Drug clearance at a given dose was slower than reported in uncooled preterm infants. No deaths or serious adverse effects were seen.

CONCLUSIONS: Epo 1000 U/kg per dose intravenously given in conjunction with hypothermia is well tolerated and produces plasma concentrations that are neuroprotective in animals. A large efficacy trial is needed to determine whether Epo add-on therapy further improves outcome in infants undergoing hypothermia for HIE. *Pediatrics* 2012;130:683–691

Perinatal hypoxic-ischemic encephalopathy (HIE), a known cause of neonatal encephalopathy, occurs in 1 to 3 per 1000 term births.[1,2] Up to 12 000 infants are affected each year in the United States. Neonatal "asphyxia" accounts for 22% of annual neonatal deaths worldwide, totaling 814 000 deaths in 2008.[3] Therapies for HIE remain limited. Hypothermia initiated within 6 hours of birth provides modest improvements in outcome.[4–9] Yet despite this therapy, over 40% of infants will die or suffer moderate to severe disabilities including cerebral palsy, intellectual impairment, and epilepsy.[10,11]

The hematopoietic cytokine erythropoietin (Epo) has neuroprotective and neuroregenerative effects in the brain.[12–19] High doses of Epo administered to neonatal rodents after hypoxic-ischemic (HI) brain injury results in improved histologic and functional outcomes including improved memory and swim speed.[17,20–27] Two clinical trials suggest that infants with HIE treated with 5 to 7 doses of Epo experience improved neurologic outcomes.[28,29] However, small patient numbers, short length of follow-up,[29] and lack of intention-to-treat analysis[28] limit conclusions from these studies. Importantly, the safety of high-dose Epo in cooled infants has not been evaluated. We performed a phase I study to determine the safety and pharmacokinetics of Epo ranging from 250 to 2500 U/kg per dose as add-on therapy to hypothermia, and to determine a dosage that would produce target concentrations associated with neuroprotection in animal models.

METHODS

In this multicenter, open-label, dose-escalation study, we enrolled 24 newborn infants $\geq$ 36 weeks' gestation with HIE. All patients underwent hypothermia therapy at 1 of the 5 centers: University of California, San Francisco (N = 10); Seattle Children's Regional Hospital and Medical Center (N = 5); Children's National Medical Center (N = 4); Children's Hospital of Oakland (N = 3); and Santa Clara Valley Medical Center (N = 2). The study received institutional review board approval at participating hospitals and was registered with the US Food and Drug Administration (Investigational New Drug 102 138) and clinicaltrials.gov (identifier NCT00719407).

Patient Selection

Similar to the Coolcap study,[5] all patients met 3 criteria: (1) altered level of consciousness with at least 1 of the following: lethargy, stupor, or coma; hyperalert state; hypotonia; abnormal reflexes including oculomotor or pupillary abnormalities; absent or weak suck; or clinical seizures[30]; (2) perinatal depression based on at least 1 of the following: 10-minute Apgar score $\leq$ 5; need for resuscitation at 10 minutes (ie, chest compressions or endotracheal or mask ventilation); pH $<$ 7.00, or base deficit $\geq$ 12, in cord or arterial blood within 60 minutes of birth; and (3) under 23.5 hours of age at time of consent.

All patients received an amplitude-integrated EEG (aEEG) before enrollment. We excluded patients with any of the following: normal aEEG voltages (upper margin $\geq$ 10 μV and lower margin $\geq$ 5 μV) and no electrographic seizures; severe aEEG abnormality (upper margin $<$ 10 μV and electrographic seizures)[5]; birth weight $<$ 1800 g; congenital anomaly, genetic syndrome, metabolic disorder, or toxoplasmosis, other infections, rubella, cytomegalovirus infection, and herpes infection; head circumference $<$ 2 SD; infant judged likely to die due to the severity of illness; hematocrit $>$ 60; or no in-dwelling line.

Epo Administration

We administered up to 6 Epo doses. The first dose was given at $<$24 hours of age, and subsequent doses were given at 48-hour intervals. We administered Epo (10 000 U/mL preservative-free solution) over 5 minutes intravenously (IV), followed by normal saline flush. Each patient received 1 of 4 dosages of Epo for all of their doses: 250 (N = 3), 500 (N = 6), 1000 (N = 7), or 2500 U/kg per dose (N = 8). We tested the lowest dose first, escalating to each subsequent dose only after data and safety monitoring board approval.

Pharmacokinetic Analysis

Blood (0.15 mL per sample) was collected as follows: 0 (predose baseline), 30 minutes, 1, 3, 6, 12, and 24 hours after the first Epo dose; 48 and 48.5 hours (pre- and postdose number 2); and predose and 30 minutes after final dose. Plasma and cerebrospinal fluid (CSF) Epo concentrations were determined by using the Quantikine IVD Human Epo Immunoassay enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). Samples were diluted so the estimated values fell within the validated range for the assay. Duplicate measurements were made and averaged to create a single measurement for each sample. Variability of the test was $<$2%, and sensitivity was 0.6 mU/mL.

First dose concentrations were used to compute pharmacokinetic parameters after plasma Epo concentrations were corrected for endogenous baseline values by subtracting the predose concentrations from subsequent values.[31–33] Data analysis was conducted by using noncompartmental pharmacokinetic techniques.[34,35] The elimination rate constant (k) for the plasma data were derived by using linear regression to compute the slope of the ln plasma Epo concentration versus time data during the terminal portion of the

curve. The trapezoidal rule was used to compute the area under plasma concentration versus time curve (area under the curve [AUC]) until the last measured value at 48 hours. The AUC was extended to infinity by taking the quotient of the 48-hour concentration and the elimination rate constant. Cmax was the maximum plasma concentration observed after the first dose.

The half-life for the plasma Epo concentration versus time curve was computed by dividing 0.693 by the elimination rate constant. Clearance (Cl), volume of distribution (using the steady-state [Vss] and area [Varea] methods), and mean residence time (MRT) were calculated by using the following formulas: Cl = D/AUC, Vss = [D (AUMC)]/AUC,[2] Varea = D/[k(AUC)], MRT = AUMC/AUC, where D is the Epo dose and AUMC is the area under the first moment curve (computed by using the trapezoidal rule to the last measured concentration and extrapolated to infinity).[34,35]

Presence of nonlinear pharmacokinetics was identified by plotting the AUC versus dose and by comparison of the dose and AUC ratios. Accumulation after multiple doses and the attainment of steady-state was assessed by comparing concentrations obtained one-half hour ($C_{0.5h}$, $C_{48.5h}$, and $C_{FinalPeak}$ representing peak concentrations) and 48 hours (C_{48h} and $C_{FinalTrough}$ representing trough concentrations) after the 3 Epo doses.

When possible, a CSF sample was collected from patients undergoing lumbar puncture for another medical indication. If this occurred, an additional blood sample was obtained within 15 minutes of the CSF sample so that the percent CSF:plasma ratio could be determined (CSF concentration/plasma concentration $\times$ 100).

Safety Monitoring

Serious adverse events included the following: (1) major venous thrombosis; (2) polycythemia (hematocrit $>$ 60, or hematocrit increase $\geq$ 15% not due to red blood cell transfusion); (3) hypertension (systemic blood pressure [SBP] $>$ 95 if 0–7 days of age; SBP $>$100 if 8–14 days; SBP $>$105 if over 2 weeks)[36,37]; (4) intraparenchymal or grade III/IV intraventricular hemorrhage; or (5) unexpected death.

We monitored blood pressure and heart rate continuously for the first 3 days and every 4 hours thereafter until 11 days of age or hospital discharge. We collected laboratory data to monitor complete blood count, electrolytes, and organ function at 1, 3, 5, and 14 days. Brain MRI scans performed in all patients as part of routine clinical care were reviewed by a single neuroradiologist (A. James Barkovich) to determine extent of brain injury and presence of hemorrhage or thrombosis.

We compared HIE comorbidity rates in our patients with the rates described in historical cooled controls from CoolCap[5]: major cardiac arrhythmia (other than sinus arrhythmia or bigeminy); hypotension requiring inotrope support; clinical bleeding and clotting studies consistent with disseminated intravascular coagulopathy; abnormal renal function (serum creatinine $>$ 0.9); hypocalcemia (serum calcium level $<$ 8 mmol/L); hypoglycemia (glucose $<$ 30 mg/dL); thrombocytopenia (platelet count $<$ 100 000 per μL); elevated liver enzymes (aspartate aminotransferase $>$ 200 IU/L or alanine aminotransferase $>$ 100 IU/L); culture proven bacteremia; hyponatremia (sodium $<$ 130 mmol/L); and hypokalemia (potassium $<$ 3.5 mmol/L).

Statistical Analysis

Pharmacokinetic data analysis was conducted by using 1-way analysis of variance (ANOVA) for between-subject measurements and repeated-measures ANOVA for comparison of consecutive peak concentrations within individual subjects. For comparison of consecutive trough concentrations, the paired *t* test was used (SPSS software; SPSS, Inc, Chicago, IL). If a significant difference ($P < .05$) was detected among groups for pharmacokinetic parameters or peak concentrations, the Tukey honestly significant difference (HSD) test (1-way ANOVA) or paired *t* tests (repeated-measures ANOVA) were used to determine differences between groups.

RESULTS

We approached parents of 26 infants for consent, and 2 declined. Twenty (83%) of the 24 enrolled infants were born at an outside hospital. Average age at consent was 15.4 ($\pm$5.7) hours (range, 1–23.5). Target cooling temperatures were achieved at 5.2 $\pm$ 4.2 hours after delivery (range, 1–18); all infants were cooled at target temperature for 72 hours, using whole body (N = 21) or head (N = 3) cooling.

Patients received an average of 4.8 ($\pm$ 1.2) doses of Epo (range, 2–6). Nine (38%) patients received all 6 doses. The remaining patients either went home before 11 days of age (N = 10), lost IV access (N = 4), or had a protocol violation that prompted discontinuation of Epo (N = 1). Average length of hospitalization was 13.5 $\pm$ 7.2 days (range, 6–36).

Clinical Characteristics

Fifteen infants (62.5%) had a 10-minute Apgar score $\leq$ 5 (Table 1). Six (25%) infants required chest compressions for an average of 12.8 $\pm$ 10.6 minutes (range, 3–30). Mean arterial or venous cord pH was 6.87 ($\pm$0.14).

Nearly half (45.8%) of infants were delivered via emergent cesarean section.

TABLE 1 Clinical Characteristics of 24 Infants With HIE Who Received Hypothermia and High-Dose Epo Therapy

	N[a]	Mean (SD) or %
Birth weight, kg	—	3.3 (0.6)
Gestational age, wk	—	39 (1.8)
Head circumference, cm	—	34.4 (1.8)
Girl	12	50
Encephalopathic		
Altered consciousness	24	100
Hypotonia	18	75
Lethargy	17	71
Poor suck	14	58
Stupor or coma	8	33
Reflex abnormality	8	33
Clinical seizures	8	33
Hyperalert	4	17
Perinatal depression	24	100
5-min Apgar ($N = 24$)		
0–3	14	58
4–6	7	29
7–10	3	13
10-min Apgar ($N = 20$)		
0–3	5	25
4–6	11	55
7–10	4	20
Resuscitation > 10 min	21	88
Chest compressions	6	25
Cord arterial or venous 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	46
Spontaneous vaginal	8	33
Vacuum or forceps delivery	4	17
Elective cesarean section	1	4
Placental histology ($N = 9$)		
Acute chorioamnionitis ($n = 3$) or funisitis ($n = 1$)	4	44
Increased perivillous fibrin deposition	1	11
Thrombosed umbilical artery	1	11
Placental infarcts	1	11
Normal	2	22

[a] All data are mean (SD), or number of patients and percent.
–, not applicable.

Perinatal complications included placental abruption ($n = 4$), preeclampsia or pregnancy-induced hypertension ($n = 3$), failed home birth ($n = 3$), prolonged rupture of membranes ($n = 2$), uterine rupture ($n = 2$), clinical chorioamnionitis ($n = 1$), decreased fetal movement ($n = 1$), prolapsed cord ($n = 1$), true cord knot ($n = 1$), cord rupture ($n = 1$), and tight nuchal cord ($n = 1$). Over half ($n = 13$) of the patients had either clinical ($n = 9$) or electrographic ($n = 7$) seizures during the hospital stay, and 12 patients received phenobarbital. Placental histology was abnormal in 7 of 9 infants (Table 1).

Pharmacokinetic Results

Epo levels (AUC and Cmax) for each dosing regimen are listed in Table 2. The AUC increased in a nonlinear fashion with increasing doses. As Epo dose increased, disproportionately large increases in AUC were noted ($P < .0001$). The mean AUC ratios were 2.7 for the 500 U/kg and 250 U/kg doses (expected value for linear pharmacokinetics = 2); 7.1 for the 1000 U/kg and 250 U/kg doses (expected value = 4); and 17.8 for the 2500 U/kg and 250 U/kg doses (expected value = 10). This pattern of change in AUC was due to a progressive decrease in clearance with dose escalation (49% decrease from 250 U/kg to 2500 U/kg, $P < .001$), a MRT increase of 164% from the lowest to the highest dose ($P < .0001$), and an increase in half-life of 146% over the dosage range ($P < .001$). Volume of distribution did not change significantly with dose escalation. Plasma Epo concentrations demonstrated fairly limited variability across individual patients, with an average coefficient of variation [coefficient of variation = (SD/mean) × 100] of 26% for Cmax.

For all dosages, Epo peak and trough concentrations did not significantly change after the first dose (Fig 1). Steady-state plasma Epo concentrations were attained by the second dose for all 4 dosages. Excessive accumulation of Epo due to nonlinear pharmacokinetics was not observed in our patients over the treated dosage range.

CSF concentrations were obtained in 3 patients after their first dose of 500 U/kg (Table 3). There was a wide range in the sampling time (1–23 hours, postdose), and the CSF:plasma ratio ranged from 1% to 9%. Of note, the enzyme-linked immunosorbent assay does not differentiate recombinant Epo from endogenous Epo.

Adverse Events

There were no serious adverse events and no neonatal deaths. The 95% confidence interval suggests that the rate of serious adverse events is at most 12%. Median percent change in hematocrit between initial and final evaluations (excluding 3 patients who received red blood cell transfusions) was −14% (interquartile range: −21% to −7%). Comorbidities were common in these critically ill newborns (Table 4). However, the frequency of systemic complications was not statistically

TABLE 2 Epo Pharmacokinetics According to Dosage (Mean ± SD)

Parameter	250 U/kg	500 U/kg	1000 U/kg	2500 U/kg
AUC ([U*h]/L)[a]	18 426 ± 8976	50 306 ± 7426[b]	131 054 ± 17 083[b]	328 002 ± 61 945[b]
Cmax (U/L)[a]	3156 ± 1615	7046 ± 814[b]	13 780 ± 2674[b]	33 316 ± 7377[b]
Cl (mL/h per kg)[c]	15.6 ± 6.3	10.1 ± 1.5[d]	7.7 ± 0.9[e]	7.9 ± 1.5[e]
t1/2 (h)[c]	7.6 ± 6.9	7.2 ± 1.9	15.0 ± 4.5[d]	18.7 ± 4.7[e]
MRT (h)[a]	8.7 ± 6.6	9.6 ± 1.7	19.1 ± 5.2[d]	23.0 ± 5.4[e]
Vss (mL/kg)	133 ± 119	95 ± 18	146 ± 38	178 ± 48
Varea (mL/kg)	170 ± 178	104 ± 25	166 ± 48	209 ± 60

Cl, clearance; t1/2, terminal half-life; Vss, steady-state volume of distribution; Varea, volume of distribution using the area method.
[a] $P < .0001$ (ANOVA).
[b] $P < .001$ (Tukey HSD, compared with 250 U/kg).
[c] $P < .001$ (ANOVA).
[d] $P < .05$ (Tukey HSD, compared with 250 U/kg).
[e] $P < .01$ (Tukey HSD, compared with 250 U/kg).

different from that reported in historical controls who received hypothermia alone (Table 4).[5]

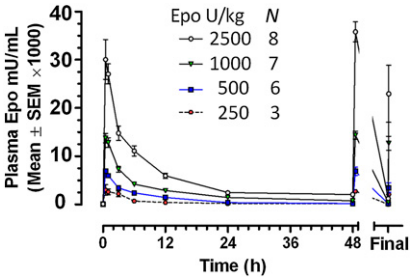

FIGURE 1
Mean plasma Epo concentrations measured in infants who received 250, 500, 1000, or 2500 U/kg Epo in conjunction with hypothermia. The dramatic rise in Epo levels seen at 0, 48 hours, and at the final time point reflect the first, second, and final Epo dose administrations. Epo followed nonlinear pharmacokinetics, but excessive accumulation did not occur after multiple doses. Steady-state plasma Epo concentrations were attained by the second dose for all 4 dosages.

TABLE 3 Epo Concentrations in CSF After the First Dose of 500 U/kg

CSF (U/L)	Plasma (U/L)	CSF/Plasma Ratio (%)	Postdose Time (h)
50.9	559.7	9.1	23
58.2	797.4	7.3	18
51.9	5229.0	1.0	1

Brain MRI performed at a median of 6 (range, 4–13) days of age and using different protocols revealed no intracranial hemorrhages or sinovenous thromboses. MRI was normal in 13 (54%) infants, demonstrated watershed injury in 9 (42%) infants, basal ganglia injury in 1 (4%) infant, and focal arterial infarction in 1 (4%) infant.

DISCUSSION

We report the first clinical study of high-dose Epo in conjunction with hypothermia. Doses up to 2500 U/kg per dose IV given every other day were well tolerated. When administered with hypothermia, Epo 1000 and 2500 U/kg per dose IV achieved or exceeded plasma concentrations that are neuroprotective in animal models.

Epo, a glycoprotein used widely in neonates to treat anemia of prematurity, is an exciting potential neuroprotective therapy for HIE. Two small trials suggest that multiple doses of Epo, 300 to 2500 U/kg per dose, administered in the first 1 to 2 weeks after birth, result in improved neurodevelopmental outcome.[28,29]

Neonatal rats with HI brain injury portray dramatic histologic and functional improvements following high-dose Epo.[23,25] Multiple doses of Epo reduce infarct volume in a dose-dependent manner.[21] Epo reduces neuronal loss and learning impairment after HI brain injury.[17,20] When initiated as late as 48 to 72 hours after injury, there is evidence of improved behavioral outcomes, enhanced neurogenesis, increased axonal sprouting, and reduced white matter injury.[19,24] Because the therapeutic window for Epo appears to be longer than for hypothermia, we chose to initiate Epo treatment at any time up to 24 hours after delivery. Of note, a study revealing that Epo improved neurologic outcomes in HIE used an even longer therapeutic window of 48 hours after birth.[28]

When Epo binds to its receptor, several intracellular signaling pathways are triggered. Activation of the Janus kinase/Stat5 pathway, along with nuclear factor kappa B (NFkB) and Akt phosphorylation, appears to be responsible for reduced apoptotic cell death after Epo.[38,39] Other acute neuroprotective mechanisms include antiinflammatory,[40,41] antiexcitotoxic,[42] and antioxidant[43] effects. Epo also stimulates growth factors[44,45] and enhances neurogenesis, angiogenesis, and long-term repair and plasticity, thus providing neuroprotective and trophic effects that last well beyond the acute period of injury.[12,13,19,23,46–48]

Hypoxia-ischemia in the brain leads to a hypoxia-inducible factor-1-mediated increase in Epo expression[49] and elevated Epo and Epo receptor levels in neurons, astrocytes, and microglia.[50,51] Newborn infants with HIE demonstrate significantly elevated CSF Epo levels even in the absence of Epo treatment.[52] Erythropoietic doses of Epo (200–400 U/kg) used to treat anemia do not raise CSF Epo concentrations.[53] Given the

TABLE 4 Comorbidities in 24 Patients Treated With Epo and Hypothermia, Compared With 112 Patients Treated With Hypothermia Alone in the Coolcap Trial[5]

	Epo + HT, *N* = 24		HT Only, *N* = 112		
	N	%	*N*	%	*P*
Intubated for respiratory distress	23	96	94	84	.13
Hypokalemia	18	75	71	63	.28
Renal dysfunction	12	50	73	65	.16
AST or ALT elevation	11	46	42	38	.45
Thrombocytopenia	11	46	36	32	.20
Hypotension	10	42	62	55	.22
Hyponatremia	9	38	49	44	.57
Hypocalcemia	9	38	49	44	.57
Disseminated intravascular coagulation	2	8	21	19	.37
Hypoglycemia	1	4	14	13	.47
Hyperkalemia[a]	1	4	0	0	NA[b]
Hyperglycemia (requiring insulin)	1	4	0	0	NA
Direct hyperbilirubinemia[c]	1	4	0	0	NA
Sepsis or bacteremia	0	0	3	3	NA
Polycythemia	0	0	3	3	NA

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HT, hypothermia; NA, not applicable.
[a] Maximum potassium = 5.5.
[b] *P* value could not be calculated due to 0 cells.
[c] Maximum total bilirubin = 19.9; maximum direct bilirubin = 4.2.

large size of the molecule (37 kD), only 1% to 2% of circulating Epo crosses the blood brain barrier, most likely via passive diffusion.[54,55] In contrast, high-dose Epo has been shown in rats, primates, and humans to achieve significant elevations in CSF and brain Epo levels.[28,33,54,55] This is particularly true in the setting of hypoxia-ischemia, presumably due to an increased permeability of the blood brain barrier.[28,56]

We obtained 3 CSF samples after a first Epo dose of 500 U/kg per day (Table 3). Although we cannot conclusively determine the origin of the Epo measured in the CSF, these CSF:plasma ratios may reflect passage of exogenously administered drug across the blood brain barrier. Because IV administered Epo demonstrated a distribution phase of 1 to 3 hours in the plasma, it is likely that Epo was still fluxing into the CSF at 1 hour after dosage administration. This is a possible explanation for the low CSF:plasma ratio found at 1 hour postdose and the higher ratios that were observed 18 to 23 hours after a dose. This finding is consistent with results found in adults where peak levels in the CSF of Epo occurred 9 to 24 hours after an intravenous dose.[57]

Similar to previous neonatal studies,[28,29,58,59] we found that high-dose Epo was well tolerated. Adverse effects reported in adults on chronic Epo therapy such as hypertension, thrombosis, polycythemia, seizures, and death have not been reported in infants. Between 1991 and 2006, over 2400 infants were enrolled in 30 randomized controlled trials of Epo for anemia of prematurity, with Epo therapy ranging from 70 to 5000 U/kg per week (35–750 U/kg per dose).[60,61] Although chronic Epo in infants under 32 weeks' gestation may increase the risk of retinopathy of prematurity,[61] this is not a concern for term infants. Additional safety data are anticipated from several ongoing studies of high-dose Epo in term and preterm neonates.

Epo appears to be primarily eliminated by receptor-mediated clearance.[31,62] As in previous reports,[32,33,58,63] we found that Epo followed nonlinear pharmacokinetics. As the dose of Epo increased twofold, fourfold, and 10-fold, the overall exposure to circulating Epo (AUC) increased 2.7, 7.1, and 17.8 times, respectively. However, peak and trough concentrations remained stable after the first dose, and steady state peak and trough concentrations were achieved after 48 hours.

Compared with premature infants given identical doses of IV Epo,[58] our patients demonstrated about a twofold reduced rate of Epo elimination. Possible explanations for the slower drug elimination observed in our patients include hypothermia treatment, hypoxia-ischemia, or both. How hypothermia affects the pharmacokinetics of Epo deserves further study.

An Epo dose of 1000 U/kg IV produced Cmax and overall exposure (AUC) levels that are most comparable with established neuroprotective levels reported in preclinical studies. In a rat model of neonatal HIE comparing several dosing regimens, multiple Epo doses of 5000 U/kg given subcutaneously (SC) or intraperitoneally (IP) afforded the greatest amount of neuroprotection.[26] At this dose, the mean AUC in treated rats ranged from 117 677 (SC) to 140 331 U*h/L (IP), and the mean Cmax ranged from 6224 U/L (SC) to 10 015 U/L (IP).[33] In our clinical study, Epo 1000 U/kg per dose given in conjunction with hypothermia provided AUC (131 054 $\pm$ 17 083 U*h/L) and Cmax (13 780 $\pm$ 2674 U/L) values that most closely reproduce these optimal neuroprotective values. In contrast, Epo 500 U/kg per dose produced insufficient plasma elevations to reach neuroprotective levels, and doses of 2500 U/kg produced AUC and Cmax values that exceeded the optimal neuroprotective range by approximately threefold.

The upper safety limit of Epo is unknown. Although doses as high as 3000 U/kg per dose are being tested in preterm infants without apparent adverse effects,[59] preclinical data suggest that too much Epo can lead to diminished efficacy[26] and that extremely high doses may in fact be harmful.[64] Epo 1000 U/kg per dose IV produces drug exposure levels that afford optimal neuroprotection in animal models, and this moderate dose is likely to minimize risks associated with giving too much Epo, especially in the setting of hypothermia, which may slow drug clearance. Therefore, Epo 1000 U/kg per dose would be a reasonable dose for future evaluation in clinical trials of add-on therapy with hypothermia.

Limitations of this study include the lack of outcome data and paucity of CSF Epo concentrations. Clinical MRI scans were performed at different times and with different protocols. It is also unknown whether the optimal range of neuroprotective plasma levels will be the same in humans as in rodents.

In the United States, hypothermia has become standard of care for HIE, and it is no longer ethical to withhold hypothermia in neuroprotection trials. Although infants who miss the window for hypothermia may benefit from Epo alone, it is not feasible to design an efficacy trial targeting this very small minority of infants, at least in this country where the vast majority of infants with HIE are currently being cooled.

CONCLUSIONS

Given the compelling preclinical data, the suggestive findings from 2 human trials, and the favorable safety and pharmacokinetic results of this study, a phase III trial is warranted to determine the neuroprotective efficacy of high-dose Epo, given as add-on

therapy to hypothermia, in newborns with HIE.

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