Erythropoietin and Brain Magnetic Resonance Imaging Findings in Hypoxic-Ischemic Encephalopathy: Volume of Acute Brain Injury and 1-Year Neurodevelopmental Outcome

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In the Neonatal Erythropoietin and Therapeutic Hypothermia Outcomes study, 9/20 erythropoietin-treated vs 12/24 placebo-treated infants with hypoxic-ischemic encephalopathy had acute brain injury. Among infants with acute brain injury, the injury volume was lower in the erythropoietin than the placebo group ($P = .004$). Higher injury volume correlated with lower 12-month neurodevelopmental scores. *(J Pediatr 2017;186:196-9).*

Trial registration ClinicalTrials.gov: NCT01913340.

Despite therapeutic hypothermia, neonatal hypoxic-ischemic encephalopathy (HIE) still results in neurologic morbidities including cerebral palsy, epilepsy, and cognitive impairment in about 40% of survivors.[1] To aid in prognostication, brain magnetic resonance imaging (MRI) is typically performed after rewarming (day 4 to 5). A normal brain MRI, along with a normal neurologic examination, provides reassurance for a favorable neurologic outcome, whereas an abnormal MRI may predict long-term impairment, depending on the severity and distribution of injury. Conventional MRI with diffusion-weighted imaging (DWI) can detect ischemic brain injury,[2,3] ranging from a few hours to 7 days following the event.[4,5] By measuring the size of DWI abnormalities, the volume of "acute" brain injury can be analyzed.[6]

Erythropoietin (Epo) is a promising neuroprotective agent for neonatal HIE, with both acute and regenerative effects.[7-10] However, the effect of Epo on volume of acute brain injury has not been studied. The purpose of this analysis was to compare the severity of acute brain injury in newborns with HIE treated with Epo vs placebo by measuring volume of injury on DWI and evaluating the correlation between volume of acute brain injury and outcome at 12 months of age.

Methods

The Neonatal Erythropoietin and Therapeutic Hypothermia Outcomes (NEATO) study[11] was a phase II, prospective, randomized, double-blind, placebo-controlled trial that enrolled 50 newborns with moderate to severe HIE from 7 US sites.[11] Infants were randomized to receive Epo 1000 units/kg or an equal volume of normal saline (placebo) on days 1, 2, 3, 5, and 7 of age in addition to hypothermia. Infants enrolled in the NEATO study underwent a clinical brain MRI following therapeutic hypothermia on a 1.5T or 3T MRI scanner based on available magnet strength at each study site. Clinical MRI protocols included T1- and T2-weighted imaging, susceptibility weighted imaging or gradient echo, DWI, and apparent diffusion coefficient (ADC).

For this study, the volume of acute brain injury seen on DWI and ADC images was quantified, as opposed to qualitatively measuring the degree of MRI injury on T1, T2, and DWI images as previously reported.[11] Because pseudo-normalization of DWI can mask areas of true DWI signal abnormalities beginning at 7 days after injury, 4 infants who had brain MRI performed at >7 days of age were excluded.[12] Two independent reviewers, who were masked to treatment group, measured the area of restricted diffusivity on DWI to determine the volume of acute brain injury. Acute brain injury was considered to be present when the volume of DWI signal abnormality was >0 cm^3. An ADC of $<0.8 \times 10^{-3}/mm^2$ was used to define areas of definite restricted diffusion seen on DWI[3] and to delineate borders between abnormal low ADC (restricted diffusivity) and noninjured adjacent parenchyma. The areas of acute brain injury were manually traced on serial axial DWI (B1000) images. Cross-sectional areas of DWI injury (ie, areas of acute brain injury) were quantified from the individual DWI slices input into a personal computer-based system as converted digital images using DT 2858 Frame Grabber (Data Translation, Wellesley, Massachusetts). Total intracranial, whole brain,

ADC	Apparent diffusion coefficient
AIMS	Alberta Infant Motor scale
DWI	Diffusion weighted imaging
Epo	Erythropoietin
HIE	Hypoxic-ischemic encephalopathy
MRI	Magnetic resonance imaging
NEATO	Neonatal Erythropoietin and Therapeutic Hypothermia Outcomes study
WIDEA	Warner Initial Developmental Evaluation

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Supported by the Thrasher Research Fund (PI: Y.W.). The authors declare no conflicts of interest.

http://dx.doi.org10.1016/j.jpeds.2017.03.053

Table I. Baseline characteristics in infants with and without acute brain injury seen on brain MRI DWI (n = 44)

		Volume of acute brain injury			
	n	None (0 cm^3) (n = 23)	>0 cm^3 (n = 21)	*P* value	All infants
Male sex	44	52.2% (12)	52.4% (11)	.99	52.3% (23)
Birth weight (g)	44	3187 (869)	3324 (574)	.85	3252 (738)
Gestational age (wk)	43	38.4 (1.4)	39.0 (1.9)	.22	38.7 (1.7)
Cesarean delivery	44	60.9% (14)	66.7% (14)	.69	63.6% (28)
5-min Apgar	42	3/ 4/ 5	2/ 3/ 5	.52	2/ 3/ 5
10-min Apgar	41	4/ 6/ 6	3/ 5/ 5	.19	3/ 5/ 6
Lowest pH	36	7.0 (0.2)	6.9 (0.2)	.13	7.0 (0.2)
Lowest base deficit	35	−14.3 (7.6)	−18.1 (6.5)	.27	−16.0 (7.3)
Encephalopathy	44			.59	
Moderate		87.0% (20)	81.0% (17)		84.1% (37)
Severe		13.0% (3)	19.0% (4)		15.9% (7)
Sentinel event	44	26.1% (6)	38.1% (8)	.39	31.8% (14)
Epo-treated	44	47.8% (11)	42.9% (9)	.74	45.5% (20)
Age at brain MRI (d)	44	4.5 (1.5)	4.2 (1.2)	.27	4.4 (1.4)
Age at discharge (d)	39	17.3 (12.1)	14.5 (9.4)	.41	16.1 (11.0)

For continuous variables, first quartile/median/third quartile or mean (SD) and Wilcoxon rank-sum test reported; for categorical variables, column % (n) and χ^2 test reported.

corpus callosum volumes (cm^3), and head circumference (cm) were also measured for each infant using T1 images as previously described.[6] Institutional review board approval was obtained at each hospital, and the study was registered with the US Food and Drug Administration (Investigational New Drug 102 138) and ClinicalTrials.gov (NCT01913340, trial registration July 29, 2013).[11]

Infants were evaluated at 12 months of age using the Warner Initial Developmental Evaluation (WIDEA), a validated 43-item parental questionnaire that assesses the 4 domains of self-care, mobility, communication, and social cognition.[13] The Alberta Infant Motor Scale (AIMS) was administered to assess motor development by observation of the infants' most mature motor movements in prone, supine, sitting, and standing positions.[14]

Statistical Analyses

Summary statistics were calculated for baseline characteristics using first quartile, median, third quartile, or mean and SD for continuous variables, and frequency and percentage for categorical variables. Differences between infants with and without acute brain injury were compared using the Wilcoxon rank-sum test and χ^2 test, for continuous and categorical variables, respectively.

Correlations between acute brain injury, Apgar scores, age at discharge, and 12-month WIDEA and AIMS scores were assessed by Pearson correlation coefficient for the whole population, as well as for Epo and placebo groups separately. All analyses were completed in R software v 3.2.2 (R Foundation, Vienna, Austria).

Results

Of 44 infants with brain MRI performed at ≤7 days, MRI occurred at a mean (±SD) of 4.4 ± 1.4 days of age (**Table I**). Twenty infants (45.5%) were randomized to the treatment group; 11 infants received 3 doses, 8 infants received 4 doses, and 1 infant received 5 doses of Epo before brain MRI.

Acute brain injury was present in 9 of 20 (45%) infants in the Epo group compared with 12 of 24 (50%) infants in the placebo group ($P = .77$). Infants with and without acute brain injury were similar for baseline characteristics (**Table I**). Acute brain injury volume was negatively correlated with base deficit ($P < .05$), 5-minute Apgar score ($P < .01$), and 10-minute Apgar score ($P < .01$) (**Table II**). Infants who received Epo treatment had a length of hospital stay of 13.0 ± 6.7 days

Table II. Correlation of Apgar scores, acute brain injury, Epo treatment, and 12-month outcome

	Base deficit	5-min Apgar	10-min Apgar	Acute corpus callosum injury	Acute brain injury	WIDEA total score	WIDEA motor score	AIMS score	Age at discharge
Base deficit									
5-min Apgar	−0.11								
10-min Apgar	.08	0.82‡							
Acute corpus callosum injury	−.01	0.16	0.13						
Acute brain injury	−0.41*	−0.40†	−0.43†	0.15					
WIDEA total score	−0.13	−.08	−0.23	−0.41*	−0.39*				
WIDEA motor score	−0.30	.07	−.09	−0.47†	−0.42†	0.80‡			
AIMS score	−0.14	−.02	−0.16	−0.51†	−0.55‡	0.88‡	0.89‡		
Age at discharge	0.33	−0.11	.08	0.18	0.34*	−0.76‡	−0.70‡	−0.76‡	
Epo treatment	−0.21	.07	−.09	−0.21	−0.17	0.27	0.40*	0.37*	−0.28

*$P < .05$.
†$P < .01$.
‡$P < .001$.