Feasibility and Safety of Erythropoietin for Neuroprotection after Perinatal Arterial Ischemic Stroke

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Objective To perform a feasibility and safety study with recombinant human erythropoietin (rhEPO) in neonates with perinatal arterial ischemic stroke.

Study design Neonates with a magnetic resonance imaging–confirmed perinatal arterial ischemic stroke (n = 21) were treated with 1000 IU/kg rhEPO immediately after diagnosis and at 24 and 48 hours after the first dose. Repeat magnetic resonance imaging was performed when the patients were 3 months of age. Coagulation and hematologic variables (red blood cells, white blood cells, platelet counts) were performed in the first week after initiation of treatment. We also compared 10 patients who were treated with rhEPO with 10 historic infants with perinatal arterial ischemic stroke matched for the involved arterial branch to investigate whether rhEPO reduces the residual size of the infarction and subsequent brain growth between first and second scan.

Results Seizures were a first symptom in 20 of 21 neonates. Heart rate, blood pressure, and coagulation function were in the normal range, as were red blood cells, white blood cells, and platelet counts. In a subgroup of 10 rhEPO-treated neonates, no differences were detected in residual infarction volumes or neurodevelopmental outcome compared with their historical nontreated counterparts.

Conclusions rhEPO in neonates with perinatal arterial ischemic stroke had no adverse effects on red blood cells, white blood cells, platelets counts, or coagulation. rhEPO, 3000 IU/kg in total, given during a 3-day period, appears to be a safe therapy. The beneficial effects remains to be demonstrated in a larger, randomized, double-blind, placebo-controlled trial. *(J Pediatr 2014;164:481-6)*.

Perinatal arterial ischemic stroke is a common neonatal complication, with an incidence of 1 per 2300 live births.[1,2] With this adjustment, the incidence of perinatal arterial ischemic stroke is greater than the annual rate of large-vessel stroke in adults.[2] Perinatal arterial ischemic stroke carries a high risk of adverse motor and cognitive outcome in neonates who have a confirmed diagnosis.[2,3]

Recombinant human erythropoietin (rhEPO) reduces hypoxia-ischemia–induced free radical formation and proinflammatory and apoptotic activity.[4,5] Models of neonatal stroke have shown a reduction of infarction volumes and improvement of cognitive function,[6-8] even after delayed treatment.[5,9,10] In adult subjects with stroke, rhEPO appears to be safe and effective.[11] High-dose rhEPO in preterm infants improves long-term outcomes without adverse effects,[12] although some clinical studies suggest that hypertension is an adverse effect of the high-dose administration of rhEPO.[13] Theoretically, multiple high doses of rhEPO could overstimulate the bone marrow, leading to high hematocrit values, induction of a procoagulant state, and increased susceptibility to ischemic disease,[9,14] although this scenario has never been reported in studies in (preterm) neonates.

We performed a feasibility and safety study in 21 neonates in whom perinatal arterial ischemic stroke was confirmed with magnetic resonance imaging (MRI) to investigate the effects of multiple high doses of rhEPO. We hypothesized that hemodynamic variables (heart rate, blood pressure), red blood cells, white blood cells, platelet counts, and coagulation would not be influenced by short-term treatment with high-dose rhEPO. We also compared infarction volume and subsequent braingrowth in 10 infants with perinatal arterial ischemic stroke who were treated with rhEPO with 10 comparable historic cases who were not treated with rhEPO to assess whether rhEPO reduced infarction volume or influenced brain growth compared with untreated infants with perinatal arterial ischemic stroke.

ADC	Apparent diffusion coefficient
DWI	Diffusion-weighted imaging
EPO	Erythropoietin
MCA	Middle cerebral artery
MRI	Magnetic resonance imaging
PCA	Posterior cerebral artery
rhEPO	Recombinant human erythropoietin
TE	Echo time
TR	Repetition time

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Methods

From September 2009 until October 2011, 21 consecutively admitted term neonates with MRI-confirmed perinatal arterial ischemic stroke who were admitted to the Wilhelmina Children's Hospital and Isala Clinics were included after written parental consent was obtained. All infants were treated in the Wilhelmina Children's Hospital. The study was approved by the Medical Ethics Committee.

After clinical and cranial ultrasound suspicion of perinatal arterial ischemic stroke, infants had a confirmed diagnosis via MRI. MRI scans were performed on a 1.5-T ACS-NT system or a 3.0-T whole-body Achieva system (Philips Medical Systems, Best, The Netherlands).[15,16] Scans were acquired in the axial plane, field of view of 180 × 180 mm, and a 256 × 256 matrix with the use of 2-mm slices for T2 (echo time [TE]/repetition time [TR]: 150/7670) and 4-mm slices for diffusion-weighted imaging (DWI; TE/TR 89/4000). For DWI, b-values of 0 and 1000 s/mm^2 were used. On the 3.0-T MRI system, scans also were acquired in the axial plane, with the same field of view, but using a 256 × 256 matrix and 2-mm slices for T2 (TE/TR: 120/6292). For DWI (TE/TR: 68/2423), a 128 × 128 matrix was used, with 4-mm slices and b-values of 0 and 800 s/mm^2. After completion of imaging, apparent diffusion coefficient (ADC) maps were created to avoid confusion with T2 effects.

Immediately after diagnosis with MRI, patients were administered 1000 IU/kg rhEPO intravenously, which was repeated at 24 and 48 hours after the first dose. The dose and duration of rhEPO was determined by earlier experimental and clinical studies in newborn animals and preterm neonates (see also the Discussion). Red blood cells, white blood cells, platelet counts, and coagulation status were performed before rhEPO and at day 1, 2, and 3 and once between days 5-7 after start of rhEPO. Heart rate and blood pressure were monitored during the administration of rhEPO. When the subjects were 3 months of age, the MRI examination was repeated to evaluate residual damage.

Neurodevelopmental Evaluation

Two authors (M.B., L.d.V.) evaluated anonymous neonatal DWI, T1, and T2 scans of all 21 infants for involvement of the posterior limb of the internal capsule and/or cerebral peduncle, which is predictive for development of hemiplegia. In 4 infants in whom no agreement was reached, the MRI scan was scored by an expert who was not involved in the study. A consensus subsequently was reached. Neurodevelopmental outcome, including development of an unilateral spastic cerebral palsy, was assessed at the age of 12-24 months and related to neonatal neuroimaging.

Quantitative MRI Comparison

For preliminary evidence of whether the administration of rhEPO resulted in less residual damage at 3 months, 10 infants (6 male) who received rhEPO were matched with 10 historical controls (7 male) born between 2005 and 2009 who received the same care as the rhEPO-treated counterpart apart from rhEPO therapy. Infants were matched for the affected arterial territory (1 posterior cerebral artery [PCA], 4 main branch middle cerebral arteries [MCAs], 2 posterior MCA, 1 anterior MCA, and 2 cortical MCA branch strokes). Infants with small cortical strokes were not included because they were difficult to match with historical identical cases, and the evaluation method would not be accurate enough to detect any differences in these infants.

A more extensive description of the methods used is given in the **Appendix** and **Figure 1** (available at www.jped.com). In brief, the volumes of both hemispheres were manually determined on the neonatal scan and on the second scan at 3 months. The stroke volume on the neonatal scan was determined by use of the DWI. Next, the final stroke volume at 3 months was estimated by comparing the difference between the observed and expected volume of the affected hemisphere, in which the expected volume of the affected hemisphere was determined using the amount of growth of the unaffected hemisphere. Finally, the percentage of the original stroke volume that was lost was estimated by dividing the percentage of the affected hemisphere that was affected by the stroke on the neonatal scan by the percentage of the expected volume of the affected hemisphere at 3 months that was lost to the stroke. Thus, if this percentage equaled 100%, the relative stroke volume was equal in both scans, suggesting that the entire ischemic area on the first scan was lost. If this percentage was greater than 100, more tissue was lost than expected on the first scan, and a percentage lower than 100 suggests recovery of ischemic tissue.

Statistical Analyses

Clinical and hematologic data are summarized as medians and ranges. Possible changes over postnatal age were evaluated by ANOVA for repeated measurements. Adjustments for multiple comparisons were made by posthoc testing (Scheffé procedure). A P value of $<.05$ was considered significant. For statistical analysis SSPS 20.0 (SSPS Inc, Chicago, Illinois) was used.

Results

Gestational age ranged from 37.1 to 41.7 weeks (median, 39.0 weeks), and birth weight ranged from 2450 to 4410 g (median, 3303 g). There was an overpresentation of male subjects (n = 13). All infants had "idiopathic" perinatal arterial ischemic stroke. All but one infant presented with hemiconvulsions, confirmed as unilateral with the use of 2-channel amplitude-integrated electroencephalography (BRM3; Natus, Seattle, Washington). All were treated with antiepileptic drugs. All infants were in stable condition on admission. None had recurrent stroke, developed hemorrhages, other severe adverse events, or died. Age at diagnosis and subsequent start of rhEPO ranged from 2 to 10 days (median, 4 days). Heart rate and blood pressure were always within normal ranges (heart rates ranged between 98 and 174 beats/min; mean blood pressure between 36 and 65 mm Hg).