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Future alternative therapies in the pipeline for mild neonatal encephalopathy: Review of evidence of neuroprotection with erythropoiesis stimulating agents

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Recent evidence reviewed in this special issue suggests that infants classified with mild neonatal encephalopathy (NE) at < 6 h of age may have more adverse outcomes compared to historical reports based a definition of encephalopathy evolution in the first week of life [1–4]. Thus it appears that the neonates with mild NE although having better survival and neurodevelopmental outcomes than those with moderate NE, have more adverse outcomes then initially reported and could benefit from additional neuroprotection. Additionally, it is becoming evident that such concern has spread into clinical practice, as therapeutic hypothermia is being used on neonates with mild NE, with seemingly limited evidence to support its use as mild NE may have a different risk benefit ratio then moderate NE [5–8].

An ideal neuroprotective agent for infants with mild NE would provide neuroprotection with minimal adverse effects. While therapeutic hypothermia could be an option for neonates with mild NE, this needs to be further studied as there are risks to applying this therapy to seemingly well appearing neonates with mild NE, and no good biomarkers to stratify those at highest risk who are most likely to benefit. Issues include that infants who receive hypothermia need to be placed on an uncomfortable cold blanket and separated from their mothers for at least 72 h, and may require transport to a higher care center. During this time the newborn cannot be held or receive skin-to-skin contact with his or her parents. Infants receiving therapeutic hypothermia are usually not fed, and central lines are required. As an alternative, Erythropoiesis stimulating agents (ESAs), will be discussed in this review as appealing agents being studied in the pipeline to provide neuroprotection in this at risk, but seemingly well population of mild NE.

The evidence will be summarized starting with 1) translational studies, 2) preterm safety and efficacy studies, 3) term infants with HIE in combination to hypothermia therapy and 4) recent studies in untreated term infants with NE.

1. ESA neuroprotection in animal models

ESAs have been evaluated as neuroprotective treatment for over 10 years. These studies have been based on the discovery that the Epo receptor is expressed throughout the human brain (in cultured neurons, astrocytes, oligodendrocytes, microglia, and endothelial cells) [9–17]. Both erythropoietin (Epo) and darbepoetin (Darbe) administered peripherally can cross the blood-brain barrier via extracellular pathways in amounts that can account for their neuroprotective actions [18]. Multiple animal studies have demonstrated significant neuroprotective effects of Epo administration, both in term and preterm models. Animal models have shown Epo to activate cellular mechanisms that promote cellular maturation, inhibition of apoptosis, neurovascular remodeling, revascularization and neurogenesis [9–17,19–26]. Exogenously administered Epo also enhances endothelial progenitor cell mobilization from the bone marrow, amplifies the production of neural progenitor cells, and stimulates oligodendrogenesis [14,15,19].

Systemic administration of Epo improves functional and histological recovery of ischemic brain injury in experimental animal models of perinatal brain injury [21–24]. Epo has been found to reduce infarct volume and improve functional and neurobehavioral performance when given immediately prior to or after neonatal brain injury using the Vannucci-Rice model [16,17,21]. Neurobehavioral testing revealed significant enhancement of muscle strength, limb placing reflexes, motor coordination and neurosensory skills [22]. In rat models of neonatal stroke Epo was shown to improve both behavioral and histological outcomes even if given one week after the induced stroke, underscoring Epo's neuroprotective properties mentioned above [21].

In addition to Epo, Darbe has been studied in animal models for neuroprotection. Darbe has comparable biological activity to Epo but with an extended circulating half-life. Darbe administration following cortical impact injury in adult rats improved cerebrovascular function and reduced histological damage in a dose and time dependent manner [27]. Weekly administration of Darbe conferred histological and behavioral neuroprotection after intracerebral hemorrhage in rats, similar to that of Epo administration [26–28]. Following focal cerebral ischemia in rats (middle cerebral artery suture-occlusion), Darbe treated rats showed decreased infarct volume and total infarct areas as well as improved neurologic scores relative to vehicle-treated animals [26].

Using cultured human fetal neuronal cells both Darbe and Epo

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increased Epo receptor gene expression and Nestin gene expression, however cells cultured with Darbe showed significantly greater Nestin gene expression than cells cultured with Epo: there was a four-fold increase in Nestin gene expression in cells cultured with Darbe compared with cells grown with no ESAs ($p < 0.05$, Darbe versus Epo; $p < 0.001$, Darbe or Epo versus control) [29].

In summary, there is convincing data in a variety of animal models that ESAs can cross the blood-brain barrier and enhance neurological recovery following stroke, NE, and trauma. Additionally, these affects can be seen even if the ESA is administered remotely from the time of injury, making it less time sensitive then other forms of neuroprotection. This is accomplished through several important mechanisms such as inhibition of apoptosis and enhanced neurogenesis.

2. ESA neuroprotection in preterm humans

The administration of human recombinant Epo in infants has been studied as an alternative to red blood cell transfusions in the treatment of anemia since the late 1980s. Between 1991 and 2009, 2723 preterm infants were enrolled in 33 randomized controlled trials to evaluate the safety and efficacy of Epo as a treatment for anemia of prematurity [30]. Treatment regimens varied widely, ranging from 70 to 5000 U/kg/week, with duration of therapy ranging from 2 weeks to several months. Halperin and colleagues reported data from the first pilot study on the treatment of anemia of prematurity in 1990 [31]. Since then, a multitude of studies have been performed examining a variety of Epo doses from low (< 500 U/kg/week) to high (> 500 U/kg/week) and treatment periods started before 8 days of age or between 8 and 28 days after birth) [32–34]. Gumy-Pause and colleagues studied higher doses of Epo and demonstrated no impact on episodes of infection, NEC, sepsis or neutropenia between low (1250 U/kg/week) and “high-dose” (up to 5000 U/kg/week) Epo [35]. The National Institute of Child Health and Human Development, Neonatal Research Network (NRN) study of early erythropoietin therapy for anemia of prematurity examined the effects of Epo treatment (400 U/kg 3 × / week) initiated by 4 days after birth and continued through 35 weeks postmenstrual age in preterm infants < 1250 g birth weight. This study revealed only a small impact in transfusion requirement, but was important in that it demonstrated no increase in adverse events with similar rates of hospital morbidities, mortality, length of hospital stay, neutropenia, hypertension and seizures in the Epo treated and placebo/control infants. Follow-up at 18–22 months corrected age revealed similar rates of neurodevelopmental impairment and need for re-hospitalization between groups [36].

Bierer and colleagues reported an association between higher Epo levels (> 500 mU/mL) and higher Mental Developmental Index scores on the Bayley Scales of Infant Development (Bayley)- II at 18–22 months' corrected age in 16 extremely low birth weight infants [37]. Similarly, Brown and colleagues reported improved neurodevelopmental outcomes in a secondary analysis of 82 preterm infants born < 1500 g and < 30 weeks' gestational age treated with Epo for anemia. They found increasing cumulative Epo exposure was associated with higher Bayley- II Mental Developmental Index scores [38]. Neubauer and colleagues reported a similar beneficial effect on 10–13 year outcome of 148 preterm infants treated with Epo under a variety of dosing regimens: the Epo group scored significantly better than untreated children in the overall developmental assessment (55% vs. 39% normally developed, $p < 0.05$) as well as in the psychological examination (mean composite HAWIK-III IQ score, 90.8 vs. 81.3, $p < 0.005$) [39]. Song and colleagues randomized 800 preterm infants to receive either 500 IU/Kg of Epo or placebo every other day for two weeks; and found the Epo treated group had less moderate to severe neurological disability at 18 months corrected age (26.9% vs 13%, $p < 0.001$) with no adverse side effects reported [40].

Regarding the administration of Darbe in preterm infants, Warwood et al. showed that a single dose of Darbe accelerated effective erythropoiesis in a pilot study of 12 preterm infants [41]. The same group of investigators reported that IV administration to neonates resulted in a shorter half-life, a larger volume of distribution, and more rapid clearance than adults [42]. Ohls and colleagues performed a randomized, placebo controlled study to assess the safety and efficacy of Darbe administered to preterm infants (500–1250 g birth weight). In this study they reported preterm infants who received ESAs (Darbe 10 μg/kg once a week or Epo 400 U/kg three times a week) had significantly better cognitive outcomes at 18–22 months corrected age and at preschool testing compared with placebo recipients, with no adverse events reported [36,44]. Natalucci and colleagues published the 2 year neurodevelopmental follow up results from 448 infants, randomized during the initial hospitalization, to assess the affects of a short duration (3 doses within 1 week of age) of early high-dose Epo (3000 U/kg) in preterm infants (26–31 weeks gestation). Investigators found that, among infants who received prophylactic Epo, there was no significant difference in neurodevelopmental outcome between the groups. No adverse events were reported, authors speculate that they were not treated long enough [45]. Most recently, Ohls and colleagues randomized preterm infants to receive Darbe (10 μg/kg, once per week), Epo (400 U/kg, 3 times/week), or placebo through 35 weeks' postconceptual age and evaluated their cognitive function at 3.5 to 4 years of age. They found former preterm infants treated with ESAs did significantly better on full scale IQ (FSIQ) and performance IQ than in the placebo group (FSIQ: 91.1 ± 17.5 vs 79.2 ± 18.5, $P = 0.036$; performance IQ: 93.0 ± 17.0 vs 79.5 ± 19.5, $P = 0.018$) [46]. The same group recently reported on MRI findings at 4 years of age, their findings suggest that ESAs may improve white matter development, however this finding was noted predominantly in female infants treated with ESAs [47].

There are two large trials currently enrolling patient to assess neurodevelopmental outcome in preterm infants treated with ESAs. The PENUT trial (NCT01378273) will assess whether early high dose Epo (1000 U/kg/dose for the first two weeks, followed by Epo 400 U/kg/dose until 32 weeks corrected age) will improve survival without neurodevelopmental impairment in preterm infants (24–28 weeks gestation) at 2 years of age. The Darbe trial (NCT03169881) is being conducted by the NRN and will assess whether early Darbe (10 μg/kg/once every week until 35 weeks corrected age) will improve neurocognitive outcome in preterm infants (23–28 weeks gestation) at 2 years of age.

Over this wide range of dosing and duration of therapy, ESAs have been quite safe in neonatal populations. No study of Epo treated newborns has reported an increase in thrombotic complications, a side effect seen in adults.

3. ESAs in term infants with NE not treated with hypothermia

Recent studies have evaluated the use of Epo in term infants with moderate/severe NE not treated with hypothermia. The first was a randomized (Not masked) prospective study of 167 term infants with moderate/severe NE conducted by Zhu and colleagues [48]. Infants were randomly assigned to receive either Epo ($N = 83$) or placebo ($N = 84$). Epo treated infants received either 300 U/kg of Epo ($N = 52$) or 500 U/kg of Epo every other day for 2 weeks starting within 48 h of birth. Study participants were followed to 18 months of age and underwent detailed neurodevelopmental assessment. Primary outcome was death or moderate/severe disability defined as cerebral palsy, severe hearing loss, blindness, gross motor function classification levels 3 through 5, and a MDI < 70 on the BSID- II. Complete outcome data were available for 91.6% of participants. Epo treated infants had significantly lower rates for death or moderate to severe disability (24.6% [18/73] infants versus 43.8% [35/80]) in control infants; $p = 0.017$). Subgroup analyses revealed benefit from Epo treatment only in babies with moderate NE. No differences in primary outcomes or side effect profiles were noted for the two studied Epo doses [48].

A pilot study by Elmahdy et al. examined the safety and efficacy of