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Neuroprotective Effects of Erythropoiesis Stimulating Agents in Term and Preterm Neonates

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Abstract

Purpose of Review—The use of erythropoiesis stimulating agents (ESAs) such as erythropoietin (Epo) and Darbepoetin (Darbe) in preterm and term infants has been studied for over 20 years. Recent investigations have explored potential neuroprotective effects of ESAs. We review recent clinical trials and experimental animal models that provide evidence in support of using ESA to improve neurodevelopmental outcomes in term and preterm infants.

Recent Findings—Continued work using animal models have confirmed neuroprotective properties of ESAs, including promotion of oligodendrocyte development in the face of neuronal injury. Clinical studies in term and preterm infants have reported neuroprotective effects following ESA administration, and improved neurodevelopmental outcomes have been reported in studies of preterm infants.

Summary—ESAs show great promise in preventing and treating brain injury in term and preterm infants.

Keywords

Neuroprotection; Neurodevelopment; Oligodendrogenesis; Erythropoiesis Stimulating Agents

Introduction

Prematurity is a significant risk factor for neurodevelopmental impairment. Associated long-term neurodevelopmental deficits, such as mental retardation, cerebral palsy, and learning disabilities are common morbidities in former premature infants [1]. While significant progress has been made in improving survival of ELBW infants, improved neurodevelopmental outcomes have not been attained. Specific targeted neuroprotective strategies are necessary and erythropoietin (Epo) is a promising neuroprotective agent, supported by both animal and human studies. This review outlines basic science data that supports Epo as a neuroprotective agent, with a specific focus on its role in preserving white matter development. Clinical studies that demonstrate efficacy in both premature infants, and in term infants with a history of hypoxic ischemic encephalopathy will be reviewed.

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Potential Neuroprotective Mechanisms of Epo

Multiple animal studies have demonstrated significant neuroprotective effects of Epo administration, both in term and preterm models. While Epo likely mediates many processes important to normal brain development, its effects on oligodendrogenesis and white matter formation are of specific interest because of premature birth associated white matter injury [2]. The Epo receptor (EpoR) is expressed throughout all stages of oligodendrocyte development; however receptor expression is significantly increased following injury [3–6]. As such, Epo is accepted as an important regulator of normal oligodendrocyte differentiation and development, and is integral to white matter neuroprotection in the preterm infant. Although the exact cellular mechanisms underlying the neuroprotective effects of Epo are not completely delineated, many studies have identified potential mechanisms of action, including improved oligodendrocyte maturation leading to white matter preservation, decreased apoptosis, anti-inflammatory effects, and anti-oxidant/anti-excitatory properties.

In the brain, Epo synthesis is regulated by several mechanisms, including hypoxia-induced activation of hypoxia-inducible factor 1 (HIF-1), insulin, hypoglycemia, and excitatory neuronal activity [7–9]. In addition, EpoR expression is upregulated in neurons by pro-inflammatory cytokines in response to tissue injury [10]. Signaling via the EpoR pathway inhibits apoptosis, minimizes excitatory damage through downregulation of glutamate release, and reduces production of pro-inflammatory cytokines [11–14]. Epo itself may be anti-inflammatory, though some speculate that inflammation is decreased secondary to an Epo-mediated reduction in apoptosis [15, 16]. In addition, Epo restricts vascular permeability across the blood brain barrier by activating EpoR dependent intracellular signaling pathways [17]. While prior studies have focused on the effects of Epo on neurons and astrocytes, more recent studies have investigated the specific neuroprotective effects on developing oligodendrocytes.

Oligodendrocyte differentiation and survival

Oligodendrocytes are generated in waves during the perinatal period and their development is tightly regulated by cell-intrinsic processes as well as the extracellular environment [18]. Any disruption in this environment renders the developing oligodendrocyte vulnerable to potential maldevelopment and thus compromises subsequent white matter formation [19, 20]. Oligodendrocyte progenitor cells (OPCs) express EpoR, and receptor expression is upregulated following perinatal brain injury [6]. Signaling via EpoR is an important trophic factor during embryonic and fetal brain development, but is not structurally critical. [4, 21–23]. In addition, multiple *in vitro* studies reveal that in times of stress; neural cells overexpress EpoR, and in the absence of Epo ligand, these cells subsequently undergo apoptosis [24, 25]. Such studies highlight an important neuroprotective role for Epo following injury and during early postnatal neurodevelopment; and have led to significant interest in how Epo may impart oligodendrocyte specific neuroprotection. In support of this, several recent animal models of perinatal brain injury have demonstrated improved myelin basic protein expression after systemic Epo administration [26–31].

Postnatal oligodendrogenesis

The subventricular zone is an accepted primary source of postnatal neurogenesis and is also an important source of astrocytes and oligodendrocytes during early neurodevelopment [32]. A recent study of neonatal stroke in the rat model demonstrated that exogenous Epo administration improved oligodendrogenesis and resulted in less apoptosis in injured striatum following injury. Mechanisms underlying these changes included increased progenitor proliferation, decreased cell death, and a cellular fate switch [33].

Anti-apoptotic effects on developing white matter

The anti-apoptotic effects of Epo are well described in erythropoietic cells and are likely similar in neural cells. Multiple pathways have been evaluated in the CNS and evidence of specific anti-apoptotic mechanisms vary according to CNS cell type. Epo is a known regulator of the Bcl-2 family of anti-apoptotic genes and shifts the Bcl-2/Bax ratio toward cell survival in microglia [34, 35]. Another potential anti-apoptotic effect of Epo in microglia is via mediation of poly (ADP-ribose) polymerase inhibitors (PARP-1) [29]. PARP-1 functions in DNA replication, transcription, and repair; and is activated by DNA damage [36, 37]. While PARP-1 may promote cell repair, its over-activation contributes to cell death. Over-activation of PARP-1 mediates microglial activation in CNS injury and can be inhibited by Epo administration, thus decreasing microglial induced neonatal WMI injury [29]. Alternatively, effects may occur in developing oligodendrocytes, thus supporting oligodendrocyte maturation. In a perinatal white matter injury model of embryonically-induced, transient systemic hypoxia-ischemia, rat pups treated with Epo demonstrated a significant reduction in cleaved caspase-3 expression at postnatal days 5 and 9 in the white matter [6], demonstrating a protective effect of oligodendrocytes themselves.

Oligodendrocyte development and maturation

In cell culture studies examining the effects of Epo administration on embryonic rat oligodendrocytes, Epo increased myelin basic protein expression and promoted oligodendrocyte process formation. These findings suggest that Epo mediates oligodendrocyte maturation during early postnatal development [38].

Similar mechanisms were recently demonstrated in an *in vitro* model of prenatal transient systemic hypoxia-ischemia. In this study, cells exposed to hypoxia-ischemic injury and glutamate excitotoxicity demonstrated a marked benefit from Epo administration [26]. This finding further delineates the potential importance of exogenous Epo administration to extremely premature infants, who not only have underdeveloped white matter, but who are also exposed to multiple insults during a critical period of neurodevelopment.

Clinical Studies

Both retrospective studies and prospective randomized trials have been performed in neonatal populations at greatest risk for long term developmental abnormalities, namely extremely low birth weight infants, and term infants with hypoxic ischemic encephalopathy.

Preterm ESA studies

A number of studies have been published supporting improved neurodevelopmental outcomes of preterm infants receiving ESAs. Neubauer and colleagues evaluated 148 former preterm infants at 10–13 years of age who were randomized to Epo or placebo/control in various multicenter Epo studies [39]. The Epo group scored significantly better than untreated children in an overall developmental assessment (55% vs 39% normally developed, $p < 0.05$) as well as in cognitive measures (mean composite HAWIK-III IQ score, 90.8 vs 81.3, $p < 0.005$). Differences were ascribable to children with IVH: infants with IVH treated with Epo scored significantly better than untreated children (52% vs 6% normally developed, composite HAWIK-III IQ score, 90.3 vs 67.0). Treated and untreated children without IVH did not differ in their outcome. Based on these data Epo appeared to be a promising treatment for ELBW infants, especially in those infants with brain injury due to intraventricular hemorrhage.

As a follow up to the high dose Epo study in ELBW infants performed by Juul and colleagues [40], investigators collected developmental outcome data on available subjects at 12–36 months corrected gestation [41]. The 30 infants in the original Epo treated cohort received daily doses of Epo at 500, 1000 or 2,500 units/kg for a total of 3 doses. Birth characteristics and growth from 4 to 36 months were similar for untreated and Epo-treated patients. Infants were evaluated with either Bayley Scales of Infant Development (BSID) II or BSID III. Analysis of neurodevelopmental follow-up scores from 17 of 25 Epo-treated and 18 of 26 control infants showed that administration of Epo correlated with improvement of cognitive and motor scores. No negative long-term effects of Epo treatment were evident.

We recently completed follow up of infants previously enrolled in a randomized, masked placebo controlled trial of ESAs administered to VLBW infants (NCT00334737; [42, 43]). Of the 99 infants evaluated during the hospital phase of the study, 80 (29 Epo, 27 Darbepoetin (Darbe, 24 placebo) returned for follow-up (Table 1). After adjustment for gender, analysis of covariance revealed significantly higher cognitive scores in Darbe (96.2 ± 7.3 ; mean $\pm$ SD) and Epo recipients (97.9 ± 14.3) compared to placebo recipients (88.7 ± 13.5 ; $p = 0.01$ vs ESA recipients). Darbe and Epo recipients also scored significantly higher on a test for object permanence ($p = 0.05$). No infants in the ESA groups had CP, compared with 5 in the placebo group ($p < 0.001$). No differences among groups were found in visual or hearing impairment, and overall neurodevelopmental impairment was significantly lower in the ESA groups compared to placebo (odds ratio 0.18; 95% confidence interval 0.05 to 0.63). This is the first study to report improved developmental outcomes in preterm infants receiving Darbe. If confirmed by a larger trial, Darbe might be more advantageous given its weekly dosing schedule.

Similar to previous large randomized trials, we saw no differences in the incidence of any stage of retinopathy of prematurity during the hospital phase, and no differences in visual impairment at 18–22 months corrected age. Meta-analyses first published in 2006 suggested an association between early (first week of life) Epo administration and retinopathy of prematurity (ROP) > stage 2 [44] compared with late Epo administration [45]. Revised meta-analyses corrected a previous misclassification of a single center Epo study into the late Epo

administration group. Close ophthalmologic evaluation for ROP continues to be a priority in clinical trials in preterm infants. A high dose Epo study is in progress in Switzerland (NCT00413946). Leuchter and colleagues randomized 420 infants during the first three hours of life to receive EPO (3,000 U/kg body weight) or placebo intravenously at 3, 12–18 and 36–42 hours after birth [46]. Enrollment is complete, and developmental follow up is in progress. There were no differences in IVH or ROP in a preliminary report of the first 100 randomized infants.

ESA studies in Term Infants

Two studies evaluating Epo for neuroprotection in term infants with HIE have been published since the previous review [13]. The first, by Elmahdy and colleagues from Egypt [47], examined biochemical and neurophysiologic parameters associated with administration Epo, 2,500 units/kg SC for 5 days (NCT00945789). 30 infants with mild to moderate HIE were randomized to Epo or control. An additional 15 term infants were included as healthy controls. Serum nitric oxide was measured at enrollment for all 45 infants and after 2 weeks for the HIE infants. EEGs were performed at enrollment and at 2–3 weeks; MRIs were performed at 3 weeks, and neurologic evaluations were performed at 6 months.

Serum nitric oxide levels were elevated in the infants with HIE, compared to healthy controls. Infants with HIE who received Epo had improvement in EEG findings and NO levels. MRIs were not different between the HIE infants, but Epo recipients had fewer neurologic and developmental abnormalities at 6 months. The authors concluded that Epo administration to infants with HIE to protect against encephalopathy was feasible.

The key issue with this study is that none of the infants were cooled. Current standard of care in the United States is to provide hypothermia for infants with moderate to severe HIE. In an effort to evaluate the safety, efficacy and pharmacokinetics of Epo given in conjunction with hypothermia, Yvonne Wu and colleagues randomized 24 infants with moderate to severe HIE to an open label dose escalation trial of Epo (NEAT Trial [Neonatal Erythropoietin in Asphyxiated Term Newborns], NCT00719407; [48]). Infants received one of 4 doses of Epo (250, 500, 1,000, or 2,500 units/kg) administered IV every other day for up to 6 doses. Pharmacokinetics were performed with the first, second and last doses, and infants were monitored closely for any adverse effects.

Epo followed non-linear pharmacokinetics, but did not accumulate with multiple dosing. The area under the curve was 50,306, 131,054 and 328,002 unit*hour/L for 500, 1,000 and 2,500 units/kg, respectively. No serious adverse effects or deaths were reported. They concluded that 1,000 units/kg Epo administered intravenously in conjunction with hypothermia was well tolerated and produced plasma concentrations that in previous studies were neuroprotective in animals.

Based on the previous studies a number of multicenter clinical trials evaluating Epo in conjunction with hypothermia in term infants with HIE are being planned. Only one study to date has evaluated Darbepoetin as a potential neuroprotective agent in term infants undergoing hypothermia as treatment for HIE. The DANCE study (Darbepoetin administered to neonates undergoing cooling for encephalopathy; NCT01471015) lead by

Mariana Baserga [49] has enrolled 29 of a planned 30 infants with moderate to severe HIE. Infants were randomized to receive placebo, Darbe 2 µg/kg or Darbe 10 µg/kg IV at < 12 hours of life and on day 7. Preliminary pharmacokinetics for the 10 µg/kg group shows an area under the curve of 163.7 ng*hour/mL and a terminal half-life of 53.4 hours, approximately 3 times the half life of Epo reported in the NEAT Trial. No side effects attributable to Darbe were reported. If developmental outcomes of these infants are similar to infants in Epo trials, Darbe may be a better choice due to its longer half-life and fewer number of doses needed.

A number of adult studies have evaluated neuroprotective properties of ESAs. Although disease processes and patient characteristics differ significantly, a number of mechanisms of neuroprotection may be relevant to neonatal neuronal injury. Messé and colleagues evaluated the effects of Darbe on adults undergoing aortic surgery (NCT0064798; [50]). The study was terminated prematurely because of the FDA hold placed on ESA studies of neuroprotection following publication of increased mortality in the Epo recipients enrolled in the European multicenter study of adult stroke patients [51], therefore only 9 adults received 1 mg/kg Darbe immediately prior to surgery. An additional cohort of 9 untreated adult patients was obtained for comparison. There were no differences in the primary outcome of death or neurologic impairment between Darbe recipients (1/9, 11%) and controls (3/9, 33%, $p=0.58$), although the small number of patients enrolled precluded appropriate statistical analyses. Patients with perioperative neurologic ischemia had significantly higher concentrations of S100β and GFAP, CSF biomarkers measured in samples obtained from lumbar drains. There were no differences in these biomarkers between the Darbe and control groups (S100β: 214 versus 260 ng/mL, $p=0.69$; GFAP: 22 versus 580 pg/mL, $p=0.45$), although all point estimates favored Darbe treatment.

In another study impacted by the FDA hold, Andropoulos and colleagues completed a randomized trial of Epo administered to neonates undergoing complex cardiac surgery (NCT0013240; [52]). Infants with D-transposition, hypoplastic left heart syndrome or aortic arch reconstruction were randomized to 1 preoperative and 2 postoperative doses Epo or placebo. Initial dosing was 1,000 units/kg for the first 33 infants, which was decreased to 500 units/kg for the last 26 infants. Magnetic resonance imaging and developmental assessments were performed.

There were no differences between groups in MRI findings of brain injury, clinical events or death. Developmental assessment at 12 months revealed no statistical differences in cognitive or language outcomes between Epo and placebo groups, although the study was not powered to detect a difference. No adverse effects of Epo were reported. The authors concluded that a larger prospective trial was required to determine efficacy of Epo for neuroprotection in this population.

Conclusion

Clinical studies in preterm and term infants have shown ESAs to be safe, and evidence of neuroprotection is growing. Preterm infants administered ESAs during their NICU stay show higher cognitive scores and a lower incidence of neurodevelopmental impairment.

Studies of ESA administration in term infants with HIE being cooled for encephalopathy are ongoing. Developmental follow up of those term infants is progressing. ESAs hold great promise in providing neuroprotection and improving neurodevelopmental outcomes of preterm neonates. In addition, we are confident that ongoing research will generate a definitive body of evidence that ESAs are neuroprotective in term infants.

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Key Points

- Recent clinical studies demonstrate evidence for significantly improved neurodevelopmental outcomes in neonates treated with ESAs, following extreme prematurity and perinatal brain injury.
- Basic science studies demonstrate that ESAs prevent apoptosis of developing oligodendrocytes and concomitantly support oligodendrocyte survival and maturation following injury.
- ESAs appear to specifically support oligodendrogenesis.

Fig 1.

Fig 1, upper panel: Oligodendrocyte progenitor cells (OPCs)/immature oligodendrocytes. In response to injury, Epo receptor expression is increased. Epo bound receptor results in improved oligodendrocyte differentiation/maturation, survival, and increased process extension. In absence of ligand, immature oligodendrocytes undergo cell death. Figure 1, lower panel: Mechanisms of Epo neuroprotection: enhanced oligodendrocyte development and decreased apoptosis

Table 1

Patient Characteristics and Neurodevelopmental outcomes

	Darbe (n=27)	Epo (n=29)	Placebo (n=24)	p	Adjusted Odds Ratio	p
Birth Weight (grams)	938.3 ± 176.5	947.2 ± 212.7	953.0 ± 210.0	NS		
Gestation (weeks)	28.1 ± 1.8	27.8 ± 1.9	27.8 ± 1.6	NS		
Corrected age at follow up (months)	20.5 ± 1.1	21.2 ± 2.0	20.6 ± 1.9	NS		
Composite cognitive	96.2±7.5	97.9±14.0	88.7±13.3	0.01		
Composite language	92.4±12.8	89.9±17.4	83.6±13.9	0.05		
Composite social/emotional	103.5±19.8	99.6±18.0	96.7±18.4	0.38		
Object permanence	2.8±0.4	2.4±0.8	2.2±1.0	0.05		
Neurodevelopmental Impairment:	3 (11%)	2 (7%)	9 (38%)		0.18 (0.05 to 0.63)	0.01
Cerebral Palsy (%)	0 (0)	0 (0)	5 (21)	0.002	0 (0 to 0)	<0.001
Visual deficit (%)	1 (4)	0 (0)	1 (4)	0.09	0.23 (0.04 to 1.54)	NS
Hearing deficit (%)	0	1 (3)	1 (4)	0.33	0.54 (0.11 to 2.65)	NS
Composite Cognitive Score<85	0	3 (10.3%)	6 (25%)	0.02	0.18 (0.04 to 0.78)	0.01
Composite Cognitive Score <70	0	1 (3.4%)	2 (8.3%)	0.29		

Odds Ratios adjusted for gender. Values represent mean ± SD or number (percent). P values represent three way comparisons among groups. (Data summarized from references 38 and 39)