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Erythropoietin (Epo) for infants with hypoxic-ischemic encephalopathy (HIE)

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Abstract

Purpose of review—Perinatal asphyxia, intraventricular hemorrhage and stroke are common causes of neonatal brain injury, with hypoxia-ischemia as the final common pathway of injury. Erythropoietin (Epo) has potential to lessen neurologic sequelae due to hypoxia-ischemia. The purpose of this review is to highlight new clinical trials and experimental evidence that expand our understanding of Epo as a potential treatment for perinatal brain injury.

Recent findings—Several trials of Epo treatment are reviewed: Two phase I/II trials of high-dose Epo given to preterm infants established pharmacokinetic and safety profiles, and a trial of Epo treatment for term infants with moderate hypoxic-ischemic encephalopathy found reduced disability. Potential risks and benefits of high-dose Epo are discussed. New evidence related to Epo receptor expression, signal transduction pathways, and mechanisms of neuroprotection are reviewed.

Summary—Cautious optimism is warranted regarding the use of high-dose Epo as a treatment option for neonatal brain injury. To date, Epo has been safe to use in neonatal populations and now studies of neuroprotective efficacy are underway.

Keywords

Neuroprotection; growth factors; newborn

Introduction

Medical advances in neonatology have significantly improved survival statistics, particularly for extremely preterm infants. Similar progress has not been made to improve neurodevelopmental outcomes for brain-injured neonates. Clinical trials of hypothermia have demonstrated benefit for term neonates with mild and moderate brain injury, but none when hypoxia-ischemia is severe or prolonged [1,2,3]. Hypothermia is also contraindicated for preterm infants, leaving this group with no proven therapeutic options when hypoxia-ischemia occurs. A neuroprotective pharmaceutical treatment to minimize neonatal brain injury is greatly needed. The optimal therapy will be safe for use in both preterm and term neonates, and effective when administered after an insult. Animal studies support the efficacy and safety of erythropoietin (Epo) as a therapeutic intervention for a variety of brain insults [4*], and Epo is now in the clinical testing phase. We highlight recent clinical trials and experimental reports that further consideration of Epo as a therapy for neonatal hypoxic-ischemic injury.

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Epo Trials in Preterm Infants

Both preventative treatment and rescue therapy are possible applications of Epo. For example, all extremely low birth weight (< 1000 g) infants could be treated prophylactically because they are at high risk for poor outcome. In contrast, rescue treatment would occur only after a neurologically deleterious event. The advantage of rescue therapy is that unnecessary exposure is eliminated, but the disadvantage is that treatment may be delayed. With either approach, Epo treatment must be safe.

To evaluate safety, we reviewed the use of Epo as an erythropoietic treatment in prospective randomized trials. Between 1991 and 2006, over 2400 infants were enrolled in 30 randomized controlled trials to evaluate the safety and efficacy of Epo for the prevention or treatment of anemia of prematurity. Treatment regimens ranged from 70 to 5,000 U/kg/week (35 to 750 U/kg/dose), with duration of therapy ranging from 2 weeks to several months [5,6]. None of these studies reported increased risk for stroke, hemorrhage, clotting, or death. At the outset, erythropoietic Epo dosing for neonates was extrapolated from adults. But that dosing was found to be too low for neonates who have a higher volume of distribution and more rapid clearance than adults. Subsequent trials in preterm infants established the safety, pharmacokinetics and efficacy of higher doses [7,8,9]. In contrast, animal Epo neuroprotection studies have generally used high doses (1,000 – 5,000 U/kg) to ensure penetration of the blood-brain barrier (BBB) [10]. The safety of high-dose Epo was recently confirmed in rats [11].

Two single-center phase I/II prospective trials examining the safety and efficacy of high-dose Epo for preterm infants have been published [12**,13**]. Table 1 compares key design details from these trials. In the study by Fauchère *et al.* [12**], newborns born 24 – 32 weeks of gestation and < 1500 g were given Epo (3000 U/kg x 3 i.v. doses, n = 30) or placebo (n = 15). The primary outcome was survival without intraventricular hemorrhage (IVH), periventricular leukomalacia (PVL), and retinopathy of prematurity (ROP). Care was withdrawn from 5 of 30 Epo-treated infants due to severe IVH (n = 3, one case diagnosed at enrollment) or severe respiratory failure (n = 2). All of these deaths occurred in infants < 26 weeks of gestation. Complication risks were not different for ROP, IVH, sepsis, necrotizing enterocolitis (NEC), and lung disease. A phase III randomized controlled study is ongoing in Switzerland.

The phase I/II study by Juul *et al.* [13**] evaluated the safety and pharmacokinetics of Epo given to newborns born 23 – 28 weeks of gestation and < 1000 g. Three Epo doses were tested (500, 1000, and 2500 U/kg, x 3 i.v. doses, n = 10/dose). There were trends towards less IVH ($p = 0.07$), less severe IVH or PVL ($p = 0.06$), and fewer infants were diagnosed with stage III NEC ($p = 0.018$). There were no Epo-related complications. Plasma Epo concentrations 30 min after injection were 5,973, 12,291 and 34,197 mU/mL after 500, 1000, and 2500 U/kg, respectively. Figure 1 compares rat [14] and human [13**] plasma Epo concentrations following a single Epo injection. The circulating concentrations of Epo in rats given 5,000 U/kg Epo (a neuroprotective dose) is comparable to that for infants given 500 or 1000 U/kg, suggesting that 500 – 1000 U/kg is a suitable dosing range. Note that plasma concentrations fall more rapidly after i.v. dosing, so more frequent dosing might be required.

While there are design differences between these two trials, there is considerable agreement regarding the short-term safety of high-dose Epo. Between the two studies, 60 preterm newborns received high-dose Epo and there were no complications, and no harmful effects on blood pressure or hematological indices. In addition, measures of liver and renal function were unaffected. These trials complement one another by documenting the short-term safety of three injections of high-dose Epo therapy for preterm infants.

Early Epo may improve cognitive function. He *et al* [15] evaluated the effect of repeated early Epo on the neurobehavioral development of preterm infants. Preterm infants given Epo 250

U/kg i.v. three times/week for 4 weeks (n = 22) were compared to 22 untreated infants. Neurologic assessments were done at term corrected gestational age (CGA), and at 6 and 12 months. At term, the neonatal behavioral neurological assessment was improved in Epo-treated infants. At 6 and 12 months, the Gesell development schedule showed benefits in gross motor, fine motor and language skills ($P<0.05$).

Two retrospective reports also suggest that early Epo treatment can improve neurodevelopmental outcome. In the first study, designed to test the effects of Epo on erythropoiesis and transfusion needs in preterm infants born ≤ 1250 g, subjects received Epo from day 4 of life until 35 weeks CGA, but there was no effect of Epo on neurodevelopmental outcome when all infants were considered [16]. In a follow-up analysis considering only infants born < 1000 g (n = 12), mental developmental index (MDI) scores were higher at 18 to 22 months for infants with peak serum Epo concentrations > 500 mU/mL [17]. More recently, a retrospective analysis of data from infants born < 1500 g (n = 82) treated with 250 – 400 U/kg/dose Epo 3 times/week for 6 weeks to prevent anemia of prematurity, found that MDI scores at median age 25 months correlated with cumulative Epo exposure [18**]. Although differences in early cognitive function due to Epo exposure are small, they are detectable. Collectively, these data are very encouraging because they indicate that even lower doses of repeated Epo can be beneficial.

Epo Trials in Term Infants

The first trial of Epo therapy for neuroprotection in term infants born > 37 weeks with moderate to severe hypoxic-ischemic encephalopathy (HIE) has now been completed [19**]. Zhu *et al*. randomized eligible babies to either Epo (n=83) or conventional (n=84) treatment. Epo-treated babies received either 300 U/kg (n=52) or 500 U/kg (n=31), every other day for 2 weeks, with the first dose administered by 48 hours of life. Epo treatment improved neurologic signs at 7, 14, and 28 days as assessed by Thompson Neurologic Assessment, reduced disability for moderate HIE, decreased the overall number of MDI scores below 70, and reduced the incidence of cerebral palsy at 18 months of age. Death or disability at 18 months was present in 43.8% of controls compared to 24.6% of Epo-treated subjects ($p < 0.02$). There were no discernable differences in outcomes based on Epo doses, and no adverse effects of Epo were identified. Consistent with trials of hypothermia for HIE, Epo was only effective for infants with moderate injury, and did not improve outcome for severely-affected infants. Considering that both hypothermia and Epo are more effective when given proximal to injury, it is possible that severe HIE cases are indicative of an early insult with protracted prenatal injury and this highlights the drawback of rescue therapy.

The observation that low doses of Epo were neuroprotective in the Zhu trial warrants further discussion. Epo concentrations were measured in a subset of patients given 500 U/kg Epo and cerebrospinal fluid (CSF) Epo concentration increased within 3 h, and in parallel with circulating Epo concentrations which were only 14 – 44 mU/mL. The elevated CSF Epo concentration indicates that Epo readily penetrated the BBB. We speculate that hypoxia-ischemia triggers a breach in the BBB that permits greater penetration of Epo, and other factors, into brain tissues like CSF and presumably neuronal structures as well. Under these conditions, perhaps Epo neuroprotection includes systemic effects such as enhanced erythropoiesis which increases iron utilization, thereby decreasing free iron and reducing oxidative brain injury [20]. In that context, we note that Epo recently decreased the systemic inflammatory response in preterm and term infants [21*]. We speculate that systemic effects of Epo such as stabilizing oxygen availability, decreasing free iron, and reducing inflammation, complement the direct neuroprotective effects of Epo and may explain why lower dosing strategies also improve outcome. Figure 2 summarizes known mechanisms that contribute to Epo neuroprotection. The net effect of the acute actions of Epo work to decrease apoptosis. Epo also improves long term

brain healing after an insult by providing increased oxygen carrying capacity through erythropoiesis and angiogenesis, and also by increasing neurogenesis.

Combination Therapies

Epo has demonstrated neuroprotection in many models of brain injury, but protection has been incomplete. This has led investigators to consider combinations of protective therapies that also deserve discussion.

Hypothermia with Epo

Multiple clinical trials examining safety and efficacy of mild hypothermia (32 – 34°C) have now been published [1,2,3,22]. Despite differences in approach (head cooling *vs*. total body cooling), there is general agreement that hypothermia improves outcomes for moderately (but not severely) asphyxiated infants, decreasing the combined outcome of death and neurologic dysfunction at 18 – 24 months from approximately 60 to 45%. Combination therapies such as Epo plus hypothermia are being considered to further improve outcomes. It will be important to thoroughly evaluate the safety of combined therapies because unanticipated complications may arise. It will also be important to consider the combined effects of hypoxia-ischemia, hypothermia, and Epo on clotting function. Hypoxia-ischemia increases the risk for disseminated intravascular coagulation. Hypothermia disrupts hemostasis in a dose-dependent manner with clotting disorders present even when hypothermia is mild [23], possibly due to decreased fibrinogen availability and delayed thrombin production [24]. Epo treatment may also influence clotting function because adults exhibit cardiovascular accidents, catheter thrombosis, and clot formation coincident with Epo. No data are available as to how these forces interact. Even though no Epo-induced clotting complications have been reported in neonates, it is important to remain vigilant as clinical studies proceed.

Insulin like Growth Factor-1 (IGF-1) with Epo

IGF-1 is another possible candidate for use with Epo because it is neurotrophic and neuroprotective [25,26*,27]. Synergistic effects of IGF-1, mediated by activation of phosphoinositol kinase (PI3-K) have been reported when Epo and IGF-1 treatment are combined in cell culture [28]. IGF-1 augmented Epo neuroprotection and reduced the threshold dose of Epo. Epo and IGF-1 co-treatment also prolonged the therapeutic window so that treatment initiated 9 h after injury was still effective. Preliminary studies are testing the possible intranasal delivery of these combined treatments in rodents [29].

Other Applications of Epo

Research into the neuroprotective properties of Epo is not limited to newborn hypoxia-ischemia. In addition to its neuroprotective effects, Epo stimulates angiogenesis and neurogenesis [30*,31*]. These properties make Epo a candidate therapy for many disorders in children and adults. For example, Epo has been recently evaluated as a treatment for traumatic brain injury [32,33*,34,35], Parkinson's disease [36], and depression [37,38].

Mechanisms of Epo Neuroprotection

Epo receptor (EpoR) activation can trigger different signaling pathways. The conventional understanding is that Epo prevents neuronal apoptosis via Janus kinase/Stat5 activation and NFκB phosphorylation [39]. However, Epo neuroprotection also involves PI3K and protein kinase B (Akt) signaling. Using Stat5 null mutation, Byts *et al*. found that activation of PI3K/Akt, but *not* Stat5, was essential for Epo-induced protection against excitotoxicity, while Stat5 and Akt were required for neurotrophic effects of Epo [40**]. Similarly, Epo prevented

excitotoxicity in neuronal cultures [41] via a PI3K/Akt-dependent mechanism. Data are beginning to associate specific effects of Epo with specific signaling pathways.

A brain-specific heterodimer composed of EpoR with common beta chain (βc) receptor was proposed as the specific receptor mediating Epo neuroprotection [42]. New data weakens this hypothesis because expression of the βc receptor does not correspond with either Epo or EpoR expression in brain [43*]. Sanchez *et al.* have definitively evaluated Epo, EpoR, and βc receptor RNA expression and receptor immunolabeling in postnatal and aged rat brain, and also examined neuronal precursor PC12 cells, and their findings refine the localization of EpoR and identify specific patterns of regulation during development.

Epo is reported to stimulate vascular endothelial growth factor secretion and angiogenesis via PI3K/Akt and extracellular-signal-regulated kinases (ERK, a.k.a. MAPK) signaling pathways [31*]. Other Epo effects are now thought to be mediated through Epo stimulation of brain-derived neurotrophic factor (BDNF) [44,45]. Epo-mediated upregulation of BNDF occurs in hippocampus [46] after experimental autoimmune encephalomyelitis [47], and after spinal ischemia [48]. Electroconvulsive shock applied to rats stimulated both Epo and BDNF expression, suggesting that antidepressive effects of electroconvulsive therapy may involve Epo and BDNF [49].

Schelshorn *et al.* found that neurons specifically express hemoglobin in response to either hypoxia or Epo, and that neuronal hemoglobin expression is neuroprotective [50**]. This is interesting because local hemoglobin expression could ensure neuronal oxygen availability in the event of hypoxia-ischemia.

Risks of Epo

Complications seen in adults (e.g. hypertension, clotting, seizures, polycythemia, and death) have not been identified in infants. Preterm infants have a long history of Epo treatment, with few reported side effects. Neutropenia was initially thought to be a complication of Epo treatment unique to preterm infants [51,52], but further experience has shown this not to be the case when erythropoietic doses are used [53,54]. If higher doses are used for neuroprotection, this will need to be watched. In the 2 pilot studies published to date, this was not a noted complication [12**,13**]. A concern unique to the preterm population remains whether Epo might increase the risk or severity of ROP. Since a prospective randomized human trial to study this is unlikely, we turn to animal models to help us answer this question. We recently reported that early high-dose Epo (5,000 U/kg/x3) did not exacerbate (or reduce) ROP in a neonatal rat model [55**]. Using a mouse model, Chen *et al.* suggested that Epo effects on ROP may depend on timing because late Epo exposure exacerbated, but early Epo exposure reduced experimental ROP [56**].

Conclusion

Consideration of Epo as a potential therapeutic agent for brain injury has advanced to a new phase. Clinical studies are ongoing to test the safety and efficacy of Epo in patient populations that span from newborns to adults. Neither term nor preterm infants have exhibited complications after Epo. Nevertheless, it is important to proceed cautiously with clinical trials because risks may vary among specific populations, ages, and disease states. We are cautiously optimistic regarding the use of repeated early high-dose Epo as a neurotherapeutic treatment for neonatal brain injury.

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* of special interest

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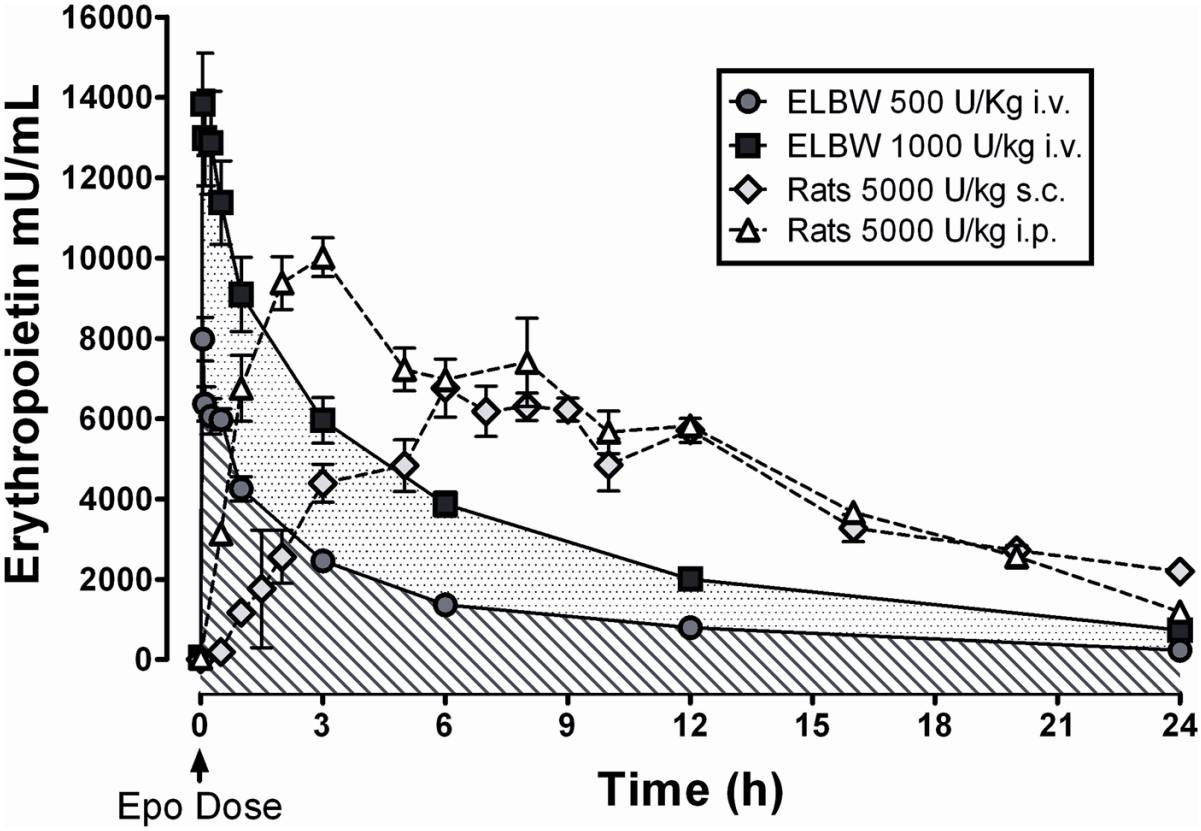

Figure 1.
A comparison of human and rat blood Epo concentrations. Data from [13] and [14]

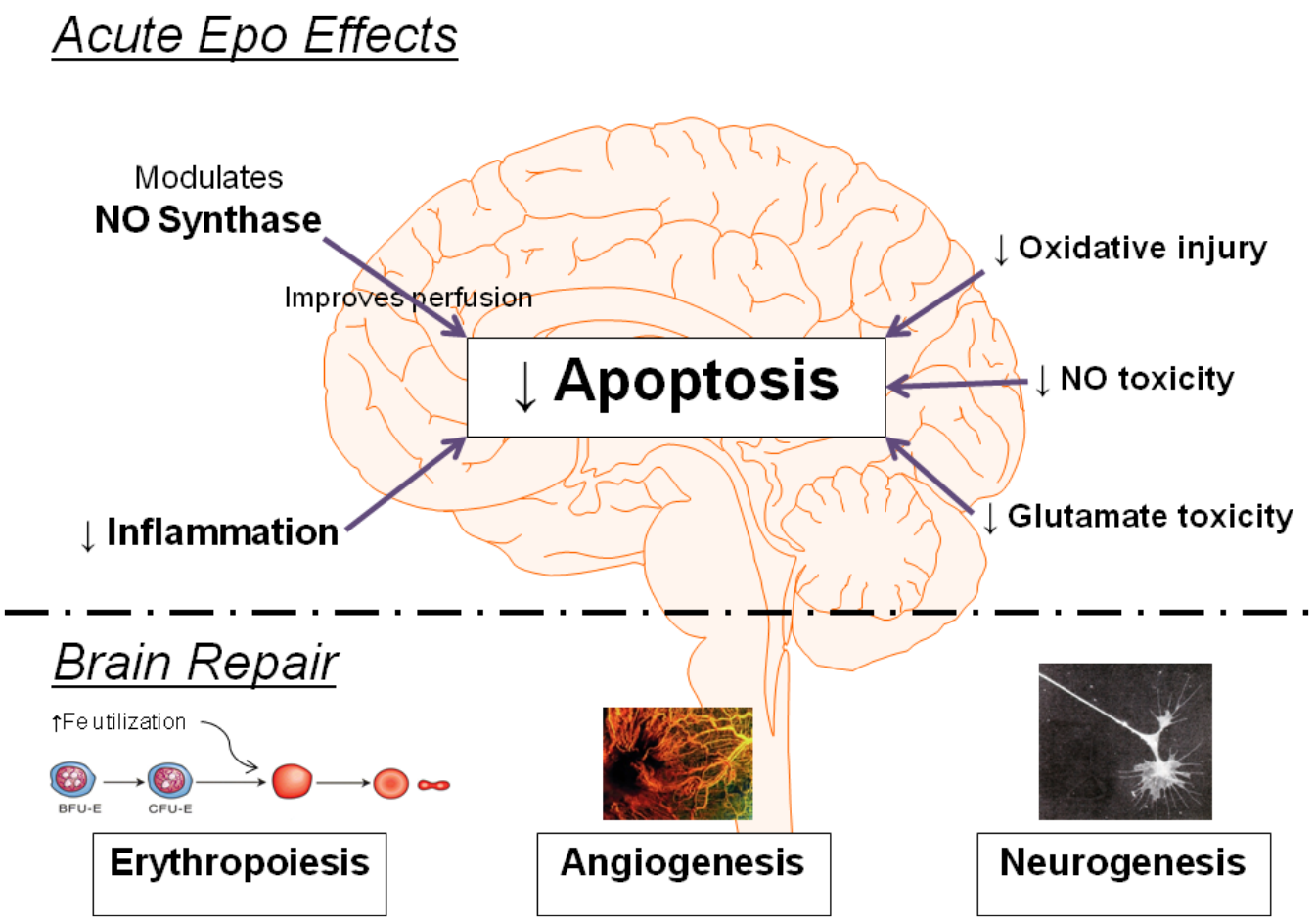

Figure 2.
Mechanisms of Epo neuroprotection.