

## HYPOTHESIS

# Does erythropoietin protect the preterm brain?

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There is a high incidence of hypoxic-ischaemic brain injury and intraventricular haemorrhage in newborn infants, particularly those born preterm. Many die during the newborn period or suffer permanent neurodevelopmental handicaps. Hypoxic brain injury develops over several hours and could potentially be influenced by intervention. At present, no drug exists that effectively prevents infant brain injury or ameliorates detrimental neurodevelopmental effects. The hypothesis is put forward that systemic administration of recombinant human erythropoietin positively affects the neurodevelopmental outcome of high risk preterm infants affected by brain injury. A multicentre, randomised, placebo controlled study is proposed to prospectively test this hypothesis.

The biological role of erythropoietin (Epo), formerly known only as a kidney derived haemopoietic growth factor, has expanded beyond haemopoiesis<sup>10–11</sup> since the observation that Epo and its receptor (EpoR) are expressed early in fetal development in human brain. Furthermore, its expression in cultured neurones and astrocytes<sup>12</sup> as well as functional effects on neuronal cells have been shown.<sup>13–15</sup> Epo exerts its effects through its receptor (EpoR), and binding of Epo to its receptor ultimately results in the activation of several downstream signals.<sup>10–11</sup> The essential importance of these pathways was shown by their targeted inhibition, which largely negated the protective effects of Epo against hypoxia induced brain injury. Increased concentrations of Epo in cord blood and amniotic fluid were identified as markers of fetal hypoxia, indicating intact Epo synthesis in the fetus.<sup>16–17</sup> Juul *et al*<sup>13</sup> reported raised concentrations of Epo in the cerebrospinal fluid of infants suffering from ischaemic brain injury. Interestingly, Epo concentrations were not raised in children with meningitis; a possible explanation may be that Epo production was inhibited by proinflammatory cytokines as reported in patients with inflammatory diseases.<sup>10–13</sup> Significant upregulation of Epo and EpoR was observed in the human hypoxic-ischaemic brain, underlining the potential of Epo/EpoR as endogenous neuroprotective factors.<sup>15</sup> Data from different models of neuronal injury suggest that Epo mediates its effects by a combination of mechanisms: (a) promotion of cell survival signalling cascades, thus reducing apoptosis<sup>18–20</sup>; (b) diminution of intracellular calcium; (c) stimulation of NO production<sup>21–22</sup>; (d) antioxidative,<sup>23–25</sup> (e) anti-inflammatory,<sup>19–26</sup> and (f) angiogenic actions.<sup>27–28</sup> Although the precise roles of the different mechanisms involved are yet to be defined, a number of promising investigations suggest that Epo administration indeed translates into neuroprotection *in vitro* as well as *in vivo*.

Despite the recent advances in neonatal intensive care, 2% of full term newborns are still affected by hypoxic-ischaemic brain injury or intraventricular haemorrhage, with a considerably higher incidence in preterm infants. Between 20% and 50% of infants affected by brain injury die during the newborn period, and 25–60% of the survivors suffer from permanent neurodevelopmental handicaps, including cerebral palsy, seizures, mental retardation, and learning disabilities.<sup>1–3</sup> The different mechanisms involved in the acute phase of hypoxic injury of the immature brain are induced by lack of oxygen, resulting in multiple events that ultimately lead to neuronal cell death. In the reperfusion phase, neuronal cell damage is mediated by the enhanced production of proinflammatory cytokines,<sup>4–7</sup> oxygen radicals, and nitric oxide (NO), and an imbalance between excitatory and inhibitory neurotransmitter systems. The net effect of this is often the stimulation of apoptosis and cell death.<sup>8</sup> As these processes develop over several hours, they can potentially be influenced by intervention even if this is instituted after the initial insult has occurred. However, intervention should occur as early as possible, and preventive action is clearly even more desirable. Currently no drug exists that effectively prevents infant brain injury or ameliorates any detrimental neurodevelopmental effects in affected infants.<sup>9</sup>

Epo crosses the blood/brain barrier after systemic administration and mediates noteworthy neuroprotective effects in animals with experimental brain injury induced by ischaemia, mechanical trauma, inflammation, and kainate toxicity (fig 1). In detail, Epo protects gerbils from ischaemia induced brain damage, and conversely neurological outcome is worsened by the application of soluble EpoR.<sup>29</sup> A reduction in infarct size in mice treated with Epo 24 hours before experimental infarction was observed.<sup>30</sup> In addition, reduction in the inflammatory infiltrate

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**Abbreviations:** Epo, erythropoietin; EpoR, erythropoietin receptor

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and diminished production of proinflammatory cytokines were reported in rats with cerebral ischaemia.<sup>31</sup> Neuroprotective properties of Epo are associated with the preservation of learning abilities after transient brain ischaemia in gerbils.<sup>32</sup> The protective effects were detected when Epo was administered before or up to six hours after the injury.<sup>26</sup> Other groups described a duration of the protective effects of at least three days even without the continued presence of Epo.<sup>19–20</sup> In experimental subarachnoid haemorrhage, Epo normalises cerebral blood flow,<sup>33–34</sup> resulting in a significant decrease in necrotic neurones after systemic application.<sup>35</sup> Rats with experimental ischaemic spinal cord injury or mechanical spinal cord trauma had a significantly better functional neurological outcome when treated with Epo. These effects were mainly attributed to the prevention of motor neurone and oligodendrocyte apoptosis, preservation of the white matter tracts, and greatly diminished secondary inflammation.<sup>36–37</sup>

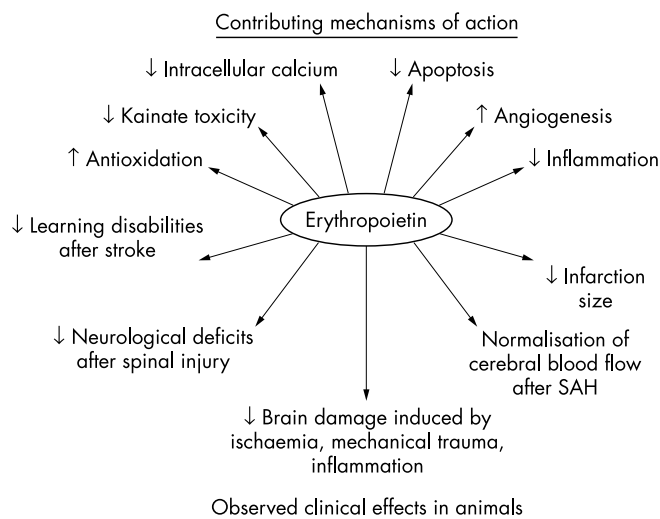
“In preterm infants, side effects of Epo administration were minimal compared with those in adults.”

Over the past 15 years, Epo has been used successfully in a large number of adult patients with a variety of diseases and shown to have a good safety and tolerability profile. In the first clinical trial assessing the safety and efficacy of Epo for the treatment of acute stroke, a reduction in infarct size and a beneficial effect on the functional neurological outcome was reported without noteworthy side effects.<sup>38</sup> In preterm infants, side effects of Epo administration were minimal compared with those in adults. Severe side effects were not reported in a number of clinical studies analysing the efficacy of Epo for the treatment of anaemia of prematurity.<sup>39–45</sup> In those studies that assessed the incidence of typical diseases of preterm neonates (intraventricular haemorrhage, periventricular leucomalacia, necrotising enterocolitis, retinopathy of prematurity, chronic lung disease, or late onset sepsis), a significant difference was only detected for necrotising enterocolitis, which was less common in preterm infants who received Epo.<sup>46</sup> However, conclusions on the neuroprotective effect of Epo cannot be drawn from these studies because they were not primarily designed to test this hypothesis. Importantly, these studies excluded severely ill patients, and, in addition, treatment was started at least 72 hours after

birth, with doses considerably smaller (100–500 U/kg) than those used in experimental studies (350–5000 U/kg). Whether Epo effectively crosses the blood/brain barrier after systemic administration is debatable. Juul *et al*<sup>47</sup> did not detect raised Epo concentrations in cerebrospinal fluid in neonates treated with Epo for prevention of anaemia of prematurity. Conversely, other groups reported that Epo effectively crosses the blood/brain barrier after systemic administration.<sup>26–37–48</sup> In summary, Epo dosage and the competence of the blood/brain barrier are the main factors that influence the crossing of Epo into the cerebrospinal fluid.

The application of Epo in high risk preterm infants raises some safety concerns. As expression of Epo and EpoR is detected very early in fetal brain, it is conceivable that Epo administration to preterm infants may also have detrimental effects on neurodevelopment.<sup>7–13–14</sup> The observed induction of proliferation of neuronal stem cells may also have a negative impact, as this may be at the expense of multipotent progenitor cells, the ultimate function of which we do not know.<sup>49</sup> In addition, Epo influences apoptosis, which may be an integral component of normal brain development, and there are no experimental or animal studies on this. Some retrospective studies have compared the neurodevelopmental outcome of children from studies evaluating Epo treatment for anaemia of prematurity with the control group and did not detect a deleterious effect.<sup>50–52</sup> Nevertheless, safety studies primarily designed to assess this concern are mandatory. An increased risk of thromboembolic events was observed in adult patients with polycythaemia.<sup>53–54</sup> However, these complications were not observed in clinical trials of the use of Epo in infants.<sup>39–45</sup> Furthermore, recent experimental data from transgenic mice overexpressing Epo indicate that thromboembolic disease may even be prevented by Epo induced erythrocytosis.<sup>55</sup> Another important issue is the long term effect on the haemopoietic system. Recently, aplastic anaemia caused by Epo antibodies has been reported in adult patients treated with Epo.<sup>56</sup> Although similar observations have not been reported in infants, careful observation must continue.

Does Epo protect the preterm brain? In view of the encouraging data and considering the high incidence of major brain injury in very preterm infants, we conclude that the possible benefit afforded by Epo should be assessed by a clinical study in high risk infants. Thus, we propose a multicentre, randomised, placebo controlled study to prospectively test the hypothesis that systemic administration of recombinant human Epo positively affects the neurodevelopmental outcome of high risk preterm infants affected by brain injury. High dose Epo (1500 U/kg subcutaneously) should be given on days 1, 2, and 4 to high risk preterm infants, as most brain injuries occur perinatally or within the first days of life. Thus, preterm infants would receive Epo as early as possible after or even before the onset of brain damage providing the best possible benefit. The primary end points of the study should be the longitudinal assessment of gross motor function tests as well as verbal and performance intelligence tests. Secondary end points include behavioural consequences and the impact on activities of daily living and health related quality of life. Epidemiological data from recent years suggest that about 30% of preterm infants with a birth weight below 1000 g will have intraventricular haemorrhage or periventricular leucomalacia. On the basis of data from experimental studies, we expect the functional differences to be about 30% between the verum and placebo groups; we have calculated with a two sided test, an  $\alpha$  error of 0.05, power 80%, and a sample size of the proposed trial of 294 preterm infants in each group. Most of the current data from experimental and animal studies, as well as the first



**Figure 1** Neuroprotective properties of erythropoietin. Contributing mechanisms as well as effects observed in animal studies are depicted. SAH, Subarachnoid haemorrhage.

clinical study in human stroke patients, indicate that Epo may indeed be a potent neuroprotective agent with an excellent tolerability and safety profile, justifying a clinical trial in high risk infants.

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