HYPOTHESIS

Does erythropoietin protect the preterm brain?

T Strunk, C Härtel, C Schultz

Arch Dis Child Fetal Neonatal Ed 2004;**89**:F364–F366. doi: 10.1136/adc.2003.041533

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.

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.^{1–3} 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,^{4–7} 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.⁸ 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.⁹

“Significant upregulation of Epo and EpoR was observed in the human hypoxic-ischaemic brain, underlining the potential of Epo/EpoR as endogenous neuroprotective factors.”

The biological role of erythropoietin (Epo), formerly known only as a kidney derived haemopoietic growth factor, has expanded beyond haemopoiesis^{10–11} 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 astrocytes¹² as well as functional effects on neuronal cells have been shown.^{13–15} Epo exerts its effects through its receptor (EpoR), and binding of Epo to its receptor ultimately results in the activation of several downstream signals.^{10–11} 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.^{16–17} Juul *et al*¹³ 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.^{10–13} Significant upregulation of Epo and EpoR was observed in the human hypoxic-ischaemic brain, underlining the potential of Epo/EpoR as endogenous neuroprotective factors.¹⁵ 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^{18–20}; (b) diminution of intracellular calcium; (c) stimulation of NO production^{21–22}; (d) antioxidative,^{23–25} (e) anti-inflammatory,^{19–26} and (f) angiogenic actions.^{27–28} 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.

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.²⁹ A reduction in infarct size in mice treated with Epo 24 hours before experimental infarction was observed.³⁰ In addition, reduction in the inflammatory infiltrate

Abbreviations: Epo, erythropoietin; EpoR, erythropoietin receptor

See end of article for authors' affiliations

Correspondence to:
Dr Schultz, University
Hospital Schleswig-
Holstein, Campus Lübeck,
Department of Paediatrics,
Ratzeburger Allee 160,
23538 Lübeck, Germany;
schultz@paedia.ukl.
mu-luebeck.de

Accepted
14 February 2004

and diminished production of proinflammatory cytokines were reported in rats with cerebral ischaemia.³¹ Neuroprotective properties of Epo are associated with the preservation of learning abilities after transient brain ischaemia in gerbils.³² The protective effects were detected when Epo was administered before or up to six hours after the injury.²⁶ Other groups described a duration of the protective effects of at least three days even without the continued presence of Epo.^{19–20} In experimental subarachnoid haemorrhage, Epo normalises cerebral blood flow,^{33–34} resulting in a significant decrease in necrotic neurones after systemic application.³⁵ 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.^{36–37}

“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.³⁸ 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.^{39–45} 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.⁴⁶ 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*⁴⁷ 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.^{26–37–48} 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.^{7–13–14} 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.⁴⁹ 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.^{50–52} Nevertheless, safety studies primarily designed to assess this concern are mandatory. An increased risk of thromboembolic events was observed in adult patients with polycythaemia.^{53–54} However, these complications were not observed in clinical trials of the use of Epo in infants.^{39–45} Furthermore, recent experimental data from transgenic mice overexpressing Epo indicate that thromboembolic disease may even be prevented by Epo induced erythrocytosis.⁵⁵ 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.⁵⁶ 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 α 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.

Authors' affiliations

T Strunk, C Härtel, C Schultz, Department of Paediatrics, University of Lübeck Medical School, Lübeck, Germany

REFERENCES

- Wood NS, Marlow N, Costeloe K, *et al.* Neurologic and developmental disability after extremely preterm birth. *N Engl J Med* 2000;**343**:378–84.
- Vannucci RC. Hypoxic-ischemic encephalopathy. *Am J Perinatol* 2000;**17**:113–20.
- Berger R, Bender S, Sefkow S, *et al.* Peri/intraventricular hemorrhage: a cranial ultrasound study on 5286 neonates. *Eur J Obstet Gynecol Reprod Biol* 1997;**75**:191–203.
- Yoon BH, Romero R, Yang SH, *et al.* Interleukin-5 concentrations in umbilical cord plasma are elevated in neonates with white matter lesions associated with periventricular leukomalacia. *J Obstet Gynecol* 1996;**174**:1433–40.
- Schultz C, Temming P, Gembruch U, *et al.* Polyclonal intravenous immunoglobulin to prevent brain injury in preterm infants. *Lancet* 2002;**359**:1522–3.
- Schultz C, Rott C, Temming P, *et al.* Enhanced interleukin-6 and interleukin-8 synthesis in term and preterm infants. *Pediatr Res* 2002;**51**:317–22.
- Dammann O, Leviton A. Brain damage in preterm newborns: biological response modification as a strategy to reduce disabilities. *J Pediatr* 2000;**136**:433–8.
- Juul SE. Erythropoietin in the central nervous system, and its use to prevent hypoxic-ischemic brain damage. *Acta Paediatr Suppl* 2002;**438**:36–42.
- Vannucci RC, Perlman JM. Interventions for perinatal hypoxic-ischemic encephalopathy. *Pediatrics* 1997;**100**:1004–14.
- Jelkmann W. The biology of erythropoietin. *Clin Invest* 1994;**72**:S3–10.
- Ebert BL, Bunn HF. Regulation of the erythropoietin gene. *Blood* 1999;**94**:184–7.
- Nagai A, Nakagawa E, Choi HB, *et al.* Erythropoietin and erythropoietin receptors in human CNS neurons, astrocytes, microglia, and oligodendrocytes grown in culture. *J Neuropathol Exp Neurol* 2001;**60**:386–92.
- Juul SE, Anderson DK, Li Y, *et al.* Erythropoietin and erythropoietin receptor in the developing human central nervous system. *Pediatr Res* 1998;**43**:40–9.
- Juul SE, Yachnis AT, Rojiani AM, *et al.* Immunohistochemical localization of erythropoietin and its receptor in the developing human brain. *Pediatr Dev Pathol* 1999;**2**:148–58.
- Sirén AL, Knerlich F, Poser W, *et al.* Erythropoietin and erythropoietin receptor in human ischemic/hypoxic brain. *Acta Neuropathol* 2001;**101**:271–6.
- Maier RF, Bohme K, Dudenhausen JW, *et al.* Cord blood erythropoietin in relation to different markers of fetal hypoxia. *Obstet Gynecol* 1993;**81**:575–80.
- Buescher U, Hertwig K, Wolf C, *et al.* Erythropoietin in amniotic fluid as a marker of chronic fetal hypoxia. *Int J Gynaecol Obstet* 1998;**60**:257–63.
- Digicaylioglu M, Lipton SA. Erythropoietin-mediated neuroprotection involves cross-talk between Jak2 and NF- κ B signalling cascades. *Nature* 2001;**412**:641–3.
- Sirén AL, Fratelli M, Brines M, *et al.* Erythropoietin prevents neuronal apoptosis after cerebral ischemia and metabolic stress. *Proc Natl Acad Sci USA* 2001;**98**:4044–9.
- Junk AK, Mammis A, Savitz SI, *et al.* Erythropoietin administration protects retinal neurons from acute ischemia-reperfusion injury. *Proc Natl Acad Sci USA* 2002;**99**:10659–64.
- Genc S, Kuralay F, Genc K, *et al.* Erythropoietin exerts neuroprotection in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated C57/BL mice via increasing nitric oxide production. *Neurosci Lett* 2001;**298**:139–41.
- Assandri R, Egger M, Gassmann M, *et al.* Erythropoietin modulates intracellular calcium in a human neuroblastoma cell line. *J Physiol* 1999;**516**:343–52.
- Chattopadhyay A, Choudhury TD, Bandyopadhyay D, *et al.* Protective effect of erythropoietin on the oxidative damage of erythrocyte membrane by hydroxyl radical. *Biochem Pharmacol* 2000;**59**:419–25.
- Kristal B, Shurtz-Swirski R, Shasha SM, *et al.* Interaction between erythropoietin and peripheral polymorphonuclear leukocytes in hemodialysis patients. *Nephron* 1999;**81**:406–13.
- Sela S, Shurtz-Swirski R, Sharon R. The polymorphonuclear leukocyte: a new target for erythropoietin. *Nephron* 2001;**88**:205–10.
- Brines ML, Ghezzi P, Keenan S, *et al.* Erythropoietin crosses the blood-brain barrier to protect against experimental brain injury. *Proc Natl Acad Sci USA* 2000;**97**:10526–31.
- Ribatti D, Presta M, Vacca A, *et al.* Human erythropoietin induces a pro-angiogenic phenotype in cultured endothelial cells and stimulates neovascularization in vivo. *Blood* 1999;**93**:2627–36.
- Yamaji R, Okada T, Moriya M, *et al.* Brain capillary endothelial cells express two forms of erythropoietin receptor mRNA. *Eur J Biochem* 1996;**239**:494–500.
- Sakanaka M, Wen TC, Matsuda S, *et al.* In vivo evidence that erythropoietin protects neurons from ischemic damage. *Proc Natl Acad Sci USA* 1998;**95**:4635–40.
- Bernaudin M, Marti HH, Roussel S, *et al.* A potential role for erythropoietin in focal permanent cerebral ischemia in mice. *J Cereb Blood Flow Metab* 1999;**19**:643–51.
- Agnello D, Bigini P, Villa P, *et al.* Erythropoietin exerts an anti-inflammatory effect on the CNS in a model of experimental autoimmune encephalomyelitis. *Brain Res* 2002;**952**:128–34.
- Catania MA, Marciano MC, Parisi A, *et al.* Erythropoietin prevents cognition impairment induced by transient brain ischemia in gerbils. *Eur J Pharmacol* 2002;**437**:147–50.
- Grasso G, Buemi M, Alafaci C, *et al.* Beneficial effects of systemic administration of recombinant human erythropoietin in rabbits subjected to subarachnoid hemorrhage. *Proc Natl Acad Sci USA* 2002;**99**:5627–31.
- Springborg JB, Ma X, Rochat P, *et al.* A single subcutaneous bolus of erythropoietin normalizes cerebral blood flow autoregulation after subarachnoid haemorrhage in rats. *Br J Pharmacol* 2002;**135**:823–9.
- Alafaci C, Salpietro F, Grasso G, *et al.* Effect of recombinant human erythropoietin on cerebral ischemia following experimental subarachnoid hemorrhage. *Eur J Pharmacol* 2000;**406**:219–25.
- Celik M, Gökmen N, Erbayraktar S, *et al.* Erythropoietin prevents motor neuron apoptosis and neurologic disability in experimental spinal cord ischemic injury. *Proc Natl Acad Sci USA* 2002;**99**:2258–63.
- Gorio A, Gökmen N, Erbayraktar S, *et al.* Recombinant erythropoietin counteracts secondary injury and markedly enhances neurological recovery from experimental spinal cord trauma. *Proc Natl Acad Sci USA* 2002;**99**:9450–5.
- Ehrenreich H, Hasselblatt M, Dembowski, *et al.* Erythropoietin therapy for acute stroke is both safe and beneficial. *Mol Med* 2002;**8**:495–505.
- Maier RF, Obladen M, Scigalla P, *et al.* The effect of epoetin beta (recombinant human erythropoietin) on the need for transfusion in very-low-birth-weight infants. *N Engl J Med* 1994;**330**:1173–8.
- Maier RF, Obladen M, Kattner E, *et al.* High- versus low-dose erythropoietin in extremely low birth weight infants. *J Pediatr* 1998;**132**:866–70.
- Maier RF, Obladen M, Müller-Hansen I, *et al.* Early treatment with erythropoietin β ameliorates anemia and reduces transfusion requirements in infants with birth weights below 1000 g. *J Pediatr* 2002;**141**:8–15.
- Meyer MP, Meyer JH, Commerford A, *et al.* Recombinant human erythropoietin in the treatment of the anemias of prematurity: results of a double-blind, placebo-controlled study. *Pediatrics* 1994;**94**:918–23.
- Shannon KM, Keith JF, Mentzner WC, *et al.* Recombinant human erythropoietin stimulate erythropoiesis and reduces erythrocyte transfusions in very low birth weight preterm infants. *Pediatrics* 1995;**95**:1–8.
- Ohls K, Veerman MW, Christensen RD. The use of erythropoietin in neonates. *Clin Perinatol* 2000;**3**:681–96.
- Ohls RK, Ehrenkranz RA, Wright LL, *et al.* Effects of early erythropoietin therapy on the transfusion requirements of preterm infants below 1250 grams birth weight: a multicenter, randomized, controlled trial. *Pediatrics* 2001;**108**:934–42.
- Ledbetter J, Juul SE. Erythropoietin and the incidence of necrotizing enterocolitis in infants with very low birth weight. *J Pediatr Surg* 2000;**35**:178–82.
- Juul SE, Harcum J, Li Y, *et al.* Erythropoietin is present in the cerebrospinal fluid of neonates. *J Pediatr* 1997;**130**:428–30.
- Farrell F, Juul SE, Elliott C, *et al.* Erythropoietin crosses the blood brain barrier: an analysis in a non-human primate model. *Blood* 2001;**98**:148b.
- Shingo T, Sorokan ST, Shimazaki T, *et al.* Erythropoietin regulates the in vitro and in vivo production of neuronal progenitors by mammalian forebrain neural stem cells. *J Neurosci* 2001;**21**:9733–43.
- Newton NR, Leonard CH, Piecuch RE, *et al.* Neurodevelopmental outcome of prematurely born children treated with recombinant human erythropoietin in infancy. *J Perinatol* 1999;**19**:403–6.
- Soubasi V, Kremenopoulos GF, Diamanti E, *et al.* Follow-up of very low birth weight infants after erythropoietin treatment to prevent anemia of prematurity. *J Pediatr* 1995;**127**:291–7.
- Ehrenkranz R, Ohls RK, DAS A, *et al.* Neurodevelopmental outcome and growth at 1822 months in extremely low birth weight infants treated with early erythropoietin and iron [abstract]. *Pediatr Res* 2002;**51**:291.
- Ambrus JL, Ambrus CM, Dembinsky W, *et al.* Thromboembolic disease susceptibility related to red cell membrane fluidity in patients with polycythemia vera and effect of phlebotomies. *J Med* 1999;**30**:299–304.
- Tefferi A, Solberg LA, Silverstein MN. A clinical update in polycythemia vera and essential thrombocythemia. *Am J Med* 2000;**109**:141–9.
- Shibata J, Hasegawa J, Siemsen HJ, *et al.* Hemostasis and coagulation at a hematocrit level of 0.85: functional consequences of erythrocytosis. *Blood* 2003;**101**:4416–22.
- Casadevall N, Nataf J, Viron B, *et al.* Pure red-cell aplasia and antierythropoietin antibodies in patients treated with recombinant erythropoietin. *N Engl J Med* 2002;**346**:469–75.