Published in final edited form as:

Pathophysiology. 2013 February ; 20(1): 31–38. doi:10.1016/j.pathophys.2012.02.005.

Erythropoietin Neuroprotection with Traumatic Brain Injury

Lucido L. Ponce, MD¹, Jovany Cruz Navarro, MD¹, Osama Ahmed, MD², and Claudia S. Robertson, MD¹

¹Department of Neurosurgery Baylor College of Medicine

²Department of Neurosurgery LSUHSC Shreveport

Abstract

Numerous experimental studies in recent years have suggested that erythropoietin (EPO) is an endogenous mediator of neuroprotection in various central nervous system disorders, including TBI. Many characteristics of EPO neuroprotection that have been defined in TBI experimental models suggest that it is an attractive candidate for a new treatment of TBI. EPO targets multiple mechanisms known to cause secondary injury after TBI, including anti-excitotoxic, antioxidant, anti-edematous, and anti-inflammatory mechanisms. EPO crosses the blood brain barrier. EPO has a known dose response and time window for neuroprotection and neurorestoration that would be practical in the clinical setting. However, EPO also stimulates erythropoiesis, which can result in thromboembolic complications. Derivatives of EPO which do not bind to the classical EPO receptor (carbamylated EPO) or that have such a brief half-life in the circulation that they do not stimulate erythropoiesis (asialo EPO and neuro EPO) have the neuroprotective activities of EPO without these potential thromboembolic adverse effects associated with EPO administration. Likewise, a peptide based on the structure of the Helix B segment of the EPO molecule that does not bind to the EPO receptor (pyruglutamate Helix B surface peptide) has promise as another alternative to EPO that may provide neuroprotection without stimulating erythropoiesis.

Introduction

Traumatic brain injury (TBI) is an important public health problem in the United States and worldwide. An estimated of 5.3 million people live with TBI-related disability in the USA (Langlois et al., 2006) and 6.2 million people in Europe (Tagliaferri et al., 2006) also face numerous challenges in their efforts to return to a full and productive life after sustaining TBI. TBI can result in long-term or lifelong physical, cognitive, behavioral, and emotional consequences. Even mild TBI, including concussion, can cause long-term cognitive problems that affect a person's ability to perform daily activities and to return to work (McAllister et al., 2001; Alves et al., 1993; Englander et al., 1992). TBI is one of the most disabling injuries in all groups of age. Currently, little can be done to reverse the primary brain damage caused by the traumatic event; however, one of the major factors influencing outcome in patients with acute brain injury is the additional brain damage that occurs from secondary injury processes. Currently, no specific pharmacological therapy for TBI is available to decrease the severity of secondary brain injury and improve outcome. The need

© 2012 Elsevier Ireland Ltd. All rights reserved.

Address correspondence to: Claudia Robertson, MD Professor, Department of Neurosurgery Baylor College of Medicine One Baylor Plaza Houston, Texas 77030 Phone 713-873-2792 FAX 713-873-6609 .

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

for an effective drug for TBI treatment is more than evident. Numerous experimental studies in recent years have suggested that erythropoietin (EPO) is an endogenous mediator of neuroprotection in various central nervous system disorders, including TBI.

EPO is a 34-kDa (165-amino acid) glycoprotein hematopoietic growth factor belonging to the type I cytokine superfamily (Lin et al., 1985). EPO has long been recognized as the hormone that regulates erythropoiesis. In 1906, the concept of hormonal regulation of hematopoiesis initially began with Carnot, a professor of medicine in Paris, and DeFlandre, his assistant. They proposed that there factor might exist that triggers the development of reticulocytes after studying the peripheral blood of rabbits; they named this factor 'hemopoietin'. The conclusion was reached by injecting anemic donor rabbit blood into normal rabbits, which led to erythropoiesis (Carnot and Deflandre, 1906). Eva Bonsdorff and Eeva Jalavisto continued to study this erythropoietic factor and later called it 'erythropoietin'. In 1953, Erslev confirmed the presence of a factor responsible for erythropoiesis and suggested its therapeutic use (Erslev 1953).

In 1957 renal production of EPO was hypothesized (Jacobson et al., 1957). This was later supported by studies conducted on isolated perfused kidneys (Erslev et al., 1974) and well established when EPO messenger ribonucleic acid was demonstrated in renal tissue (Bondurant et al., 1986; Beru et al., 1986; Schuster et al., 1987). EPO gene expression is induced in the kidney by the hypoxia-induced transcription factor (HIF) in response to tissue hypoxia (Jones et al., 2001). Subsequent production of EPO stimulates erythroid cell production which increases oxygen delivery to tissues. These hematopoietic effects of EPO are mediated by transmembrane receptors named EPO receptors (EPOR) (Jelkmann, 2004). EPO and EPOR expression change significantly during development. During fetal development, EPO is initially produced in the liver, but shortly after birth, EPO production is subsequently shifted to the kidney (Jelkmann, 1992).

The discovery of EPO and EPOR in many non-erythroid organs and tissues such as endothelial cells, reproductive organs, heart, gastrointestinal tract, muscle cells, and the central nervous system (Sytkowski et al., 2004) suggested that EPO has other roles. Extrarenal production of EPO accounts for approximately 15 to 20 percent of total EPO secretion in adult rodents (Erslev et al., 1980). In humans, very low but still detectable levels of EPO are found in severely anemic anephric individuals (Caro et al., 1979) consistent with the presence of extrarenal synthesis of EPO. Different cell types (neurons, glial cells and endothelial cells) in the nervous system produce EPO and express EPOR. EPO has multiple actions in the nervous system.

The expression of EPO in the brain in astrocytes and neurons is hypoxia inducible. Up to a 100-fold increase in astrocytes results when incubated under hypoxic conditions (Marti et al., 1996) and lasts for up to 24 hours or more (Chimuka et al., 2000). Adult human brain has shown EPO expression in biopsies of the hippocampus, amygdala, and temporal cortex. In addition, HIF-2 α , rather than HIF-1 α , is the major regulator of EPO expression during hypoxia in the brain (Warnecke et al., 2004).

EPO has been demonstrated to have neuroprotective effects after ischemic, hypoxic, metabolic, neurotoxic and excitotoxic stress in the nervous system. EPO operates at several levels within the central nervous system, including limiting the production of reactive oxygen species and glutamate, neurotransmission modulation, reversal of vasospasm, promoting angiogenesis, preventing apoptosis, reduction of inflammation and stem cells recruitment.

Signaling Pathways Involved in Neuroprotection with EPO

Multiple studies have explored the multi-step EPO-EPOR complex signaling routes involved in the tissue-protective effects of EPO (Figure 1). Endogenous and exogenous EPO can bind the EPOR causing its homodimerization and subsequent JAK-2 phosphorylation causing an intricate downstream signaling activation process (Kawakami et al., 2001; Sola et al., 2005a). JAK-2 phosphorylation in turn activates phosphatidylinositol 3-kinase (PI3-K), induces the activation of (nuclear factor) NF- κ B and stimulates STAT-5 homodimerization (Sola et al., 2005b). Additionally, JAK-2 phosphorylation has been shown to activate the Ras-Mitogen activated protein kinase (MAPK) signaling pathways, and to modulate the intracellular calcium concentration in excitable cells, electrical activity, and neurotransmitter release by activating phospholipase C- γ (Kawakami et al., 2001; Koshimura et al., 1999). Best investigated from these signaling pathways are the PI3-K/v-akt murine thymoma viral oncogene homolog (Akt) and Ras/MAPK pathways; both of which are important for the antiapoptotic and trophic effects of EPO (Kilic et al., 2005; Chong et al., 2003a; Chong et al., 2003b; Byts et al., 2008; Wu et al., 2007a). The Janus-tyrosine-kinase-2 (JAK2)-PI3K pathway is crucial for the neuroprotective abilities of EPO. *In vivo* studies showed that inhibition of either JAK-2 or PI3-K abolished the neuroprotective effects of EPO (Zhang et al., 2006; Zhang et al., 2007). PI3-K mediated activation of Akt modulates several intracellular signaling routes involved in apoptosis, synaptic signaling, and synthesis of glycogen. The main target molecules of AKT are p53, GSK-3 and cytochrome c; which modulate important processes such as glycogen synthesis, cellular cycle and cell death (Culmsee et al., 2005; Datta et al., 1999).

The Ras/Raf/MEK/extracellular signal-regulated kinase (ERK)-1/2 pathway is also involved in the neuroprotective response, presumably contributing to the antiapoptotic effects of EPO, by increasing transcription of anti-apoptotic genes (Bcl-2, Bcl-xL). Kilic et al. found that EPO dramatically reduced the volume of infarction and induced activation of JAK-2, ERK-1/2, and PI3-K/Akt pathways associated with elevated anti-apoptotic Bcl-xL protein (Kilic et al., 2005). Finally, the JAK2-STAT pathway is likely to be important for the anti-apoptotic properties of EPO. STAT-5 may be phosphorylated upon EPO/EPOR coupling via JAK-2 (Sola et al., 2005a). Phosphorylated STAT-5 homodimerizes and enters the cell nucleus where the anti-apoptotic genes Bcl-2 and Bcl-xL are transcribed. Bcl-2 and Bcl-xL in turn are capable of preventing the release of cytochrome c from mitochondria. EPO has been found to increase STAT-5 and the concentrations of anti-apoptotic genes (Siren et al., 2001; Sola et al., 2005a; Wei et al., 2006). The precise role of the STAT-5 pathway remains to be determined, but would likely involve antiapoptotic, and neurogenesis effects.

A number of novel pathways that may mediate the ability of EPO to prevent cellular apoptosis are intimately tied to Akt-1. After activation of the PI3-K pathway, EPO modulated the pro-apoptotic forkhead transcription factor O3 (FOXO3a), which would normally activate the transcription of apoptotic genes (Chong & Maiese., 2007). Furthermore, after β -amyloid peptide exposure in neural cells, EPO uses the NF κ -B to prevent apoptosis (Chong et al., 2005).

Rationale for Treatment of TBI with EPO

TBI induces multiple injury processes in the brain which continue to add to the damage caused by the primary traumatic event for days to weeks after injury. The main goal of neuroprotection is to avoid and/or limit this secondary damage and to promote repair. Based on the activity of EPO in multiple injury processes known to be involved in secondary injury after trauma, it is a promising therapeutic strategy for TBI. In addition, a recent large clinical trial in critically ill patients suggested that EPO may reduce mortality in trauma patients

(Corwin et al., 2007). A metaanalysis of EPO clinical trials, subgroup analyses of trials, and case-matched observational studies have supported this finding (Zarychanski, et al., 2007; Napolitano et al., 2008; Talving et al., 2010).

TBI is a heterogeneous disorder with complex pathophysiology. TBI also encompasses a wide spectrum of injury severities, ranging from mild TBI where the major problems are post-concussive symptoms and cognitive impairment, to severe TBI where a fraction of the patients may die of refractory brain swelling and many will survive with permanent disabilities. Recommendations regarding preclinical data necessary to bring a new drug treatment forward to a TBI clinical study include: 1-use an agent that targets a mechanism (or even better multiple mechanisms) known to be active in TBI secondary injury, 2-demonstrate that the agent crosses the blood brain barrier, 3-determine specific dose response and time window, 4-determine necessary duration of treatment, 5-demonstrate effectiveness in more than one species and experimental model. Preclinical studies using EPO in experimental models of TBI have provided much of this data needed for translation of EPO to clinical trials.

EPO targets multiple mechanisms known to cause secondary injury after TBI

including anti-excitotoxic, antioxidant, anti-edematous, and anti-inflammatory mechanisms. EPO has the ability to protect cells against oxidative damage (Solaroglu et al., 2003; Wu et al., 2007b), and inhibits lipid peroxidation by increasing the activities of cytosolic antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (Chattopadhyay et al., 2000; Kumral et al., 2005). Several experimental studies have shown that EPO increases neuronal survival in cell cultures exposed to excitotoxic stress; likely, this enhanced resistance to glutamate toxicity was due to a diminished calcium dependant glutamate release (Kawakami et al. 2000).

In addition to its direct effect on neural cells, EPO-induced neuroprotection may involve an improvement in brain perfusion. EPO protects vascular bed integrity and stimulates angiogenesis (Wang et al., 2004; Ribatti et al., 2003; Marti et al., 2000; Jaquet et al., 2002) by acting indirectly on endothelial cells via activation of the vascular endothelial growth factor VEGF/VEGF receptor system, which is the most important regulator of endothelial growth and angiogenesis. Furthermore, EPO may have a positive effect on cerebral vasculature through alteration in nitric oxide (NO) production, which is mainly derived from arginine in two steps catalyzed by endothelial nitric oxide synthase (eNOS). Pre-injury treatment with EPO in a rodent TBI model resulted in a significant enhancement of post-injury CBF recovery with L-arginine administration, possibly by increasing eNOS activity (Cherian et al., 2007). Although such a vascular mechanism is not often considered to be the primary neuroprotective mechanism for EPO, studies using transgenic mice deficient in the neural EPOR have suggested that vascular protection may be important (Xiong et al, 2010b).

Impaired neurogenesis after TBI in an experimental model in mice null for EPOR has been described (Xiong et al., 2008). Delayed administration of EPO reduced hippocampal cell loss while enhancing angiogenesis and neurogenesis after TBI in rats (Xiong et al., 2010a). The same neurorestorative effects have been reproduced with EPO administration in rats after TBI in a dosedependent manner (Meng et al., 2011). The coupling of angiogenesis and neurogenesis has been demonstrated in rodents after brain injuries, mainly in models of ischemic injuries. The effects of EPO on post-ischemic progenitor migration are likely related to matrix metalloproteinase 2 (MMP2) and MMP9 secretion via the PI-3K/Akt-1 and ERK-1/2 signaling pathways (Wang et al., 2006).

EPO crosses the blood brain barrier

The molecular weight of EPO (≈ 34 kD) is larger than the molecular threshold for lipid-mediated transport across the brain-blood barrier (BBB), which raises some concern about the ability of EPO to provide protection to brain tissue. In 2000, Brines et al. demonstrated using labeled EPO that 5 hours after IP injection of 5000 units/kg, EPO could be demonstrated within the brain parenchyma (Brines et al., 2000). Single intravenous (IV) and intraperitoneal administrations of EPO in non-human primates achieved high levels of EPO in the cerebrospinal fluid (CSF) as early as 1 hour after administration (Juul et al., 2004). However no confirmation of receptor-mediated transport across the BBB has been found. Administration of radio-labeled EPO in mice with intact BBB showed a very slow rate of transport similar to albumin and was not saturable, suggesting that EPO crosses the BBB by extracellular pathways (Banks et al., 2004). The percent of the IV injected dose taken up per gram of brain in these studies ranged from 0.05 to 0.1%/g. Thus, passage of EPO across the BBB in levels sufficient to produce cytoprotection has been demonstrated following IV administration of EPO.

EPO has a known dose response and time window for neuroprotection and neurorestoration

In doses ranging from 1000 to 7000 units/kg given IP to rats, EPO at a dose of 5000 units/kg provided the best recovery of neurological function and preservation of hippocampal neurons (Meng et al., 2011). For optimal neuroprotection, administration of EPO must be started within 6 hours of injury. When treatment with EPO is given this early in cortical impact injury models of TBI, significant reduction in contusion volume, preservation of hippocampal neurons, and improved functional recovery have been observed (Brines et al., 2000; Cherian et al., 2007; Xiong et al., 2007). When given as long as 24 hours after injury, EPO still shows significant effects in preserving hippocampal neurons and improving functional recovery, although contusion volume is not reduced (Lu et al., 2005; Meng et al. 2011).

Multiple daily doses if EPO are more effective than a single dose

Many dose regimens for EPO have been used in experimental models of TBI, including a single dose at times ranging from 5 minutes to 24 hours post-injury, and repeated doses at daily or longer intervals. Xiong et al. showed that a dose regimen of daily doses for 3 days post-injury provided significantly better recovery of function and preservation of hippocampal neurons than a single dose, although both treatment regimens were significantly neuroprotective compared to saline (Xiong et al., 2010a).

EPO has demonstrated efficacy in multiple TBI models

TBI is a heterogeneous disorder and no single experimental model has all of the features of human TBI. Much of the preclinical work with EPO has been done in the cortical impact injury model in the rat, which produces a focal contusion. Weight drop and impact acceleration models, which produce diffuse brain injury, as well as *in vitro* models have also shown efficacy with EPO (Jin et al., 2011; Zhu et al., 2009; Adembri et al., 2004). However, in spinal cord injury, the findings have not been consistent and some investigators have not observed efficacy (Pinzon et al., 2008; Mann et al., 2008).

Dose Limiting Adverse Effects of EPO

EPO has been used safely in the treatment for anemia in chronic renal failure patients for many years. However, when EPO has been used in other patient groups and especially when hemoglobin levels greater than 12 gm/dl have been targeted, a number of serious adverse effects have occurred including a higher mortality rate and higher risk of thromboembolic

complications (Bohlius et al., 2009; Hadland & Longmore, 2009). Of greatest importance for TBI patients, increased thromboembolic complications with EPO administration are related to increased hematocrit and blood viscosity (Besarab et al., 2008), and to alteration in function and formation of platelets, inducing functional hyperreactivity (Wolf et al., 1997) and increased platelet adherence to injured endothelial surfaces (Fuste et al., 2002). The dose of EPO approved for use in patients, approximately 500 units/kg, is just at the lower limit of the single dose regimen that has been effective in TBI experimental models. This approved dose is much lower than the 5000 units/kg which has been found to be optimal after cortical impact injury (Meng et al., 2011).

Other potential complications are not as relevant for the TBI patient. EPO has the potential to promote tumor growth and to confer resistance to neoplastic therapy (Ribatti et al., 2009). EPO also may induce arterial hypertension, usually in patients with chronic renal failure, by inducing excessive *endothelin-1* release (Carlini et al., 1993; Miyashita et al., 2004). Like any protein, EPO has the potential to induce autoimmunity when administered chronically (Kharagjitsingh et al., 2005).

Non-erythropoietic derivatives of EPO

Because of the potential thromboembolic complications caused by EPO, it may be difficult to achieve neuroprotection with EPO in TBI patients without risking potentially life-threatening complications. Development of derivatives of EPO that do not bind to the classical EPOR (carbamylated erythropoietin or CEPO) or that have such a short half-life in the circulation that erythropoiesis is not significantly stimulated (asialoerythropoietin or neuroepoietin) have clearly demonstrated that the neuroprotective effects of EPO can be separated from the hematopoietic effects (Leist et al., 2004; Erbayraktar et al., 2003; Mattio et al., 2011). In 2004, Brines et al. showed that mice deficient in the common beta (CD131) receptor exhibited no neuroprotection with EPO, and suggested that these activities might be mediated by a heteroreceptor complex of the classical EPOR and the CD131 receptor (Brines et al., 2004). However, other studies have not found the CD131 receptor necessary for nonerythropoietic activities of EPO in some tissues (Kanellakis et al., 2010).

Short peptide sequences mimicking sections of the Helix B section of the EPO molecule, which does not interact with the EPOR, have been studied to try to determine the region responsible for neuroprotective activities. An 11 amino acid peptide, called the pyroglutamate Helix B surface peptide (pHBSP), was found to retain the cytoprotective activities (Brines et al., 2008). This peptide has subsequently been found to have cytoprotection in the brain after trauma and in other tissues (Robertson et al., 2011; Ueba et al., 2010).

Conclusion

Abundant experimental evidence and some clinical studies suggest that EPO should have neuroprotective activities after TBI. The characteristics of EPO which include activity against multiple pathogenic pathways known to cause secondary injury after TBI, and a time window sufficiently long to be practical in the clinical setting make EPO attractive as a neuroprotective agent. Potential thromboembolic complications associated with the hematopoietic effects may be avoided by using derivatives of EPO or EPO-mimetic peptides that do not bind to the EPOR or that have a very short half-life.

Acknowledgments

This work was supported by NIH Grant #P01-NS36680 and Congressionally Directed Medical Research Program Award #W81XWH-08-2-0132.

REFERENCES

- Adembri C, Bechi A, Meli E, Gramigni E, Venturi L, Moroni F, De Gaudio AR, Pellegrini-Giampietro DE. Erythropoietin attenuates post-traumatic injury in organotypic hippocampal slices. *J Neurotrauma*. 2004; 21:1103–1112. [PubMed: 15319009]
- Alves W, Macciocchi SN, Barth JT. Postconcussive symptoms after uncomplicated mild head injury. *J Head Trauma Rehabil*. 1993; 8:48–59.
- Banks WA, Jumbe NL, Farrell CL, Niehoff ML, Heatherington AC. Passage of erythropoietic agents across the blood–brain barrier: a comparison of human and murine erythropoietin and the analog darbepoetin alfa. *Eur J Pharmacol*. 2004; 505:93–101. [PubMed: 15556141]
- Beru N, McDonald J, Lacombe C, Goldwasser E. Expression of the erythropoietin gene. *Mol Cell Biol*. 1986; 6:2571–5. [PubMed: 3466025]
- Besarab A, Goodkin DA, Nissenson AR. Normal Hematocrit Cardiac Trial Authors. The normal hematocrit study–follow-up. *N Engl J Med*. 2008; 358:433–434. [PubMed: 18216370]
- Bohlius J, Schmidlin K, Brillant C, Schwarzer G, Trelle S, Seidenfeld J, Zwahlen M, Clarke MJ, Weingart O, Kluge S, Piper M, Napoli M, Rades D, Steensma D, Djulbegovic B, Fey MF, Ray-Coquard I, Moebus V, Thomas G, Untch M, Schumacher M, Egger M, Engert A. Erythropoietin or darbepoetin for patients with cancer — meta-analysis based on individual patient data. *Cochrane Database Syst Rev*. 2009; 3:CD007303. [PubMed: 19588423]
- Bondurant MC, Koury M. Anemia induces accumulation of erythropoietin mRNA in the kidney and liver. *Mol Cell Biol*. 1986; 6:2731–2733. [PubMed: 3785209]
- Brines ML, Ghezzi P, Keenan S, Agnello D, de Lanerolle NC, Cerami C, Itri LM, Cerami A. Erythropoietin crosses the blood-brain barrier to protect against experimental brain injury. *Proc Natl Acad Sci USA*. 2000; 97:10526–10531. [PubMed: 10984541]
- Brines M, Grasso G, Fiordaliso F, Sfacteria A, Ghezzi P, Fratelli M, Latini R, Xie QW, Smart J, Su-Rick CJ, Pobre E, Diaz D, Gomez D, Hand C, Coleman T, Cerami A. Erythropoietin mediates tissue protection through an erythropoietin and common beta-subunit heteroreceptor. *Proc Natl Acad Sci USA*. 2004; 101:14907–14912. [PubMed: 15456912]
- Brines M, Patel NS, Villa P, Brines C, Mennini T, De PM, Erbayraktar Z, Erbayraktar S, Sepodes B, Thiernemann C, Ghezzi P, Yamin M, Hand CC, Xie QW, Coleman T, Cerami A. Nonerythropoietic, tissue (protective peptides derived from the tertiary structure of erythropoietin. *Proc Natl Acad Sci USA*. 2008; 105:10925–10930. [PubMed: 18676614]
- Byts N, Samoylenko A, Fasshauer T, Ivanisevic M, Hennighausen L, Ehrenreich H, Sirén AL. Essential role for Stat5 in the neurotrophic but not in the neuroprotective effect of erythropoietin. *cell death and differentiation*. 2008; 15:783–792. [PubMed: 18259195]
- Carlini RG, Dusso AS, Obialo CI, Alvarez UM, Rothstein M. Recombinant human erythropoietin (rHuEPO) increases endothelin-1 release by endothelial cells. *Kidney Int*. 1993; 43:1010–1014. [PubMed: 8510379]
- Carnot M, Deflandre C. *Comptes Rendus Hebdomadaires des Seances de l'Acad des Sci*. 1906; 143:384–386.
- Caro J, Brown S, Miller O, Murray T, Erslev AJ. Erythropoietin levels in uremic nephric and anephric patients. *J Lab Clin Med*. 1979; 93:449–458. [PubMed: 570997]
- Chattopadhyay A, Choudhury TD, Bandyopadhyay D, Datta AG. Protective effect of erythropoietin on the oxidative damage of erythrocyte membrane by hydroxyl radical. *Biochem Pharmacol*. 2000; 59:419–425. [PubMed: 10644050]
- Cherian L, Goodman JC, Robertson C. Neuroprotection with erythropoietin administration following controlled cortical impact injury in rats. *J Pharmacol Exp Ther*. 2007; 322:789–794. [PubMed: 17470644]
- Chikuma M, Masuda S, Kobayashi T, Nagao M, Sasaki R. Tissue specific regulation of erythropoietin production in the murine kidney, brain, and uterus. *Am J Physiol Endocrinol Metab*. 2000; 279:E1242–8. [PubMed: 11093910]
- Chong ZZ, Kang JQ, Maiese K. Erythropoietin fosters both intrinsic and extrinsic neuronal protection through modulation of microglia. *Ak*. 2003a; 1:1107–1118.

- Chong ZZ, Lin SH, Kang JQ, Maiese K. Erythropoietin prevents early and late neuronal demise through modulation of Akt1 and induction of caspase 1, 3, and 8. *J Neurosci Res*. 2003b; 71:659–669. [PubMed: 12584724]
- Chong ZZ, Li F, Maiese K. Erythropoietin requires NF-kappaB and its nuclear translocation to prevent early and late apoptotic neuronal injury during beta-amyloid toxicity. *Curr Neurovasc Res*. 2005; 2:387–399. [PubMed: 16375720]
- Chong ZZ, Maiese K. Erythropoietin involves the phosphatidylinositol 3-kinase pathway, 14-3-3 protein and FOXO3a nuclear trafficking to preserve endothelial cell integrity. *Br J Pharmacol*. 2007; 150:839–850. [PubMed: 17339844]
- Corwin HL, Gettinger A, Fabian TC, May A, Pearl RG, Heard S, An R, Bowers PJ, Burton P, Klausner MA, Corwin MJ. Efficacy and safety of epoetin alfa in critically ill patients. *N Engl J Med*. 2007; 357:965–976. [PubMed: 17804841]
- Culmsee C, Mattson MP. p53 in neuronal apoptosis. *Biochem Biophys Res Commun*. 2005; 331:761–777. [PubMed: 15865932]
- Datta SR, Brunet A, Greenberg ME. Cellular survival: a play in three Akts. *Genes Dev*. 1999; 13:2905–2927. [PubMed: 10579998]
- Englander J, Hall K, Stimpson T, Chaffin S. Mild traumatic brain injury in an insured population: subjective complaints and return to employment. *Brain Inj*. 1992; 6:161–166. [PubMed: 1571720]
- Erbayraktar S, Grasso G, Sfacteria A, Xie QW, Coleman T, Kreilgaard M, Torup L, Sager T, Erbayraktar Z, Gokmen N, Yilmaz O, Ghezzi P, Villa P, Fratelli M, Casagrande S, Leist M, Helboe L, Gerwein J, Christensen S, Geist MA, Pedersen LO, Cerami-Hand C, Wuerth JP, Cerami A, Brines M. Asialoerythropoietin is a nonerythropoietic cytokine with broad neuroprotective activity in vivo. *Proc Natl Acad Sci USA*. 2003; 100:6741–6746. [PubMed: 12746497]
- Erslev AJ. Humoral regulation of red cell production. *Blood*. 1953; 8:349–57. [PubMed: 13032205]
- Erslev AJ, Caro J, Kansu E, Silver R. Renal and extrarenal erythropoietin production in anemic rats. *Br J Haematol*. 1980; 45:65–72. [PubMed: 7378330]
- Erslev AJ. In vitro production of erythropoietin by kidneys perfused with a serum-free solution. *Blood*. 1974; 44:77–80. [PubMed: 4834517]
- Fusté B, Serradell M, Escolar G, Cases A, Mazzara R, Castillo R, Ordinas A, Diaz-Ricart M. Erythropoietin triggers a signaling pathway in endothelial cells and increases the thrombogenicity of their extracellular matrices in vitro. *Thromb Haemost*. 2002; 88:678–685. [PubMed: 12362243]
- Hadland BK, Longmore GD. Erythroid-stimulating agents in cancer therapy: potential dangers and biologic mechanisms. *J Clin Oncol*. 2009; 27:4217–4226. [PubMed: 19636005]
- Jacobson LO, Goldwasser E, Fried W, Plazak L. Role of the kidney in erythropoiesis. *Nature*. 1957; 179:633–634. [PubMed: 13418752]
- Jaquet K, Krause K, Tawakol-Khodai M, Geidel S, Kuck KH. Erythropoietin and VEGF exhibit equal angiogenic potential. *Microvasc Res*. 2002; 64:326–333. [PubMed: 12204656]
- Jelkmann W. Erythropoietin: structure, control of production, and function. *Physiol. Rev*. 1992; 72:449–489. [PubMed: 1557429]
- Jelkmann W. Molecular biology of erythropoietin. *Intern Med*. 2004; 43:649–659. [PubMed: 15468961]
- Jin W, Kong J, Lu T, Wang H, Ni H, Wu J, Jiang J, Liang W. Erythropoietin prevents secondary brain injury induced by cortical lesion in mice: possible involvement of Nrf2 signaling pathway. *Ann Clin Lab Sci*. 2011; 41:25–32. [PubMed: 21325251]
- Jones NM, Bergeron M. Hypoxic preconditioning induces changes in HIF-1 target genes in neonatal rat brain. *J Cereb Blood Flow Metab*. 2001; 21:1105–1114. [PubMed: 11524615]
- Juul SE, McPherson RJ, Farrell FX, Jolliffe L, Ness DJ, Gleason CA. Erythropoietin concentrations in cerebrospinal fluid of nonhuman primates and fetal sheep following high-dose recombinant erythropoietin. *Biol Neonate*. 2004; 85:138–44. [PubMed: 14639039]
- Kanellakis P, Pomilio G, Agrotis A, Gao X, Du XJ, Curtis D, Bobik A. Darbepoetin-mediated cardioprotection after myocardial infarction involves multiple mechanisms independent of erythropoietin receptor-common beta-chain heteroreceptor. *Br J Pharmacol*. 2010; 160:2085–96. [PubMed: 20649603]

- Kawakami M, Iwasaki S, Sato K, Takahashi M. Erythropoietin inhibits calcium-induced neurotransmitter release from clonal neuronal cells. *Biochem Biophys Res Commun.* 2000; 279:293–297. [PubMed: 11112455]
- Kawakami M, Sekiguchi M, Sato K, Kozaki S, Takahashi M. Erythropoietin receptor-mediated inhibition of exocytotic glutamate release confers neuroprotection during chemical ischemia. *J Biol Chem.* 2001; 276:39469–39475. [PubMed: 11504731]
- Kharagjitsingh AV, Korevaar JC, Vandenbroucke JP, Boeschoten EW, Krediet RT, Daha MR, Dekker FW. Incidence of recombinant erythropoietin (EPO) hyporesponse, EPO-associated antibodies, and pure red cell aplasia in dialysis patients. *Kidney Int.* 2005; 68:1215–1222. [PubMed: 16105053]
- Kilic E, Kilic U, Soliz J, Bassetti CL, Gassmann M, Hermann DM. Brain-derived erythropoietin protects from focal cerebral ischemia by dual activation of ERK-1/-2 and Akt pathways. *Faseb J.* 2005; 19:2026–2028. [PubMed: 16207820]
- Kilic U, Kilic E, Soliz J, Bassetti CI, Gassmann M, Hermann DM. Erythropoietin protects from axotomy-induced degeneration of retinal ganglion cells by activating ERK-1/-2. *Faseb J.* 2005; 19:249–251. [PubMed: 15556972]
- Koshimura K, Murakami Y, Sohmiya M, Tanaka J, Kato Y. Effects of erythropoietin on neuronal activity. *J Neurochem.* 1999; 72:2565–2572. [PubMed: 10349868]
- Kumral A, Gonenc S, Acikgoz O, Sonmez A, Genc K, Yilmaz O, Gokmen N, Duman N, Ozkan H. Erythropoietin increases glutathione peroxidase enzyme activity and decreases lipid peroxidation levels in hypoxic-ischemic brain injury in neonatal rats. *Biol Neonate.* 2005; 87:15–18. [PubMed: 15334031]
- Langlois JA, Rutland-Brown W, Wald MM. The epidemiology and impact of traumatic brain injury: a brief overview. *J Head Trauma Rehabil.* 2006; 21:375–378. [PubMed: 16983222]
- Leist M, Ghezzi P, Grasso G, Bianchi R, Villa P, Fratelli M, Savino C, Bianchi M, Nielsen J, Gerwien J, Kallunki P, Larsen AK, Helboe L, Christensen S, Pedersen LO, Nielsen M, Torup L, Sager T, Sfacteria A, Erbayraktar S, Erbayraktar Z, Gokmen N, Yilmaz O, Cerami-Hand C, Xie QW, Coleman T, Cerami A, Brines M. Derivatives of erythropoietin that are tissue protective but not erythropoietic. *Science.* 2004; 305:239–242. [PubMed: 15247477]
- Lin FK, Suggs S, Lin CH, Browne JK, Smalling R, Egrie JC, Chen KK, Fox GM, Martin F, Stabinsky Z, Badrawi SM, Lai P-H, Goldwasser E. Cloning and expression of the human erythropoietin gene. *Proc Natl Acad Sci USA.* 1985; 82:7580–7584. [PubMed: 3865178]
- Lu D, Mahmood A, Goussev A, Schallert T, Chopp M. Erythropoietin enhances neurogenesis and restores spatial memory in rats after traumatic brain injury. *J Neurotrauma.* 2005; 22:1011–1017. [PubMed: 16156716]
- Mann C, Lee JH, Liu J, Stammers AM, Sohn HM, Tetzlaff W, Kwon BK. Delayed treatment of spinal cord injury with erythropoietin or darbepoetin-a lack of neuroprotective efficacy in a contusion model of cord injury. *Exp Neurol.* 2008; 211:34–40. [PubMed: 18313052]
- Marti HH, Bernaudin M, Petit E, Bauer C. Neuroprotection and Angiogenesis: Dual Role of Erythropoietin in Brain Ischemia. *News Physiol Sci.* 2000; 15:225–229. [PubMed: 11390915]
- Marti HH, Wenger RH, Rivas LA, Straumann U, Digicaylioglu M, Henn V, Tonekawa T, Bauer C, Gassmann M. Erythropoietin gene expression in human, monkey and murine brain. *Eur J Neurosci.* 1996; 8:666–76. [PubMed: 9081618]
- Mattio M, Ceaglio N, Oggero M, Perotti N, Amadeo I, Orozco G, Forno G, Kratje R, Etcheverrigaray M. Isolation and characterization of a subset of erythropoietin glycoforms with cytoprotective but minimal erythropoietic activity. *Biotechnol Prog.* Apr 20.2011 doi: 10.1002/btpr.633. [Epub ahead of print].
- McAllister TW, Sparling MB, Flashman LA, Guerin SJ, Mamourian AC, Saykin AJ. Differential working memory load effects after mild traumatic brain injury. *Neuroimage.* 2001; 14:1004–1012. [PubMed: 11697932]
- Meng Y, Xiong Y, Mahmood A, Zhang Y, Qu C, Chopp M. Dose-dependent neurorestorative effects of delayed treatment of traumatic brain injury with recombinant human erythropoietin in rats. *J Neurosurg.* Apr 15.2011 [Epub ahead of print] DOI: 10.3171/2011.3.JNS101721.

- Miyashita K, Tojo A, Kimura K, Goto A, Omata M, Nishiyama K, Fujita T. Blood pressure response to erythropoietin injection in hemodialysis and predialysis patients. *Hypertens Res*. 2004; 27:79–84. [PubMed: 15005270]
- Napolitano LM, Fabian TC, Kelly KM, Bailey JA, Block EF, Langholff W, Enny C, Corwin HL. Improved survival of critically ill trauma patients treated with recombinant human erythropoietin. *J Trauma*. 2008; 65:285–297. [PubMed: 18695463]
- Pinzon A, Marcillo A, Pabon D, Bramlett HM, Bunge MB, Dietrich WD. A re-assessment of erythropoietin as a neuroprotective agent following rat spinal cord compression or contusion injury. *Exp Neurol*. 2008; 213:120–136.
- Ribatti D, Vacca A, Roccaro AM, Crivellato E, Presta M. Erythropoietin as an angiogenic factor. *Eur J Clin Invest*. 2003; 33:891–896. [PubMed: 14511361]
- Ribatti D. Erythropoietin and cancer, a double-edged sword. *Leuk Res*. 2009; 33:1–4. [PubMed: 18620752]
- Robertson C, Cherian L, Shah M, Garcia R, Cruz-Navarro J, Grill RJ, Hand CC, Tian TS. Neuroprotection with an erythropoietin mimetic peptide (pHBSP) in a model of mild traumatic brain injury complicated by hemorrhagic shock. *J Neurotrauma*. May 5.2011 [epub ahead of print] doi:10.1089/neu.2011.1827.
- Schuster SJ, Wilson J, Erslev AJ, Caro J. Physiologic regulation and tissue localization of renal erythropoietin mRNA. *Blood*. 1987; 70:316–318. [PubMed: 3593969]
- Sirén AL, Fratelli M, Brines M, Goemans C, Casagrande S, Lewczuk P, Keenan S, Gleiter C, Pasquali C, Capobianco A, Mennini T, Heumann R, Cerami A, Ehrenreich H, Ghezzi P. Erythropoietin prevents neuronal apoptosis after cerebral ischemia and metabolic stress. *Proc Natl Acad Sci USA*. 2001; 98:4044–4049. [PubMed: 11259643]
- Sola A, Rogido M, Lee BH, Genetta T, Wen TC. Erythropoietin after focal cerebral ischemia activates the Janus kinase-signal transducer and activator of transcription signaling pathway and improves brain injury in postnatal day 7 rats. *Pediatr Res*. 2005a; 57:481–487. [PubMed: 15718373]
- Sola A, Wen TC, Hamrick SE, Ferriero DM. Potential for protection and repair following injury to the developing brain: a role for erythropoietin? *Pediatr Res*. 2005b; 57:110R–117R.
- Solaroglu I, Solaroglu A, Kaptanoglu E, Dede S, Haberal A, Beskonakli E, Kilinc K. Erythropoietin prevents ischemia-reperfusion from inducing oxidative damage in fetal rat brain. *Childs Nerv Syst*. 2003; 19:19–22. [PubMed: 12541081]
- Sytkowski, AJ. Erythropoietin: blood, brain and beyond. Wiley; Weinheim: 2004.
- Tagliaferri F, Compagnone C, Korsic M, Servadei F, Draus J. A systematic review of brain injury epidemiology in Europe. *Acta Neurochir (Wien)*. 2006; 148:255–268. [PubMed: 16311842]
- Talving P, Lustenberger T, Kobayashi L, Inaba K, Barmparas G, Schnuriger B, Lam L, Chan LS, Demetriades D. Erythropoiesis stimulating agent administration improves survival after severe traumatic brain injury: a matched case control study. *Ann Surg*. 2010; 251:1–4. [PubMed: 19779323]
- Ueba H, Brines M, Yamin M, Umemoto T, Ako J, Momomura S, Cerami A, Kawakami M. Cardioprotection by a nonerythropoietic, tissue-protective peptide mimicking the 3D structure of erythropoietin. *Proc Natl Acad Sci USA*. 2010; 107:14357–14362. [PubMed: 20660739]
- Wang L, Zhang Z, Wang Y, Zhang R, Chopp M. Treatment of stroke with erythropoietin enhances neurogenesis and angiogenesis and improves neurological function in rats. *Stroke*. 2004; 35:1732–1737. [PubMed: 15178821]
- Wang L, Zhang ZG, Zhang RL, Gregg SR, Hozeska-Solgot A, LeTourneau Y, Wang Y, Chopp M. Matrix metalloproteinase 2 (MMP2) and MMP9 secreted by erythropoietin-activated endothelial cells promote neural progenitor cell migration. *J Neurosci*. 2006; 26:5996–6003. [PubMed: 16738242]
- Warnecke C, Zaborowska Z, Kurreck J. Differentiating the functional role of hypoxia-inducible factor (HIF)-1 α and HIF-2 α (EPAS-1) by the use of RNA interference: erythropoietin is a HIF-2 α target gene in Hep3B and Kelly cells. *FASEB J*. 2004; 18:1462–1464. [PubMed: 15240563]

- Wei L, Han BH, Li Y, Keogh CL, Holtzman DM, Yu SP. Cell death mechanism and protective effect of erythropoietin after focal ischemia in the whisker-barrel cortex of neonatal rats. *J Pharmacol Exp Ther*. 2006; 317:109–116. [PubMed: 16357210]
- Wolf RF, Peng J, Friese P, Gilmore LS, Burstein SA, Dale GL. Erythropoietin administration increases production and reactivity of platelets in dogs. *Thromb Haemost*. 1997; 78:1505–1509. [PubMed: 9423803]
- Wu Y, Shang Y, Sun S, Liang H, Liu R R. Erythropoietin prevents PC12 cells from 1-methyl-4-phenylpyridinium ion-induced apoptosis via the Akt/GSK-3 β /caspase-3 mediated signaling pathway. *Apoptosis*. 2007a; 12:1365–1375. [PubMed: 17508273]
- Wu Y, Shang Y, Sun S, Liu R. Antioxidant effect of erythropoietin on 1-methyl-4-phenylpyridinium-induced neurotoxicity in PC12 cells. *Eur J Pharmacol*. 2007b; 564:47–56. [PubMed: 17362920]
- Xiong Y, Mahmood A, Lu D, Qu C, Goussev A, Schallert T, Chopp M. Role of gender in outcome after traumatic brain injury and therapeutic effect of erythropoietin in mice. *Brain Res*. 2007; 1175:301–312. [PubMed: 17976541]
- Xiong Y, Mahmood A, Lu D, Qu C, Kazmi H, Goussev A, Zhang ZG, Noguchi CT, Schallert T, Chopp M. Histological and functional outcomes after traumatic brain injury in mice null for the erythropoietin receptor in the central nervous system. *Brain Res*. 2008; 1230:247–257. [PubMed: 18657521]
- Xiong Y, Mahmood A, Meng Y, Zhang Y, Qu C, Schallert T, Chopp M. Delayed administration of erythropoietin reducing hippocampal cell loss, enhancing angiogenesis and neurogenesis, and improving functional outcome following traumatic brain injury in rats: comparison of treatment with single and triple dose. *J Neurosurg*. 2010a; 113:598–608. [PubMed: 19817538]
- Xiong Y, Mahmood A, Qu C, Kazmi H, Zhang ZG, Noguchi CT, Schallert T, Chopp M. Erythropoietin improves histological and functional outcomes after traumatic brain injury in mice in the absence of the neural erythropoietin receptor. *J Neurotrauma*. 2010b; 27:205–215. [PubMed: 19715391]
- Zarychanski R, Turgeon AF, McIntyre L, Fergusson DA. Erythropoietin-receptor-agonists in critically ill patients: a meta-analysis of randomized clinical trials. *CMAJ*. 2007; 77:725–34. [PubMed: 17823140]
- Zhang F, Signore AP, Zhou Z, Wang S, Cao G, Chen J. Erythropoietin protects CA1 neurons against global cerebral ischemia in rat: potential signaling mechanisms. *J Neurosci Res*. 2006; 83:1241–1251. [PubMed: 16511866]
- Zhang F, Wang S, Cao G, Gao Y, Chen J. Signal transducers and activators of transcription 5 contributes to erythropoietin-mediated neuroprotection against hippocampal neuronal death after transient global cerebral ischemia. *Neurobiol Dis*. 2007; 25:45–53. [PubMed: 17008107]
- Zhu L, Wang HD, Yu XG, Jin W, Qiao L, Lu TJ, Hu ZL, Zhou J. Erythropoietin prevents zinc accumulation and neuronal death after traumatic brain injury in rat hippocampus: in vitro and in vivo studies. *Brain Res*. 2009; 1289:96–105. [PubMed: 19615349]

Figure 1.

Neuroprotective signaling pathways employed by Erythropoietin. During a hypoxic event, HIF-1 α stabilization is promoted, which in turn activates hypoxia-sensitive genes (including EPO) inducing transcription of endogenous EPO. Endogenous and exogenous EPO are capable of binding the extracellular EPO receptor (EPOR), causing its homodimerization. Once EPO has coupled with EPOR, JAK2 is phosphorylated leading to several downstream signaling pathways including Ras-mitogen activated protein kinase (MAPK), phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) and the transcription factor signal transducers and activators of transcription-5 (STAT-5). PI3-K activation leads to AKT phosphorylation which may inhibit GSK3 and subsequent caspase formation. Moreover, AKT inhibits p53 activity through mdm2 activation (p53 acts as pro-apoptotic by stimulating cytochrome c release from mitochondria via Bad/Bax) leading to a diminished cytochrome c release through inhibition of Bad/Bax functioning. Additionally, EPO has been shown to enhance the antioxidant defense mechanisms, which are possible after unbounding of NF κ -B from its inactive complex, which in turn induces transcription of p53, x-linked (xIAP) and cellular (cIAP) inhibitors of apoptosis, superoxide dismutase and anti-apoptotic genes (Bcl-2 and Bcl-xL). Nuclear transcription of Bcl-2 and Bcl-xL is also possible via the STAT-5 pathway induced by JAK2 phosphorylation. Binding of EPO to its receptor (EPOR) on the endothelial cells leads to phosphorylation of JAK2, which activates PI3-K/AKT pathway, causing endothelial nitric oxide (NO) synthase phosphorylation; then increasing NO production resulting in cerebral vasodilation. Finally, EPO also induces Ras-Raf pathway, activating MAPK leading to phosphorylation of ERK-1/2 inducing the transcription of anti-apoptotic genes.