Neuroprotective effects of erythropoietin on neurodegenerative and ischemic brain diseases: the role of erythropoietin receptor

Carolina Castillo Hernández, Carlos Felipe Burgos, Angela Hidalgo Gajardo, Tiare Silva-Grecchi, Javiera Gavilan, Jorge Roberto Toledo, Jorge Fuentealba

1 Laboratory of Screening of Neuroactive Compounds, Department of Physiology, School of Biological Sciences, University of Concepción, Concepción, Chile
2 Laboratory of Biotechnology and Biopharmaceutical, Department of Pathophysiology, School of Biological Sciences, University of Concepción, Concepción, Chile

How to cite this article: Castillo C, Burgos CF, Hidalgo A, Silva-Grecchi T, Gavilan J, Toledo JR, Fuentealba J (2017) Neuroprotective effects of erythropoietin on neurodegenerative and ischemic brain diseases: the role of erythropoietin receptor. Neural Regen Res 12(9):1381-1389.

Funding: This work was supported by the Innova Proyect, No. 13IDL218688, Fondecyt Proyect, No. 1130747, 1161078, PhD CONICYT Grant, No. 21130386.

Abstract

Erythropoietin (Epo) is a fundamental hormone in the regulation of hematopoiesis, and other secondary roles mediated by the binding of the hormone to its specific receptor (EpoR), which leads to an activation of key signaling pathways that induce an increase in cell differentiation, apoptosis control and neuroprotection. It has been suggested that their function depends on final conformation of glycosylations, related with affinity to the receptor and its half-life. The presence of EpoR has been reported in different tissues including central nervous system, where it has been demonstrated to exert a neuroprotective function against oxidative stress conditions, such as ischemic injury and neurodegenerative diseases. There is also evidence of an increase in EpoR expression in brain cell lysates of Alzheimer’s patients with respect to healthy patients. These results are related with extensive in vitro experimental data of neuroprotection obtained from cell lines, primary cell cultures and hippocampal slices. Additionally, this data is correlated with in vivo experiments (water maze test) in mouse models of Alzheimer’s disease where Epo treatment improved cognitive function. These studies support the idea that receptor activation induces a neuroprotective effect in neurodegenerative disorders including dementias, and especially Alzheimer’s disease. Taken together, available evidence suggests that Epo appears to be a central element for EpoR activation and neuroprotective properties in the central nervous system. In this review, we will describe the mechanisms associated with neuroprotection and its relation with the activation of EpoR in order to identify new targets to develop pharmacological strategies.

Key Words: erythropoietin; erythropoietin receptor; neuroprotection; anti-apoptosis; Alzheimer’s disease

Erythropoietin (Epo) and Its Physiological Role

Epo is a glycoprotein hormone belonging to the superfamily of type I cytokines and is mainly responsible for the proliferation, differentiation and maturation of erythroid cells, in both embryonic and adult stages (Bunn, 2013). Epo is composed of 165 amino acids arranged into a globular three-dimensional structure, consisting of four amphipathic helices connected by loops and stabilized by two internal disulfide bridges between cysteines 7–161 and 29–33, which are important to maintain its biological activity, and this basic conformation represents about 60% of its molecular mass (~ 34 kDa) (Batmunkh et al., 2006; Bunn, 2013). This hormone is a glycoprotein characterized by having a glycosylation pattern conformed by 4 glycosylated chains, 3 of them being N-glycosylations located at positions Asn-24, Asn-38 and Asn-83, and has a fourth string O-glycosylated at residue Ser-126 (Sinclair, 2013), responsible for 40% of its molecular weight (Figure 1). Glycosylation distribution gives a great heterogeneity to the mature protein, while the absence of glycosylation reduces the stability of intermediate species and causes changes in the binding kinetics of the Epo receptor (EpoR) (Jiang et al., 2014). Epo glycosylations are mainly conformed by tetra-antennas with α(2-6) N-acetyleneuraminic acid (Neu5Ac α2-6) and N-acetylgalcosamine (GlcNAc) terminations attached to the mannose core (Toledo et al., 2006; Montesino et al., 2008; Parra and Rodriguez, 2012). Neu5Ac terminals have a direct relation to molecular half-life, while also protecting it from free radical degradation (Ingley, 2012; Parra and Rodriguez, 2012). On the other hand, the negative charge contributed by the sialic acid is responsible for the acidic properties of the molecule, having an isoelectric point between 3.31 and 4.11 (Morimoto et al., 1996).

Additionally, the oligosaccharide chains attached to Epo increase its molecular size and thereby prevent glomerular filtration, while the presence of terminal neuraminic acids prevent the exposure of galactose residues, which are taken up by hepatic transporters, increasing the plasma half-life of the hormone (Jelkmann, 2008). Epo glycosylations are necessary to facilitate its transport in plasma, and for its passage from blood to bone marrow through endothelial cells in the
blood-bone marrow barrier (Jelkmann and Wagner, 2004). During fetal development, Epo is produced in the liver, while during adulthood it is mainly synthesized in the peritubular cells of the kidney (80%) in order to compensate for oxygen decrease during hypoxia (Bunn, 2013). This function is developed through the hypoxia inducible factor (HIF-1) transcription factor (Toledo et al., 2006; Parra and Rodriguez, 2012), which stimulates Epo gene transcription by binding to an enhancer that flanks the 3′ region of the gene, increasing hormonal production and release into the plasma. Epo effects are produced by Epo/EpoR interaction, which activates signaling cascades that act on the control of apoptosis, decreasing the rate of cell death in the bone marrow during the final stages of the development of erythroid progenitor cells, especially during the colony forming unit-erythroid (CFU-E) stage (Fisher, 2003). This activation induces cell proliferation and maturation from normoblasts to reticulocytes, which are characterized by elimination of the nucleus and release from the bone marrow to blood circulation where final differentiation to erythrocytes occurs, having a half-life of approximately 120 days in normal adults (Sinclair, 2013). The circulating hormone promotes the proliferation, differentiation and maturation of erythroid progenitors to erythrocytes, which ultimately improves the oxygen transport as their key function (Maiese et al., 2012; Bunn, 2013). The normal plasma range of Epo in healthy individuals is 10–20 (mIU/mL) with a half-life of 7–8 hours (Toledo et al., 2006; Bunn, 2013).

Furthermore, in addition to being produced in the kidney, secondary Epo production sites have been described in retinal cells and astrocytes, suggesting that this hormone has a function not only in hematopoiesis, but also in different tissue types participating in cellular protection from stress and preventing apoptosis. Epo expression in non-erythroid tissue accounts for about 15–20% of the total production throughout the organism (Ponce et al., 2013).

Epo deficiency induces severe anemia in patients with chronic renal failure and renal failure associated with trauma, intensive drug treatments (patients with human immunodeficiency virus (HIV) or chemotherapy) or in patients with kidney transplants (Rabie and Marti, 2008). In these cases, external supply of the hormone is a key treatment. Epo was the first hematopoietic growth factor cloned and is currently one of the most sold biopharmaceutical products worldwide, according to the Food and Drug Administration (FDA). Recombinant human Epo (rhEpo) is indicated in the treatment of patients with anemia and diseases associated with low concentrations of Epo in plasma; additionally, it has been described that these patients show significant cognitive improvement (Sargin et al., 2011; Jiang et al., 2014).

**EpoR**

EpoR is a transmembrane receptor member of the type I cytokine superfamily, with a molecular weight of 66 kDa, which is pre-formed as homodimers on the cell surface with a density of about 1,000/cell (CFU-E) in bone marrow (Bunn, 2013). The binding of Epo to its receptor induces a conformational change that results in the autophosphorylation by Janus kinase-2 (JAK-2). The protein trans-phosphorylates, which induce phosphorylation on tyrosine residues located in the cytoplasmic region of EpoR, create a binding site for other proteins during the amplification of the signaling cascade (Losdih et al, 2013). Signaling pathways activated by EpoR include signal transducer and activator of transcription 5 (STAT-5), phosphoinositol 3′-kinase (PI3K), mitogen-activated protein kinase (MAPK), and protein kinase c (PKC) (Figure 2). This eventually leads to the regulation of cell cycle progression through phosphorylated protein kinase B (Akt), and also positively regulates anti-apoptotic proteins such as B-cell lymphoma 2 (Bcl2) and B-cell lymphoma-extra large (Bclxl) (Shen et al., 2010). The activation of EpoR also acts by directly inhibiting the action of pro-apoptotic proteins such as cytochrome c, or through p53 activation (Brines and Cerami, 2005). The Epo region that binds with higher affinity to EpoR is characterized by possession of several highly hydrophobic residues.

![Figure 1 Glycosylation of erythropoietin.](image)

**Figure 1 Glycosylation of erythropoietin.**

(A) Structure of human erythropoietin obtained from the PDB: 1EER. (B) N-glycosylated model of erythropoietin generated combining each tetra-antennas glycan to their respective amino acids in erythropoietin sequence, N24, N38 and N83. The presence of N-glycosylations did not compromise the binding site to the erythropoietin receptor. All amino acids involved in erythropoietin-erythropoietin receptor interaction were colored magenta and define regions which remain largely conserved between both glycosylation states.
Figure 2 Pathways involved in EpoR activation.
Principal signal pathways activated to induce neuroprotection when Epo interacts with EpoR or EpoRβ (expressed in CNS) in response to hypoxia or different types of stress, such as ischemic brain events or inflammation induced by Alzheimer’s disease. Epo or EPO: Erythropoietin; EpoR: Epo receptor; CNS: central nervous system; HiF-1: hypoxia inducible factor; Aβ: beta amyloid peptide; ATP: adenosine triphosphate; JAK: Janus kinase; ROS: reactive oxygen species; GSK-3β: glycogen synthase kinase 3 beta; AKT: protein kinase B; PI3K: phosphoinositol 3′-kinase; STAT5: signal transducer and activator of transcription 5; MeK-1: mitogen-activated protein kinase; Erk1,2: extracellular-regulated kinases 1,2; Bcl2: B-cell lymphoma 2; BclXL: B-cell lymphoma-extra large.

...such as Phe-138, Phe-142, Tyr-145, Phe-148, Leu-153 and Tyr-156, that are attached to the nonpolar chains of Epo, forming a hydrophobic core which interacts with a specific intrasubunit receptor motif (“WSEWS motif”, residues 209–213) directly related to receptor expression, translocation to cell membrane and its activation (Cheetham et al., 1998). It was shown that there are two regions of the Epo molecule that bind to the receptor: the first binds to a region that has a high affinity (K_M of 1 nM), and a second region that has a lower affinity, one thousand times smaller (K_M 1 μM) (Cheetham et al., 1998).

EpoR production is inefficient, only 10% of the receptor is transported to the cell membrane, and its transport requires the binding of the N-terminal portion of JAK-2 to the cytoplasmic region of the receptor in the endoplasmic reticulum in order to promote proper folding of the protein and sorting to the surface of the cell membrane. Hence, JAK-2 is essential for EpoR signaling when it is activated (Javadi et al., 2012).

However, expression of EpoR has also been observed in non-erythroid tissues, explaining the pleiotropic effects described for Epo (Lappin et al., 2002; Jelkmann and Wagner, 2004; Wagner et al., 2004; Brines and Cerami, 2005). The Epo non-canonical receptor expressed in non-erythroid tissues is structurally different from the classical EpoR because it is a heterodimer composed of a monomer of the canonical EpoR and another subunit of the “β-common receptor”, also known as CD131 (EpoR-β comm), which is identical to the beta region of cytokine receptors such as interleukin-3 (IL3) receptors or granulocyte macrophage colony-stimulating factor (GMSCF) receptors (Brines et al., 2004; Su et al., 2011) (Figure 3). It is important to mention that heterodimers of the non-canonical receptor are involved in the neuroprotective effects exerted by Epo on in vitro assays, similar to the ones observed with classical homodimer EpoR conformations. These results reinforce the idea that the activation of EpoRs on homodimer or heterodimer conformations is necessary to obtain neuro-
protective effects (Jelkmann and Wagner, 2004; Wei et al., 2006; Yu et al., 2016).

EpoR is expressed in the central nervous system (CNS) as a heterodimer (EpoR-β comm), suggesting that Epo may play a specific role in this tissue (Brines and Cerami, 2005; Rabie and Marti, 2008). It has been reported that the involvement of EpoR is critical for in vitro neuroprotection. For example, a neuroprotective effect was observed in PC12 cells pretreated with Epo for 1 hour previous to treatment with beta amyloid peptide (Aβ)25–35 peptide during 6 or 12 hours, and this effect was lost when an inhibitor of STAT-5 (a major signaling pathway activated by EpoR) was used (Ma et al., 2014). Similarly, in vivo neuroprotection was observed in rats pretreated with Epo for 10 minutes before inducing ischemia by a middle cerebral artery occlusion model (MCAO), where the neuroprotective effect was evaluated by measuring the area of damaged tissue in histological sections, showing a decrease in necrotic tissue and in the percentage of edema in animals pretreated with Epo (Ratilal et al., 2014). Also, an increased expression of EpoR has been reported in ischemic brain regions in animals treated with different doses of Epo (Yu et al., 2005; Castañeda-Arellano et al., 2014). Similar neuroprotective effects with pretreatments of Epo were reported in other animal models, such as Drosophila melanogaster that were subjected to stress periods, under conditions of hypoxia, which also showed that this effect is dependent on activation of EpoR (Miljus et al., 2014; Yu et al., 2016).

**Different Isoforms of Epo**

One disadvantage of Epo as a neuroprotective agent is its natural capacity to increase the hematocrit, which can quickly elevate blood influx to brain tissue, increasing the risk of cerebrovascular reperfusion. Thus, it is necessary to increase the range of doses to obtain neuroprotective effects when Epo is used as a peripheral treatment. Therefore, different ways to activate EpoR with minimal hematopoietic effects have been described (Pankratova et al., 2010). Since then, different isoforms of Epo have been designed, especially with chemical modifications to increase its efficacy. Masuda et al. (1999) demonstrated that when sialic acids are removed, the molecule increases its affinity for EpoR. Furthermore, a difference has been observed between systemic-Epo and CNS-Epo due to a modification in the glycosylation tree (Masuda et al., 1993). Epo produced in the retina and brain also differs from the variant produced by the peritubular cells in kidney, with respect to its sialic acid substitutions; the former almost completely devoid of sialic acid substituents called neuro-EPO (Moon et al., 2006; Parra and Rodriguez, 2012).

Another chemical modification reported to reduce the hematopoietic effect is carbamylation of lysine residues that cause a conformational and functional change on Epo because all lysines are replaced by homocitrulline, totally depleting the hematopoietic effect (Leist et al., 2004; Wang et al., 2004). On the other hand, the synthesis of peptides that mimic the interaction zone between Epo and EpoR, promoting its activation without hematopoietic effects, has been described, but the half-life of the hormone is extremely short (around 4 minutes) (Brines and Cerami, 2008; Brines et al., 2008). These peptides were modified to improve stability and to increase its half-life, obtaining a new peptide-version with a high affinity to EpoR, converting them into potential agonists of the EpoR that can produce a decrease in inflammation, providing cytoprotection (Pankratova et al., 2010; Zellinger et al., 2011; Liu et al., 2015).

**Neuroprotective Effects of Epo on Ischemic Injury**

Ischemic injury is characterized by a decrease in adenosine triphosphate (ATP), disruption of the Na+/K+ pump and ATPase, which results in severe ionic dyshomeostasis. In the brain, those events are followed by an enhanced excitotoxic tone mediated by glutamate, acidosis, calcium channel dysfunction, release of nitric oxide (NO), plasma membrane disruption, and release of pro-inflammatory cytokines, finally leading to activation of “death receptors” and apoptosis. Thus, caspase activation, especially caspase 3 which is overexpressed in neuronal apoptosis processes, could be the cause of cell death by ischemic injury (Lu et al., 2014; Grupke et al., 2015; Siddiqui et al., 2015).

Epo treatment (in vitro or in vivo) induces neuroprotection against different forms of injury, including ischemic toxicity (Gui et al., 2011). It has been reported that Epo and EpoR expression in the brain is minimal, but in the case of injury, such as ischemia, an increase in EpoR occurs, especially on endothelial cells, astrocytes, and even neurons (Sargin et al., 2011; Wu et al., 2011; Zhang et al., 2011; Ott et al., 2015). In fact, it has been reported that Epo can protect neurons in vitro during hypoxia events, where EpoR is up-regulated by those same events (Parra and Rodriguez, 2012). On the other hand, it has been reported that Epo can modulate the activity of channels by regulating the cell volume and neurogenesis on areas affected by ischemic injury and reperfusion (Iwai et al., 2007, 2010). The neuroprotective effects of Epo in vivo have been reported in the MCAO model, where a 35% reduction was observed for the edema and necrotic zones, versus the healthy zone (Zhang et al., 2014). In addition, Epo treated animals showed an increase in EpoR expression in brain ischemic zones (Yu et al., 2005, 2016; Castañeda-Arellano et al., 2014; Ratilal et al., 2014).

In hypoxia models developed on cardiomyocytes, different doses of Epo induced a dose-dependent upregulation of peroxisome proliferator-activated receptor γ co-activator 1 α (PGC1α) mRNA, a key regulator of mitochondria biogenesis, and also increased the number of mitochondria. These results suggest a positive effect of the hormone in mitochondrial biogenesis, and anti-apoptotic mechanisms (Qin et al., 2014). Epo has been associated with an inhibition of mitochondrial permeability transition pore opening, reduction in brain edema and caspase-3 expression in rats with traumatic brain injury, suggesting a direct relationship between neuro-
Epo and AD

The Aβ in AD is responsible for the loss in ionic homeostasis, due to its ability to perforate the cell membrane, forming a pore which allows the massive entrance of cations, especially calcium, inducing apoptosis (Kumar et al., 2015). It has been described the existence of molecules that are able to prevent the activation of the apoptotic pathway in cellular models pretreated with amyloid beta peptide, Epo being one of these. Activation of EpoR initiates transcription of Bcl2 genes that block apoptosis and prevent cell death (Sargin et al., 2010; Esmaeili Tazangi et al., 2015). It has been proposed that Epo-induced neuroprotection is mediated by EpoR activation, followed by an increase in nuclear factor kappa-light-chain-enhancer activity of activated B cells (NFκB), causing an inhibition of apoptotic proteins and blocking the activation of caspases induced after the typical accumulation of TNFa observed in AD patients (Stefani et al., 2006). The blockade of caspase activity is directly related with other pathways activated by EpoR, e.g., the activation of Bcl-XL that causes a decrease in ROS (Stefani et al., 2006; Pasqualetti et al., 2015; Buendia et al., 2016). The relationship between these two effects and the activation of EpoR has been demonstrated through the silencing of EpoR in astroglia, using siRNA techniques (Lourhamati et al., 2013; Zhou and Yu, 2013), and also, in vitro experiments using PC12 cells demonstrated the neuroprotective effect of Epo against toxicity induced by Aβ. Studies have shown that the protective effects of Epo depend on its concentration, the activation of its receptor and also the activation of JAK/STAT, PI3K, and RAS pathways that increase phosphorylation.
ed AKT, which was significantly increased in cells treated with Aβ25–35 and Epo, and confirmed by the use of the PI3K inhibitor LY294002 (Ma et al., 2009; Shen et al., 2010; Shang et al., 2012). Also, it has been found that Epo increases the phosphorylation of glycogen synthase kinase 3 beta (GSK-3β) via the PI3K/AKT pathway (Zhou and Yu, 2013), causing its inhibition. This event may represent a downstream mechanism by which Epo exerts its protective effects against Aβ25–35-induced apoptosis (Ma et al., 2014). Furthermore, it was demonstrated that Epo treatments induced up-regulation of Bcl-2, contributing to the neuroprotection against Aβ toxicity (Chong et al., 2013; Jia et al., 2014; Ma et al., 2014).

The cytoprotective and anti-apoptotic effects of Epo have also been described in studies using the microglial cell line EOC2, where these effects could be associated with Wnt1 and PI3K-mediated pathways, also suggesting the involvement of the mammalian target of rapamycin (mTOR) pathway in the protection of microglial cells during Aβ toxicity (Ott et al., 2015). Thus, it has been shown that Wnt1 is a central component in the promotion of microglial integrity, preventing the loss of these cells during early and late apoptotic injury by Aβ exposure (Ott et al., 2015). Wnt1 regulates the apoptotic cascade by maintaining the mitochondrial membrane potential, phosphorylating and fostering the translocation of Bad from the mitochondria to the cytosol, reducing the formation of Bad/Bcl-xL complex, and increasing the formation of the Bcl-xL/Bax complex, and blocking the activation of caspase 1 and caspase 3 through Bcl-xL (Maiese et al., 2012). Application of Epo (10 ng/mL) in microglia significantly maintained the expression of Wnt1 at 6 hours after Aβ exposure, which may suggest another probable mechanism to prevent Aβ-induced toxicity (Shang et al., 2012).

In addition, this study also shows that Epo treatments can maintain mitochondrial membrane potential during Aβ exposure in microglial models, indicating that Epo can block apoptotic signaling like caspase activity during brain injury and neurodegeneration (Shang et al., 2012).

On the other hand, an interesting role has been suggested for EpoR regarding neurite growth during neurogenesis of hippocampal cells, and also in decreasing apoptosis in cells from the dentate gyrus in an AD model, via STAT-5 activation pathway, among others (Pankratova et al., 2010; Zellinger et al., 2011; Arabpoor et al., 2012). In the parallel, several studies have assessed the improvement in cognitive functions and spatial memory in animal AD models treated with several intraperitoneal doses of Epo (Adamcio et al., 2008; Pankratova et al., 2010). In the hippocampus, the effect of Epo was shown to be selective for memory, since no effect was seen on anxiety, spontaneous activity, exploratory behavior, or motor performance (Adamcio et al., 2008). All this evidence suggests the exciting idea that the selective effect of Epo on the enhancement of memory could be medi-

---

**Figure 3 In silico prediction of interaction between Epo and EpoR/EpoRβ.**

(A) Complex Epo/EpoR generated by molecular docking where amino acids on the surface of EpoR involved in the interaction with Epo have been highlighted and colored according to their corresponding chain. For the classical EpoR, its functional conformation corresponds to a homodimer structure, and the two colors used (gray and light blue) for each chain differ only for schematic purposes. (B) Complex Epo/EpoRβ generated in a manner similar to the previous complex, with the exception that EpoRβ is a functional heterodimer, incorporating the beta common subunit formed by two interlaced chains colored red and orange, respectively. The number of amino acids in the interface is slightly lower than Epo/EpoR, however, both receptors are able to interact with Epo in a stable and energetically favorable manner. Epo: Erythropoietin; EpoR: Epo receptor.
ated by the activation of EpoR on hippocampus, representing a novel target for the treatment of neurodegenerative diseases.

Other studies have found a decrease of lipid peroxidation in brain samples from AD animals treated with Epo, which correlates with an increase of Bax levels (Chen et al., 2015). Also, it has been shown that Epo selectively induces the synthesis of the neuroglobin protein in damaged regions, promoting angiogenesis and protection of the vascular endothelium, which are key factors that contribute to neurogenesis and neuroplasticity in injured brain tissue (Maurice et al., 2013; Esmaeili Tazangi et al., 2015; Maiese, 2016a, b; Ding et al., 2017).

According to the previously commented results, the Epo treatment on AβPP/PS1 transgenic mice showed reduction of the cortical areas positive for Aβ plaques in around 20%, after treatment during 3 days/week for 4 weeks, correlating this evidence with results showing an enhancement in the performance of these transgenic mice on V-maze trials (Armand-Ugón et al., 2015; Ott et al., 2015).

In agreement with results for the requirement of EpoR activation, other studies have reinforced this idea with more data using carbamylated Epo (CEPO), and histological analysis found that the area covered by Aβ plaques in the cerebral cortex (calculated with respect to the total cerebral cortex area) of AβPP/PS1 transgenic mice was 1.3%, and that this value was reduced by 20% after treatment with Epo during 3 days/week for 4 weeks. Later, these mice were evaluated by the V-maze test to assess memory function and these data indicate that chronic treatment with EPO and CEPO improved memory of AβPP/PS1 animals to levels similar to wild type animals (Armand-Ugón et al., 2015; Ott et al., 2015).

Using field stimulation experiments, it has been demonstrated that Epo treatments on the Schaffer collateral-CA1 hippocampal areas are able to revert the extreme inhibition in long-term potentiation (LTP) induced by Aβ23-35 also showing a decrease in glutamate release in the same animal model of AD (Esmaeili Tazangi et al., 2015). Other research suggests Epo is able to recover GSK-3β activity, which is inhibited by Aβ23 (Gan et al., 2012; Ponce et al., 2013; Li et al., 2015).

Finally, current evidence suggests that Epo exerts its neuroprotective effects through activation of its receptor (EpoR), resulting in the activation of several pathways that block apoptosis and thereby reducing the damage induced by different types of stress, such as inflammatory or ischemic brain processes or neurodegenerative diseases that produce synaptic disconnection and/or silencing. Therefore, the development of new Epo isoforms lacking hematopoietic effects could be considered new potential targets, able to exert neuroprotection without the risk of vascular damage.

Author contributions: CC, IRT, and JF wrote, corrected and edited the main text. AH made a focused bibliographics revision in ischemia. CFB made the docking analyses. JG, TSG, and CC built the final scheme.

Conflicts of interest: None declared.
Chong ZZ, Shang YC, Mu Y, Cui S, Yao Q, Maiese K (2013) Targeting erythropoietin for chronic neurodegenerative diseases. Expert Opin Ther Targets 17:707-720.

Ding J, Wang J, Li QY, Yu JZ, Ma CG, Wang X, Lu CZ, Xiao BG (2017) Neuroprotection and CD131/GDNF/AKT pathway of carboxamylated erythropoietin in hypoxic neurons. Mol Neurobiol 54:5051-5060.

Ehrenreich H, Weissend B, Prange H, Schneider D, Weimar C, Wartenberg K, Schellinger PD, Bohn M, Becker H, Wegrzyn M, Jahng P, Herrmann K, Knauth M, Bähr M, Heide W, Wagner A, Schwab S, Reichmann H, Schwendemann G, Dengler R, et al. (2009) Reombinant human erythropoietin in the treatment of acute ischemic stroke. Europ 40:647-656.

Ehrenreich H, Hasselblatt M, Dembowski C, Cepek L, Lewczuk P, Stiefel M, Rustenberg HH, Breiter N, Jacob S, Kernlich F, Bohn M, Poser W, Ruther E, Kochen M, Gefeller O, Gleiter C, Wessel TC, De Ryck M, Itri L, Prange H, et al. (2002) Erythropoietin therapy for acute stroke is both safe and beneficial. Mol Med 8:495-505.

Esmaeili Tazangi P, Moosavi SM, Shabani M, Haghani M (2015) Erythropoietin improves synaptic plasticity and memory deficits by decrease of the neurotransmitter release probability in the rat model of Alzheimer's disease. Pharmacol Biochem Behav 130:15-21.

Fisher JW (2003) Erythropoietin: physiology and pharmacology update. Exp Biol Med (Maywood) 228:1-14.

Gan Y, Xing J, Jing Z, Stetler RA, Zhang F, Luo Y, Ji X, Gao Y, Cao G (2012) Mutant erythropoietin without erythropoietic activity is neuroprotective against ischemic brain injury. Stroke 43:3071-3077.

Grupke S, Hall J, Dobbs M, Bix GJ, Fraser JF (2015) Understanding history and, not repeating it. Neuroprotection for acute ischemic stroke: from review to perspective. Clin Neurol Neurosurg 129:1-9.

Gui DM, Yang Y, Li X, Gao DW (2011) Effect of erythropoietin on the expression of HIF-1 and iNOS in retina in chronic ocular hypertension rats. Int J Ophthalmol 4:40-43.

Ingleby J (2012) Integrating novel signaling pathways involved in erythropoiesis. IUBMB Life 64:402-410.

Iwai M, Cao G, Yin W, Stetler RA, Liu J, Chen J (2007) Erythropoietin promotes neuronal replacement through revascularization and neurogenesis after neonatal hypoxia-ischemia in rats. Stroke 38:2795-2803.

Maiese K (2016a) Charting a course for erythropoietin in traumatic brain injury. J Transl Sci 2:140-144.

Maiese K (2016b) Regeneration in the nervous system with erythropoietin. Front Biosci (Landmark Ed) 21:561-596.

Maiese K, Li F, Chong ZZ (2005) New avenues for exploration for erythropoietin. JAMA 293:90-95.

Maiese K, Chong ZZ, Shang YC, Wang S (2012) Erythropoietin: new directions for the nervous system. Int J Mol Sci 13:11102-11129.

Masuda S, Kada E, Nagao M, Sasaki R (1999) In vitro neuroprotective action of recombinant rat erythropoietin produced by astrocyte cell lines and comparative study with erythropoietin produced by Chinese hamster ovary cells. Cytotechnology 31:179-184.

Miller L, Bouzat P, Trouve-Buisson T, Batandier C, Pernet-Gallay K, Gaide-Chevronnay L, Barber EL, Deblonn T, Fontaine E, Payen FJ (2016) Erythropoietin and its derivatives modulate mitochondrial dysfunction after diffuse traumatic brain injury. J Neurotrauma 33:1625-1633.

Montesino R, Toledo JR, Sanchez O, Sanchez A, Harvey DJ, Royle L, Dwek RA, Rudd PM, Gerwig GJ, Kamerling JP, Cremata JA (2008) Monosialylated biantennary N-glycories containing GalNAc-GlcNAc antennae predominates when human EPO is expressed in goat milk. Arch Biochem Biophys 470:163-175.

Moon C, Krawczyk M, Paik D, Coleman T, Brines M, Juhaszova M, Sollott SJ, Lakatta EG, Talan MI (2006) Erythropoietin, modified to NAc antennae predominate when human EPO is expressed in goat milk. Arch Biochem Biophys 470:163-175.

Montesino R, Toledo JR, Sanchez O, Sanchez A, Harvey DJ, Royle L, Dwek RA, Rudd PM, Gerwig GJ, Kamerling JP, Cremata JA (2008) Monosialylated biantennary N-glycories containing GalNAc-GlcNAc antennae predominates when human EPO is expressed in goat milk. Arch Biochem Biophys 470:163-175.

Moon C, Krawczyk M, Paik D, Coleman T, Brines M, Juhaszova M, Sollott SJ, Lakatta EG, Talan MI (2006) Erythropoietin, modified to NAc antennae predominate when human EPO is expressed in goat milk. Arch Biochem Biophys 470:163-175.

Montesino R, Toledo JR, Sanchez O, Sanchez A, Harvey DJ, Royle L, Dwek RA, Rudd PM, Gerwig GJ, Kamerling JP, Cremata JA (2008) Monosialylated biantennary N-glycories containing GalNAc-GlcNAc antennae predominates when human EPO is expressed in goat milk. Arch Biochem Biophys 470:163-175.

Moore JG, Libby P, Libby P (2004) Inflammation in atherosclerosis. N Engl J Med 350:1684-1695.
Nagaiškė E, Tarazewska A, Matyja E, Grieb P, Rafalowska J (2010) Neuroprotective effect of erythropoietin in amyotrophic lateral sclerosis (ALS) model in vitro. Ultrasructural study. Folia Neuropathol 48:35-44.

Noh MY, Cho KA, Kim H, Kim SM, Kim SH (2014) Erythropoietin modulates the immune-inflammatory response of a SOD1(G93A) transgenic mouse model of amyotrophic lateral sclerosis (ALS). Neurosci Lett 574:53-58.

Ott C, Martens H, Hassouna I, Oliveira B, Erck C, Zafeiriou MP, Peteri UK, Hesse D, Gerhart S, Altas B, Kolbow T, Studler H, Kawabe H, Zimmermann WH, Nave KA, Schulz-Schaeffer W, Jahn O, Ehrenreich H (2015) Widespread expression of erythropoietin receptor in brain and its induction by injury. Mol Med doi: 10.2119/molmed.2015.00192.

Pankratova S, Kryushko D, Sonn K, Soroka V, Kohler LB, Rathje M, Gu B, Gottfried K, Clausen O, Zharovskiy A, Bock E, Berezin V (2010) Neuroprotective properties of a novel, non-haematopoietic agonist of the erythropoietin receptor, Brain 133:2281-2294.

Park KH, Choi NY, Koh SH, Park HH, Kim YS, Kim MJ, Lee SJ, Yu HJ, Lee KY, Lee YJ, Kim HT (2011) L-DOPA neurotoxicity is prevented by neuroprotective effects of erythropoietin. Neurototoxicology 32:879-887.

Park SS, Park J, Ko J, Chen L, Meriage D, Crousse-Zeineddini J, Wong W, Kerwin BA (2009) Biochemical assessment of erythropoietin products from Asia versus US Epoetin alfa manufactured by Amgen. J Pharm Sci 98:1688-1699.

Parra AL, Rodríguez JC (2012) Nasal neuro EPO could be a reliable choice for neuroprotective stroke treatment. Cent Nerv Syst Agents Med Chem 12:60-68.

Pasqualetti G, Brooks DJ, Edison P (2015) The role of neuroinflammation in dementia. Curr Neurol Neurosci Rep 15:17.

Ponce LL, Navarro JC, Ahmed O, Robertson CS (2013) Erythropoietin neuroprotection with traumatic brain injury. Pathophysiology 20:31-38.

Qin C, Zhou S, Xiao Y, Chen L (2014) Erythropoietin enhances mitochondrial biogenesis in cardiomyocytes exposed to chronic hypoxia through Akt/eNOS signalling pathway. Cell Biol Int 38:335-342.

Rabie T, Marti HH (2008) Brain protection by erythropoietin: a manifold task. Physiology (Bethesda) 23:263-274.

Ratlal BO, Arroja MM, Rocha JP, Fernandes AM, Barateiro AP, Brites DM, Pinto RM, Sepodes BM, Mota-Filipe HD (2014) Neuroprotective effects of erythropoietin pretreatment in a rodent model of transient middle cerebral artery occlusion. J Neurosurg 121:55-62.

Sargin D, Friedriehs H, El-Kordi A, Ehrenreich H (2010) Erythropoietin as neuroprotective and neuroregenerative treatment strategy: comprehensive overview of 12 years of preclinical and clinical research. Best Pract Res Clin Anaesthesiol 24:573-594.

Sargin D, El-Kordi A, Agarwal A, Muller M, Wojcik SM, Hassouna I, Sperling S, Nave KA, Ehrenreich H (2011) Expression of constitutively active erythropoietin receptor in pyramidal neurons of cortex and hippocampus boosts higher cognitive functions in mice. BMC Biol 9:27.

Shang YC, Chong ZZ, Wang S, Maiest K (2012) Prevention of beta-amylloid degeneration of microglia by erythropoietin depends on Wnt1, the PI 3-K/mTOR pathway, Bad, and Bcl-xL. Aging (Albany NY) 4:187-201.

Shen J, Wu Y, Xu JY, Zhang J, Sinclair SH, Yanoff M, Xu G, Li W, Xu GT (2010) ERK- and Akt-dependent neuroprotection by erythropoietin (EPO) against glyoxal-AGEs via modulation of Bcl-xL, Bax, and BAD. Invest Ophthalmol Vis Sci 51:35-46.

Siddiqui WA, Ahad A, Ahsan H (2015) The mystery of BCL2 family: Bcl-2 proteins and apoptosis: an update. Arch Toxicol 89:289-317.

Sinclair AM (2013) Erythropoiesis stimulating agents: approaches to modulate activity. Biologics 7:161-174.

Stefani A, Martorana A, Bernardini S, Panella M, Mercati F, Orlacchio A, Pierantozzi M (2006) CSF markers in Alzheimer disease patients are not related to the different degree of cognitive impairment. J Neurol Sci 251:124-128.

Su KH, Shyu YK, Koo YR, Ching LC, Chiang AN, Yu YB, Chen CY, Pan CC, Lee TS (2011) Common receptor integrates the erythropoietin signaling in activation of endothelial nitric oxide synthase. J Cell Physiol 226:3330-3339.

Toledo JR, Sánchez O, Seguí RM, García G, Montañez M, Zamora PA, Rodríguez MP, Cremata JA (2006) High expression level of recombinant human erythropoietin in the milk of non-transgenic goats. J Biotechnol 123:225-235.

Wagner LM, Billups CA, Furman WL, Rao BN, Santana VM (2004) Combined use of erythropoietin and granulocyte colony-stimulating factor does not decrease blood transfusion requirements during induction therapy for high-risk neuroblastoma: a randomized controlled trial. J Clin Oncol 22:1886-1893.

Wang R, Wu X, Liang J, Qi Z, Liu X, Min L, Ji X, Luo Y, Zhao H (2015) Intra-artery infusion of recombinant human erythropoietin reduces blood-brain barrier disruption in rats following cerebral ischemia and reperfusion. Int J Neurosci 125:693-702.

Wang X, Zhu C, Wang X, Gerwien JG, Schrattenholz A, Sandberg M, Leist M, Blomgren K (2004) The nonerythropoietic asialoerythropoietin protects against neonatal hypoxia-ischemia as potently as erythropoietin. J Neurochem 91:900-910.

Wei L, Han BH, Li Y, Keogh CL, Holtzman DM, Yu SP (2006) Cell death mechanism and protective effect of erythropoietin after focal ischemia in the whisker-barrel cortex of neonatal rats. J Pharmacol Exp Ther 317:109-116.

Won HH, Park J, Lee E, Kim JW, Lee D (2009) Comparative analysis of the JAK/STAT signaling through erythropoietin receptor and thrombopoietin receptor using a systems approach. BMC Bioinformatics 10 Suppl 1:S53.

World Health Organization (2011) World Health Statistics 2011. http://www.who.int/whosis/whostat/2011/en/.

Wu JH, Gao Y, Ren AJ, Zhong M, Liu L (2011) Erythropoietin receptor antibody inhibits oxidative stress induced retinal neovascularization in mice. Int J Ophthalmol 4:243-246.

Yu D, Fan Y, Sun X, Yao L, Choi W (2016) Effects of erythropoietin preconditioning on rat cerebral ischemia-reperfusion injury and the GLT-1/GLAST pathway. Exp Ther Med 11:513-518.

Yu YP, Xu QQ, Zhang Q, Zhang WP, Zhang LH, Wei EQ (2005) Intranasal recombinant human erythropoietin protects rats against focal cerebral ischemia. Neurosci Lett 387:5-10.

Zellinger C, Seeger N, Hadamitzky M, Fischborn S, Russmann V, Wendt H, Pankratova S, Bock E, Berezin V, Potschka H (2011) Impact of the erythropoietin-derived peptide mimetic Epotris on the histopathological consequences of status epilepticus. Epilepsy Res 96:241-249.

Zhang G, Lehmam HC, Bogdanova N, Gao T, Zhang J, Sheikha KA (2011) Erythropoietin enhances nerve repair in anti-ganglioside antibody-mediated models of immune neuropathy. PLoS One 6:e27067.

Zhang J, Wang Q, Xiang H, Xin Y, Chang M, Lu H (2014) Neuroprotection with traumatic brain injury. Pathophysiology 20:31-38.

Zellinger C, Seeger N, Hadamitzky M, Fischborn S, Russmann V, Wendt H, Pankratova S, Bock E, Berezin V, Potschka H (2011) Impact of the erythropoietin-derived peptide mimetic Epotris on the histopathological consequences of status epilepticus. Epilepsy Res 96:241-249.

Zhang G, Lehmam HC, Bogdanova N, Gao T, Zhang J, Sheikha KA (2011) Erythropoietin enhances nerve repair in anti-ganglioside antibody-mediated models of immune neuropathy. PLoS One 6:e27067.

Zhang J, Wang Q, Xiang H, Xin Y, Chang M, Lu H (2014) Neuroprotection with erythropoietin in preterm and/or low birth weight infants. J Clin Neurosci 21:1283-1287.

Zhou TF, Yu JG (2013) Recombinant human erythropoietin alleviates neuronal apoptosis and cognitive defects via JAK2/STAT3 signaling in experimental endotoxemia. J Surg Res 183:304-312.