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REVIEW ARTICLE

Unravelling the potential neuroprotective facets of erythropoietin for the treatment of Alzheimer's disease

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Abstract

During the last three decades, recombinant DNA technology has produced a wide range of hematopoietic and neurotrophic growth factors, including erythropoietin (EPO), which has emerged as a promising protein drug in the treatment of several diseases. Cumulative studies have recently indicated the neuroprotective role of EPO in preclinical models of acute and chronic neurodegenerative disorders, including Alzheimer's disease (AD). AD is one of the most prevalent neurodegenerative illnesses in the elderly, characterized by the accumulation of extracellular amyloid-ß (Aß) plaques and intracellular neurofibrillary tangles (NFTs), which serve as the disease's two hallmarks. Unfortunately, AD lacks a successful treatment strategy due to its multifaceted and complex pathology. Various clinical studies, both in vitro and in vivo, have been conducted to identify the various mechanisms by which erythropoietin exerts its neuroprotective effects. The results of clinical trials in patients with AD are also promising. Herein, it is summarized and reviews all such studies demonstrating erythropoietin's potential therapeutic benefits as a pleiotropic neuroprotective agent in the treatment of Alzheimer's disease.

Keywords Neurodegeneration · Neuroprotection · Erythropoietin · Neuroinflammation · Oxidative stress

Introduction

Alzheimer's disease (AD) is the most prevalent cause of senile dementia and is characterized by a progressive decline in cognition. AD is considered the sixth leading cause of death with approximately 5.8 million cases worldwide. Due to the lack of a suitable treatment for AD, this number is projected to increase to 13.8 million by 2050 (Gaugler et al. 2019). Amyloid-beta (Aß) plaque deposition is demonstrated as one of the major pathological hallmarks of AD, which can even initiate 20 years before the appearance of clinical symptoms of AD. AD dementia corresponds largely with neuronal loss, and hence, reduction of neuropathological lesions (Aß plaques and NFTs) in the AD brain alone cannot reverse the disease progression. Thus, it was suggested that a therapy that combines the reduction of neuropathological lesions of AD along with neuronal repair and neurogenesis may be successful in treating AD. For this reason, it is essential to assess the therapeutic potential of distinct novel agents, and erythropoietin (EPO) is considered among one such agent. Erythropoietin (EPO), a class 1 cytokine and growth factor, is a principal regulator of erythropoiesis

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and is broadly utilized in the treatment of anemia and diseases related to low EPO levels (Rey et al. 2019; Sun et al. 2019a). Except for its role in hematopoiesis, the presence of EPO and its receptor (EPOR) in non-erythropoietic tissues, including the brain, suggested a role besides its hematopoietic effects (Rey et al. 2019; Sun et al. 2019a). Now, various studies show the neuroprotective functions of EPO in various neurodegenerative diseases like AD (Rey et al. 2019; Sun et al. 2019a). In recent clinical research, EPO remarkably improved neuropsychological test scores in chronic kidney disease patients with cognitive deficits (Rey et al. 2019). The actions underlying the neuroprotective functions of EPO in AD are pleiotropic (Sun et al. 2019a). The clinical studies of Armand-Ugón et al. (2015) showed that EPO (2500 IU/kg, 3 days a week for 4 weeks, i.p.) improved memory and decreases Aß load in AD transgenic mice (Sun et al. 2019b). Similar effects of EPO have also been observed in animal models of AD wherein EPO decreased memory loss by reducing neurodegeneration, neuroinflammation, and cholinergic deficits and increasing hippocampal neurogenesis (Rey et al. 2019). Overall, the neuroprotective functions of EPO include increased angiogenesis and neurogenesis, decreased oxidative stress, neuroinflammation, apoptosis, and mitochondrial dysfunction (Sumbria 2020). Such positive results of EPO in AD clinical trials together with the observations that EPOR is highly expressed in the hippocampal region propose the probability of pleiotropic effects of EPO in AD (Lee et al. 2012a, b). All-inclusive, EPO has been regarded as a robust remedy for AD due to its non-erythropoietic neuroprotective functions. This review targets the potential role of EPO in reversing the major neurodegenerative processes associated with AD. Consequently, it is unfolding new approaches for the expansion of novel treatment strategies for AD. The authors aim to represent the significance of EPO as a possible therapeutic target and the role of EPORs in the pathology of AD, along with diverse factors interacting with EPO in one or another way, thus facilitating the advancement of a suitable therapeutic approach for AD (Figs. 1, 2 and 3, Table 1.

Erythropoietin and its physiological roles

Erythropoietin (EPO) is a glycoprotein hormone, a member of the superfamily of type I cytokines, and is largely responsible for the multiplication, differentiation, and growth of red blood cells, in adults as well as in embryonic phases (Bunn 2013). EPO comprises 165 amino acids organized into a globular 3-D configuration, including 4 amphipathic helices linked through loops and stabilized by 2 disulfide bonds in-between, which are essential to sustain its physiological activity, and this fundamental configuration constitutes about 60% of its molecular weight (Batmunkh et al. 2006). Also, the oligosaccharide

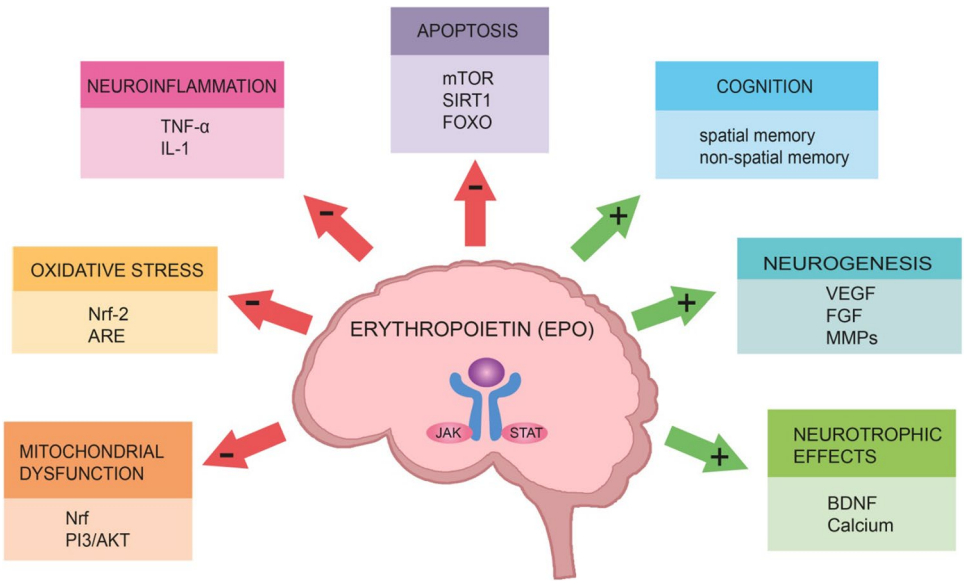

Fig. 1 Principal signaling pathways are activated or inhibited to induce neuroprotection when EPO interacts with EPOR. Conversely, the binding leads to a decrease in apoptosis, neuroinflammation, mitochondrial dysfunction, and oxidative stress and an increase in neurogenesis, cognition, and neurotrophic effects. EPOR = erythropoietin receptor; EPO = erythropoietin; Nrf = nuclear erythroid 2-related factor 2; PI3/AkT = phosphoinositide-3-kinase–protein kinase B; TNF-α = Tumour Necrosis Factor alpha; IL-1 = Interleukins-1; mTOR = Mammalian target of rapamycin; SIRT1 = sirtuin (silent mating type information regulation 2 homolog) 1; FOXO = forkhead box transcription factors; VEGF = Vascular endothelial growth factor; FGF = Fibroblast growth factor; MMPs = Matrix metalloproteinases; BDNF = Brain-derived neurotrophic factor

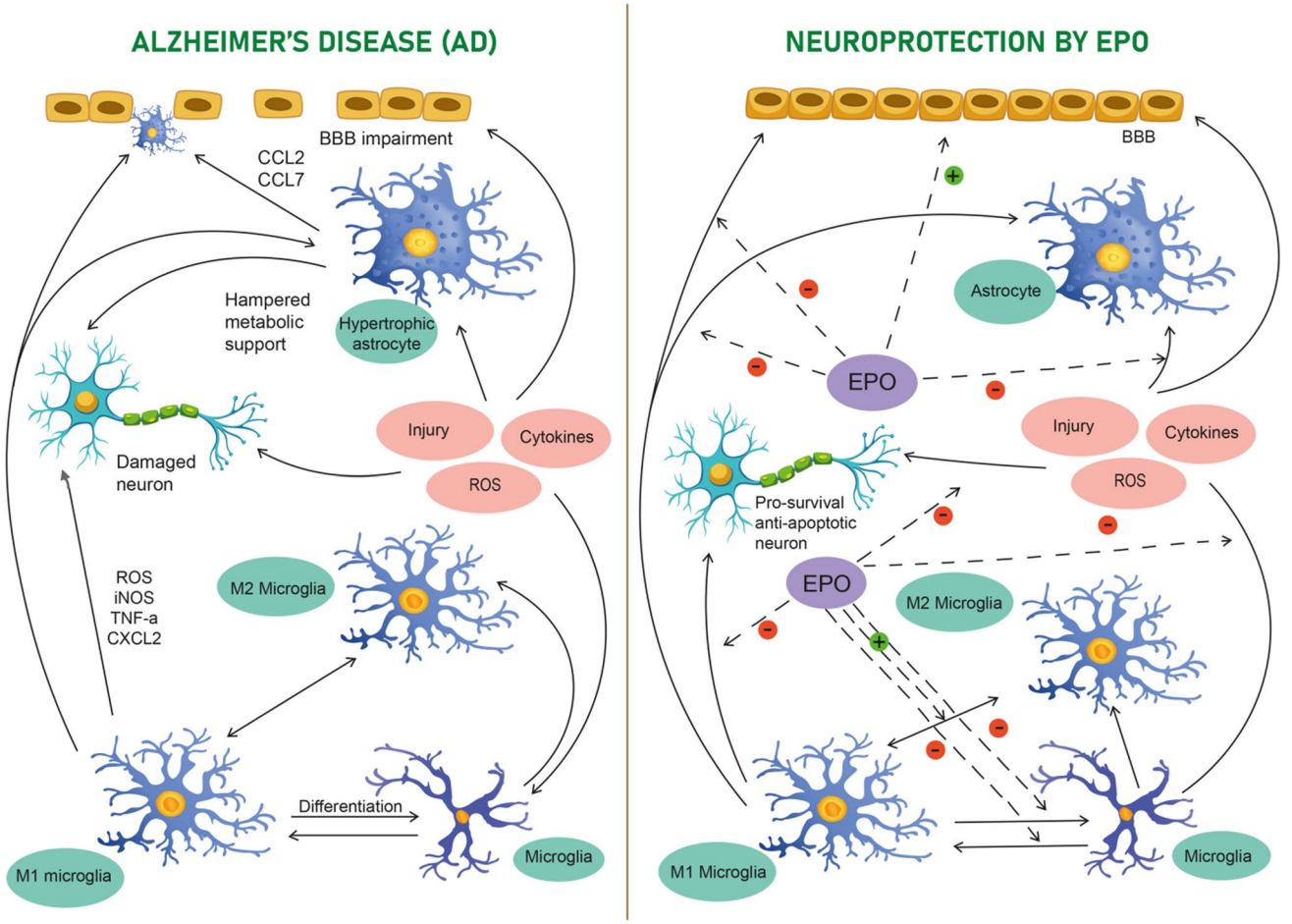

Fig. 2 Comparison between the neuroglia interactions in AD brain tissue and how EPO may protect neurons by modulating neuroinflammation and oxidative stress. In a diseased state, the BBB breaks down due to astrocyte hypertrophy and endothelial cell death, immune cells infiltrate CNS, microglia become reactive, and neurons undergo apoptosis. EPO directly blocks apoptosis of neurons and preserves the BBB by blocking apoptosis of endothelial cells and decreasing astrocyte hypertrophy, thus, decreasing infiltration of immune cells. EPO also has a direct effect on microglia by affecting their proliferative capability and influencing their M1/M2 reactive state

chains connected to EPO enhance its size and thus reduce its renal filtration, whereas the occurrence of terminal neuraminic acids prevents the exposure of galactose residues, which are taken up via hepatic transporters, increasing the plasma half-life of the cytokine (Jelkmann 2008). During fetal growth, EPO is produced in the liver, whereas during later stages it is majorly produced in the peritubular cells of the kidney to compensate for the oxygen decline during hypoxia. This process is initiated by the hypoxia-inducible factor (HIF-1) Parra and Rodriguez 2012, which further induces EPO gene transcription by binding with a stimulator and increasing its production and secretion into the plasma. The interaction between EPO and EPOR is responsible for distinct actions such as induction of signaling cascades controlling apoptosis and reducing the rate of necrosis in the bone marrow (Fisher 2003). Such activation induces cell development from normoblasts to reticulocytes, which are remarked by nucleus destruction and secretion from the bone marrow to the vasculature where terminal distinction to RBCs takes place, having a half-life of about 120 days in healthy adults (Sinclair 2013). The circulating hormone enhances the multiplication and development of erythroid progenitors into erythrocytes, which eventually ameliorates O_2 transport as their central role (Maiese et al. 2012). The standard plasma level of EPO in normal adults is 10–20 (mIU/mL) with a half-life of 7–8 h (Toledo et al. 2006). Besides being produced in the kidney, secondary production regions of EPO have been observed in astrocytes and retinal cells, indicating that this hormone has a role not only in hematopoiesis but also in multiple tissues involved in cell protection from stress and reversing cell death. EPO expression in non-erythroid cells accounts for approximately 15–20% of the total generation throughout the human body (Ponce et al. 2013). Recombinant human EPO (rhEPO) is recommended in the therapy of anemia and disorders linked with reduced

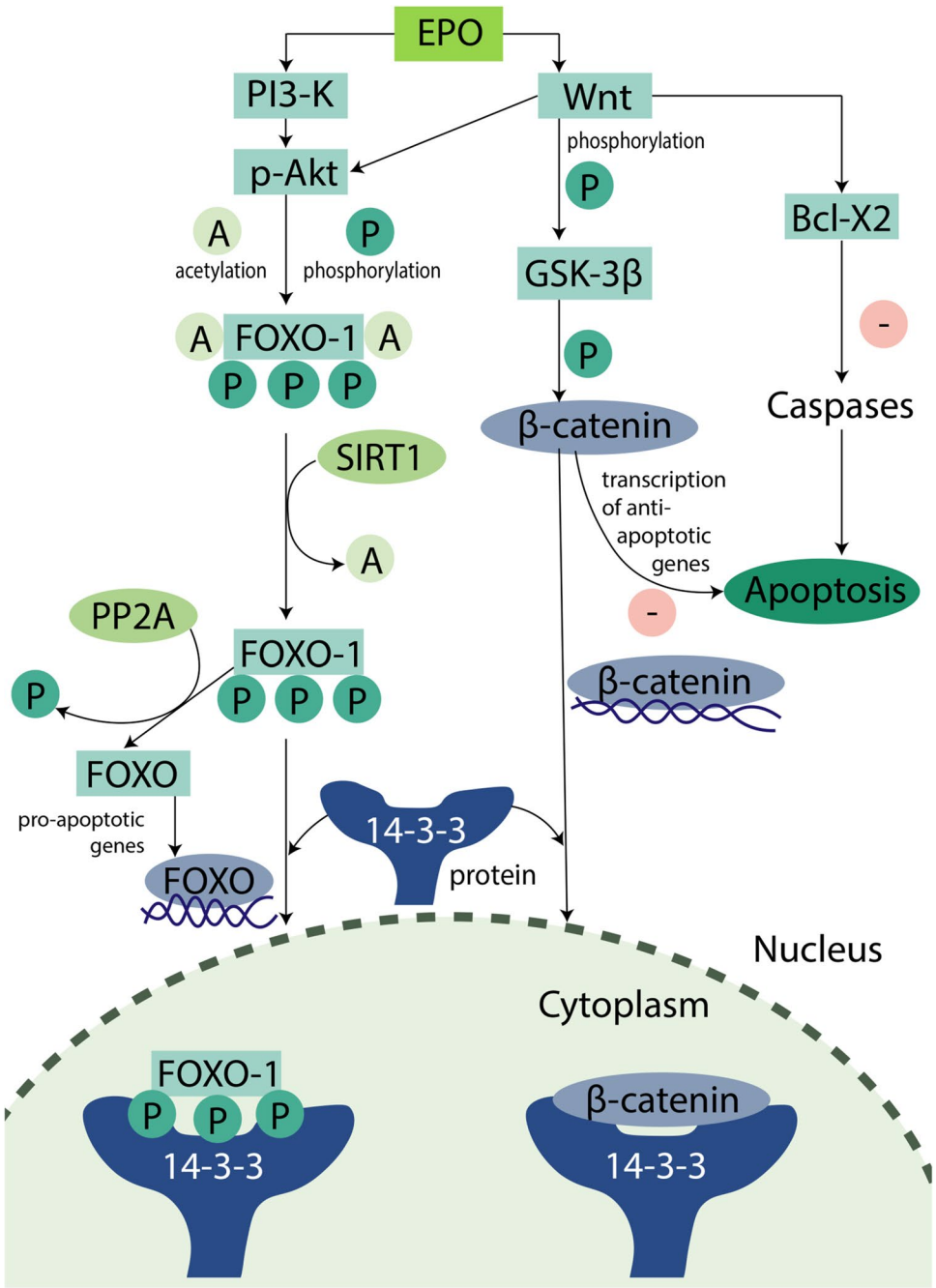

Fig. 3 Erythropoietin (EPO) utilizes novel signaling pathways to prevent apoptosis. EPO stimulates PI 3-K and subsequently leads to the activation of Akt. Akt can phosphorylate FOXO-1 to prevent its nuclear translocation and transcription of "pro-apoptotic" genes. EPO through Wnt1 phosphorylates Akt and GSK-3ß to prevent β-catenin phosphorylation by GSK-3ß and promotes the nuclear translocation of β-catenin to increase transcription of "anti-apoptotic genes". Phosphorylated FOXO-1 and ß-catenin are recruited and bound by the cytoplasmic docking protein 14–3-3. Additionally, EPO also integrates with Wnt1 to regulate the expression of anti-apoptotic protein Bcl-xL to prevent caspase activation and the induction of apoptosis. GSK-3ß = glycogen synthase kinase-3ß; PI3-K = phosphoinositide-3-kinase; FOXO-1 = forkhead transcription factor-1; Akt = protein kinase B

levels of EPO in blood plasma. Also, it has been indicated that these patients show substantial cognitive advancement (Jiang et al. 2014).

EPOR is a laminal receptor, a member of the superfamily of type 1 cytokines, with a molecular mass of 66 kDa, which is pre-determined as homodimers on the cell membrane with reliability of approximately 1000/cell (CFU-E) in the bone marrow. The binding of EPO to its receptor causes a configurational alteration that induces auto-phosphorylation through Janus kinase-2 (JAK-2). The protein trans-phosphorylates, which initiates phosphorylation on Tyr residues present in the matrix of EPOR, generating an active region for other proteins during the activity of the signaling pathways (Lodish et al. 2008). Signaling cascades stimulated by EPOR consist of protein kinase C (PKC) and phosphoinositol 3-kinase (PI3K). This ultimately leads to the regulation of cell cycle advancement through phosphorylated protein kinase B (AKT), along with the control of 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 retards the effects of pro-apoptotic agents like cytochrome

Table 1 Pre-clinical evidence for Neuroprotective Functions of Erythropoietin

Experimental Model	Ad Induction Stimulus	EPO Type	Dose of Administration	Neuro-Protective Actions	References
Primary cortical neurons of a rat model	1 mM EGTA	HrEPO	4 mg/ml	Enhanced calcium in erythroid cells and neurons Activation of transient receptor potential channels (TRCs) Ca^{2+} released from calcium stores.	(Andoh et al. 2011)
Adult male mice	Lipo-polysaccharide (LPS) and 1% tween 80	EPO	4 mg/ml	Increased ambulation frequency Enhanced cognition Reduced plaque frequency Decreased choline acetyltransferase activity	(Khairallah et al. 2014; Arai et al. 2001)
PC12	Aß25–35 peptide	RhEPO	100 mg/ml	Reduction in apoptotic cells Reduce oxidative stress Increased anti-apoptotic gene expression	(Castillo et al. 2019; Jarero-Basulto et al. 2020)
Tg2576	Overexpression of APP	RhEPO	5000 IU/kg i.p.	Reduction in amyloid ß plaques Enhanced cognition Hampered microglial activation	(Cruz et al. 2017)
Sprague-Dawley adult male rats	Streptozotocin	rhEPO	5000 IU/kg/day i.p.	Increased latency time Lower brain TNF-α level A higher number of neurons in the hippocampal region	(Cevik et al. 2017)
Male Wistar rats	Streptozotocin	EPO	5000 IU/kg	Induction of learning and memory impairments Enhanced neurogenesis in the granular layer of the dentate gyrus Increased expression of BDNF in the hippocampus	(Arabpoor et al. 2012; Ishrat et al. 2009).

C (Brines and Cerami 2005). Only 10% of the receptor is translocated to the cell surface, which needs the binding of the N-terminal site of JAK-2 to the cytoplasmic site of the receptor in the endoplasmic reticulum to allow the precise arrangement of the protein molecule (Lu et al. 2012). Therefore, JAK-2 is considered crucial for EPOR expression when it is stimulated (Javadi et al. 2012). Nevertheless, EPOR signaling has also been noticed in non-hematopoietic cells, thus demonstrating the multifaceted functions of EPO (Wagner et al. 2004). It was observed that heterodimers of the non-canonical receptor are associated with the neuroprotective effects of EPO during in vitro studies, similar to the ones obtained with typical homodimer EPOR configurations. These outcomes strengthen the objective that the stimulation of EPORs in homodimer or heterodimer configurations is vital for their neuroprotective functions (Yu et al. 2016). EPOR is expressed as a heterodimer (EPOR-ß comm) in the central nervous system (CNS), proposing specific functions of EPO in this tissue (Rabie and Marti 2008). It has been acknowledged that the involvement of EPOR is crucial for in vitro neuroprotection. For example, a neuroprotective activity was noticed in PC12 cells pre-administered with EPO for 1 h before injecting with amyloid-ß peptide for 6 to 12 h, and this activity was diminished when an antagonist of STAT-5 (a key signaling passage induced by EPOR) was administered (Ma et al. 2014). Also, upregulation of EPOR expression has been described in ischemic neuronal tissues in models treated with varying doses of EPO (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).

A drawback to EPO as a neuroprotective agent is its natural capability to enhance the erythrocyte volume fraction (EVF) or hematocrit, which rapidly promotes blood inrush to cerebral tissue, raising the possibility of cerebrovascular reperfusion. Hence, it is necessary to expand the range of doses of EPO for its neuroprotective actions when used in peripheral therapy (Pankratova et al. 2010). Ever since several isoforms of EPO have been developed with chemical modifications to improve its effectiveness. Moreover, a difference has been observed between CNS-EPO and systemic-EPO due to a modification in the glycosylation tree. The EPO produced in the cerebral and retinal cells also differs from the EPO synthesized in the peritubular cells of the kidney in respect of its sialic acid alternatives (Moon et al. 2006). Contrastingly, the production of proteins that induce the binding between EPO and EPOR, catalyzing its stimulation minus hematopoietic effects, has been developed, but with an exceptionally short half-life (about 4 min) (Brines and Cerami 2008). Such agents were modified to improve stability and to enhance their half-life, thus obtaining a novel peptide variant with increased affinity for EPOR that may reduce neuroinflammation and contribute to neuroprotection (Liu et al. 2015a, b).

Pleiotropic neuroprotective profile of erythropoietin

EPO has been observed to possess a wide range of neuronal actions in the CNS, majorly aimed at tissue injury and repair. EPO is known to be cytoprotective in a variety of experimental models of excitotoxic, hypoglycaemic, and hypoxic conditions (Chen et al. 2007). The primary neuroprotective function of EPO in cultured neuronal cells is its potential to inhibit apoptotic cell death, reducing DNA damage and cell membrane destruction (Wu et al. 2007a, b). Necrotic cell death is also reduced on exposure to EPO (Byts et al. 2008). An additional neuroprotective action of EPO is its anti-oxidative property, which defends cells against oxidative stress (Wu et al. 2007a, b) by increasing the activity of intrinsic antioxidant enzymes like glutathione peroxidase and superoxide dismutase (Kumral et al. 2005). EPO inhibits neuroinflammation by decreasing activation of microglia and astrocytes due to its inhibiting property of immune cells' entry into the traumatic region (Diem et al. 2005). In cerebrovascular endothelial cell lines, EPO attenuates TNF-α induced gene expression of interleukins (ILs) (Avasarala and Konduru 2005). It also prevents lipopolysaccharide-interferon-Γ-induced neurotoxicity in oligodendrocytes, conserving substantia alba (Genc et al. 2006) and decreasing expression of TNF-α (Campana et al. 2006). EPO also supports neurovascular stability along with vasculogenesis activation (Wang et al. 2004a, b). It protects the integrity of the BBB during trauma by protecting the proteins underlying tight junctions (Li et al. 2007) and by hampering free radical expression (Ozturk et al. 2005). During angiogenesis, EPO promotes endothelial precursor cell proliferation, translocation of endothelial cells into vascular regions, generation of matrix metalloproteinase-2 (MMPs-2), and capillary tube production (Müller-Ehmsen et al. 2006; Wang et al. 2006). EPO possesses constant anti-apoptotic properties during an oxidative injury in neuronal endothelial cells as well as during ischemic cerebral hemorrhage (Marti et al. 2000). Stimulating the activity of endothelial nitric oxide synthase (eNOS) has been reported to support the neuroprotection by EPO following experimental CNS injury (Santhanam et al. 2006). Surprisingly, tissue and plasma concentrations of nitric oxide (NO) is distinctly raised in animal models of EPO (Ruschitzka et al. 2000), although the neuronal protection by EPO is terminated in the eNOS-deficit model (d'Uscio et al. 2007). The neuronal proliferation of adult neural precursor culture by EPO appears to command the stimulation of suppressor of cytokine signaling 2 (SOCS2) (Wang et al. 2004a, b). EPO increases the differentiation of oligodendrocyte precursors and increases oligodendrocyte growth in the media (Sugawa et al. 2002). Furthermore, the EPOR-deficit model has been remarkably observed with lower neurogenesis in the subventricular zone (SVZ). Even so, EPO/EPOR expression in neurons is not essential for cerebral growth (Yu et al. 2002). The outlined neurotrophic actions of EPO comprise the potential to activate axonal outgrowth, dendritic budding, neurite production, electrical effects, and regulate Ca^{2+} and neurotransmitter production and secretion (Lipton 2004). Modern research expressed Ca-dependent stimulation of cAMP response element-binding protein (CREB) and activation of gene expression of brain-derived neurotrophic factor (BDNF) by EPO in primary hippocampal neurons (Viviani et al. 2005). In the hippocampal region of mice, EPO ameliorates neurotransmission after oxygen deprivation (Weber et al. 2002). The preclinical data providing the basis for the utilization of EPO in human neurodegenerative disorders has strongly expanded since the earliest uncovering of its neuroprotective activity, particularly, when the preclinical results of analysis of EPO in clinical acute ischemic stroke satisfy most of the STAIR criteria (Roundtable 1999).

Also, similar to AD, neuroprotective effects have been observed in various models of neurodegenerative diseases. In particular, Parkinson's disease (PD), in which apoptosis inhibition through activation of EPOR and the 1,4,5-triphosphate (IP3) pathway, was seen in different in vitro studies (Park et al. 2009, 2011; Won et al. 2009). Similar effects were observed in other neurodegenerative diseases, such as

amyotrophic lateral sclerosis (ALS), where there is the selective death of superior motoneurons (motor cortex and brain stem) and inferior motoneurons (spinal cord). This causes a gradual loss of muscle innervation, leading to paralysis and atrophy-like symptoms in aged patients. It has been observed that only 10% of these cases are related to a familial component, while 20% are associated with mutations in the superoxide dismutase 1 (SOD1) gene (Noh et al. 2014). In animal models of ALS treatment with EPO for 120 days prevented the death of motoneurons (Noh et al. 2014). Also, pretreatment with EPO inhibited the release of proinflammatory cytokines and promoted the synthesis and release of anti-inflammatory cytokines (Naganska et al. 2010; Noh et al. 2014). Such data support the neuroprotective role of EPO against several diseases related to the CNS, especially AD and PD, suggesting that EPOR could represent an interesting target for developing new therapeutic strategies (Noh et al. 2014).

Erythropoietin inhibits neuroinflammation and oxidative stress

Oxidative stress results in the abnormal destruction of DNA, lipids, and proteins, which is primarily believed to have emerged from mitochondrial dysfunction (Duchen 2004). Reactive oxidative by-products like peroxynitrite (from nitric oxide and superoxide) may catalyze DNA impairment and lead to necrosis, which eventually induces an inflammatory cascade that involves microglial sensitization. Reactive microglia are specifically known for the generation and release of reactive oxygen species/reactive nitrogen species (ROS/RNS) (Brook 2003). Neuroinflammation and oxidative stress have been proposed to play a role in several disorders, for example, Alzheimer's disease (AD), Parkinson's disease (PD), and cerebral trauma (Federico et al. 2012). EPO treatment reduces the neuronal impairment caused due to ROS/RNS generation as it was observed to protect the integrity of the mitochondrial membrane in an Aß-induced animal model of AD (Li et al. 2008). It also increases the levels of antioxidant enzymes by promoting the migration of nuclear factor erythroid 2-related factor 2 (Nrf-2) towards the nucleus where it binds and stimulates the activity of antioxidant response element (ARE) (Jin et al. 2014). In neuronal cells, the increased nuclear translocation of Nrf-2 seems to be induced by various factors. The levels and intracellular activity of several antioxidant proteins are augmented by EPO, such as catalase (Yazihan et al. 2008), superoxide dismutase (Barichello et al. 2014), and glutathione peroxidase (Kumral et al. 2005). On the other hand, EPO shows no effect on the activity of the stimulated nitric oxide synthase (iNOS) enzyme in transgenic reactive microglia (Wenker et al. 2013), despite it decreasing retinal concentrations of iNOS in the glaucoma model (Gui et al. 2011). Such studies suggest that the actions of EPO on the activity of antioxidant enzymes could be cell-specific (Al-Qahtani et al. 2014). The 2 possible pathways were proposed for EPO-mediated reduction of oxidative stress. Firstly, the growing evidence suggests that EPO is a potent scavenger of ROS/RNS (Bailey et al. 2014) based on studies revealing that EPO preserves paraquat-treated astrocytes in a superoxide dismutase knockout model (Liu et al. 2006). Secondly, in various diseases in which iron deposition is believed to be a chief regulator of oxidative stress, for example, AD (Weinreb et al. 2013), EPO may secondarily support an antioxidant action by enhancing erythrocyte generation accompanied by a reduction in blood iron levels.

In neurological disorders, immune cells are regarded as one of the damaged regions due to the increase in the expression of chemokines (Rezai-Zadeh et al. 2009). The expression of 2 of them, namely, chemokine ligand-2 (CXCL2) and chemokine ligand-7 (CCL7), is reduced upon EPO treatment in the ischemic cortex of rats after cerebral ischemia (Mengozzi et al. 2012). The above data suggest that EPO can regulate the involvement of immune cells, which may successively reduce the expression and translocation of proinflammatory cytokines into the brain. The translocation of these cells into the brain takes place as a result of BBB disruption after cerebral injury or in the case of neurodegenerative disorders like AD (Rizzo and Leaver 2010). EPO is known to protect the BBB in various models. For example, the changes in BBB and EPOR along with expression of AQP4 were examined after intraperitoneal administration of EPO in wild-type (AQP4+/+) and AQP4 knock-out (AQP4−/−) mice (Chu et al. 2014). The results showed that EPO protects the BBB by inhibiting astrocyte hyperplasia and microvascular endothelial cell death (Maiese et al. 2012). EPO also upregulates the expression of tight junction proteins in the above cells by stimulating the mitogen-activated protein kinase (MAPK) signaling pathway.

In the damaged or traumatic brain, intrinsic microglia transform into reactive forms, namely, M1 (pro-inflammatory) and M2 (alternative) phases like vascular macrophages (Boche et al. 2013). EPO may directly affect the overactive phase of the cerebral microglia by reducing the concentration of the phosphatidylserine receptors (PSR) on the microglial membrane (Chong et al. 2003), thus suggesting that treatment of EPO reduces the capability of microglia to phagocytose dead neuronal cells. Contrastingly, the in vitro study of EPO on macrophages evaluates the reactive condition instead of cell survival. As in microglia, EPO exposure stimulates the Akt/mTOR/NFκB signaling which is involved in migrating the reactive macrophage phase from M1 to M2 (Xu et al. 2013). The in vitro exposure to EPO on macrophages induces a dose-dependent decline in TNF-α and an accompanying incline in phagocytosis, indicating that EPO translocates such cells to a phagocytic

stage from a pro-inflammatory stage without reverting them to a non-reactive stage (Liu et al. 2014). Also, unlike in microglia, EPO blocks NF-κB p65 in macrophages, resulting in low levels of NO and TNF-α (Nairz et al. 2011). Studies are required to acknowledge the way in which EPO enhances NF-κB expression in microglia but reduces it in macrophages.

Erythropoietin reduces apoptotic cell death

EPO can reduce apoptotic neuronal death through several signaling pathways, such as stimulation of Akt (protein-kinase B), Wnt (Wing-less related integration site), and mTOR (mammalian target of rapamycin) signaling, and inhibition of nuclear transcription and translocation of FOXO proteins. Apoptosis takes place in two steps, an initial phase involving loss of cell membrane lipid phosphatidylserine (PS) symmetry (Fong et al. 2014) which is followed by a latter phase involving DNA degeneration (Shao et al. 2013). EPO depends on various signaling pathways for its apoptosis inhibiting property, which also may catalyze tissue restoration and neuronal preservation. Phosphoinositide 3-kinase (PI3-K) and protein kinase B (Akt) are the primary signaling pathways that provide EPO-mediated neuroprotection (Shang et al. 2012). PI3-K regulates the laminal translocation of Akt through phosphorylation of membranal lipids (Chong et al. 2005). The phosphorylation of Akt is regulated by the activity of phosphoinositide-dependent kinase (PDK) (Chong and Maiese 2007). In addition, Akt can provide neuroprotection during estrogen expression, in the presence of pro-apoptotic proteins (Bahia et al. 2012), spinal muscular degeneration, amyloid-ß (Aß) neuro-toxicity (Zeldich et al. 2014), oxygen-glucose disturbances, and hypoxia (Rong et al. 2013).

EPO also regulates the signaling pathways of the silent mating type information regulation 2 homolog 1 (SIRT1), Wnt proteins, and mammalian forkhead transcription factors of the O class (FOXO). As they are transcription factors, FOXO proteins bind with DNA to affect protein transcription that is generally "pro-apoptotic" (Maiese et al. 2009a, b). Several pathways regulate the expression of FOXO proteins (Maiese et al. 2007). For example, Akt stimulation catalyzes FOXO phosphorylation, resulting in the binding of 14–3-3 proteins to FOXOs, blocking nuclear migration, and thus inhibiting apoptotic target gene transcription (Kaushal et al. 2011). Furthermore, additional post-translational alterations in FOXO proteins involve acetylation (Peng et al. 2015), ubiquitylation (Tanaka and Iino 2014), and phosphorylation (Zeldich et al. 2014). In neuronal cells, FOXO-3a stimulation and p27 (kip1) transcription may induce apoptosis (Xu et al. 2014). In microglial cells, downregulation of FOXO-3a and blockade of FOXO-3a migration to the nucleus results in increased resistance to oxidative stress (Shang et al. 2010). Phosphorylation and nuclear migration of FOXO-3a serve as neuroprotection and endothelial protection in some experimental models of diabetes mellitus (Hou et al. 2011). SIRT1 was known to play a significant role in the regulation of FOXO protein (Maiese 2015a, b, c). As a histone deacetylase, SIRT1 variably deacetylates FOXOs, thus preserving neuronal integrity during deprived phases through multiple pathways like autophagy (Hariharan et al. 2010). SIRT1 and FOXOs process simultaneously to protect neurons. Multiple studies have also proposed that alteration of FOXO signaling, which requires SIRT1 activity, may enhance the lifespan of neurons. SIRT1 can increase senescence in mammals and provide neuroprotection during hyperoxia (Balan et al. 2008), primarily by blocking the signaling pathway of FOXO proteins (Paraíso et al. 2013). During this process, an increase in nuclear translocation of SIRT1 provides neuronal survival. Some apoptotic proteins related to p38 (Ferrara et al. 2008) and c-Jun N-terminal kinase −1 (JNK1) (Hong et al. 2010) may decrease SIRT1 signaling and enhance caspase activity, thus leading to the degeneration of SIRT1 (Gao et al. 2011). Whereas the Wnt signaling pathway inhibits the degeneration of SIRT1 supports its stimulation and inhibits stimulation of caspases (Kozako et al. 2012). Wnt proteins are multiplicative in nature as they regulate vascular cell growth (Qi et al. 2013), stem cell growth (Lee et al. 2012a, 2012b), immunity (Shah et al. 2014), malignancy (Marchetti and Pluchino 2013), and neuronal plasticity (Carbajo-Pescador et al. 2014). In the CNS, loss of Wnt activity may be linked with cognitive impairment, oxidative injury, long-term memory decline, neurological diseases, depression, and neuronal ischemia (Bayod et al. 2015). In neuronal cells, Wnt activity stimulates Akt, inhibits FOXO3a deacetylation, and stimulates FOXO3a translocation to prevent the loss of porosity of the mitochondrial membrane, cytC discharge, and caspase stimulation (Shang et al. 2010). The target of Wnt1 activity, Wnt1 inducible signaling pathway protein 1 (WISP1), significantly controls extracellular matrix generation, apoptosis, cellular translocation, and mitosis (Berschneider et al. 2014). Like Wnt1 activity, WISP1 also protects the neurons by phosphorylating FOXO3a, or by seizing FOXO3a in the matrix with protein 14–3-3. WISP1 also increases the nuclear translocation and signaling of SIRT1. Contrarily, FOXOs can inhibit the Wnt signaling pathway by reducing the activity of target genes of ß-catenin (Liu et al. 2015a, b).

EPO regulates the post-translational production and nuclear migration of FOXOs to increase neuronal stability (Maiese et al. 2010). EPO-mediated stimulation of Akt catalyzes phosphorylation and deactivation of FOXO proteins (Kashii et al. 2000), majorly FOXO3a (Bouscary et al. 2003). EPO may stimulate the binding of 14–3-3 proteins with FOXO3a to seize FOXOs in the matrix of cells, thus preventing nuclear migration and transcription of

"pro-apoptotic" agents (Chong et al. 2012). EPO increases the growth of erythroid progenitor cells through the regulation of the FOXO3a signaling pathway (Bakker et al. 2004). Besides FOXO3a phosphorylation, EPO also downregulates the protein p27 (kip1), involved in the inhibition of cell cycle control (Chamorro et al. 2013). EPO can also protect cerebral endothelial cells against hypoxia through FOXO3a phosphorylation, inhibiting protein signaling and nuclear migration (Maiese et al. 2009a, b). In experimental models of cerebral ischemia, EPO inhibits the expression of FOXO1 to prevent ischemic stroke (Zhao et al. 2015). In addition, EPO may utilize Wnt activity to decrease the expression of FOXO proteins to increase neuron survival (Maiese 2015a, b, c).

Erythropoietin blocks the autophagic cell death

Autophagy is another mechanism of cell death that is controlled by EPO. Autophagy reprocesses cell components in the cell matrix for the removal of unwanted tissues for cellular rearrangement (Nakka et al. 2016). Autophagy is classified as chaperone-mediated autophagy, macroautophagy, and microautophagy (Maiese 2015a, b, c). Macroautophagy is the primary type of autophagy that comprises the autophagosomes of cytosolic organelles and proteins, which then combine with lysosomes for degeneration and reprocessing (Maiese 2014a, b). Microautophagy causes the folding of lysosomal layers for the integration and ingestion of cytosolic constituents. Chaperone-moderated apoptosis uses cytoplasmic chaperones for the transfer of cytosolic constituents across lysosomal membranes (Bargiela et al. 2015). EPO can block autophagic damage by activating the mTOR (mechanistic target of rapamycin) signaling pathway (Maiese 2014a, b). Stimulation of mTOR inhibits autophagy through phosphorylation of autophagic-related proteins and genes such as ULKs (Unc51-like autophagy activating kinase 1) (Sanghera et al. 2011). EPOR, Akt, and mTOR stimulation protects against the enhanced activity of autophagy in epithelial cells and supports defense mechanisms against oxidative stress and hypoxia in retinal precursor cell lines (Yu et al. 2013). EPO regulates abnormal autophagy that induces apoptotic damage in preclinical neonatal necrotizing enterocolitis (Bendix et al. 2012). EPO also controls autophagic activity and may inhibit infantile cerebral injury in hyperoxic conditions (Jang et al. 2016). In experimental studies, EPO can suppress apoptosis by stimulating the activity of AMPK in neuronal cell lines (AMP-activated protein kinase) (Neasta et al. 2014). The EPO-mediated Wnt and Akt signaling pathways also intersect with the mTOR protein kinase signaling pathway (Maiese et al. 2013).

The hamartin (tuberous sclerosis 1)/tuberin (tuberous sclerosis 2) (TSC1/TSC2) complex are mTORC1 suppressors (Chong et al. 2010). Despite various active sites are acknowledged for TSC1, regulation of the TSC1/TSC2 complex can be done through pathways that involve AMPK and Akt (Saha et al. 2010). Significantly, a restricted depletion in TSC2 expression was considered as an essential step in neuroprotection against Aß to allow mTOR activation, as complete downregulation of TSC2 can decrease neuroprotection (Chen et al. 2013). AMPK also phosphorylates TSC2 to inhibit mTORC1 signaling (Morentin et al. 2014). In the brain, AMPK stimulation can reduce Aß generation and secretion (Cai et al. 2012), decrease oxidative stress variables in models with diabetic neuropathy (Du et al. 2015), reduce infarction in animals with cerebral ischemia, be responsible for increased survival (Moroz et al. 2014), regulate neuroinflammation (Russo et al. 2014), and prevent memory loss (Zhu et al. 2013). Still, AMPK stimulation is not progressively advantageous. Although, abnormal AMPK stimulation may lead to abnormal Aß generation (Cai et al. 2012) and neuronal damage (Salminen et al. 2011). Signaling pathways including Wnt can prevent the deleterious effects of AMPK. For example, WISP1 (WNT1-inducible signaling pathway protein 1) causes decreased signaling of AMPK and TSC2 and disturbs neuronal survival. The ability of WISP1 to regulate AMPK expression is an essential component in the regulation of neuronal integrity (Morentin et al. 2014).

EPO depends on mTOR signaling for cerebral cell growth and development. EPO requires mTOR for the growth of neuronal progenitor cells and their protection against oxidative damage (Wang et al. 2014a, b). EPO regulates mTOR and its downstream signaling, which includes PRAS40 (protein-rich AKT substrate) to enhance neuronal lifespan against oxygen-glucose disturbance. EPO was observed to maintain the microglial integrity against oxidative stress through mTOR and Wnt signaling. EPO can reduce Aß toxicity by regulating Wnt and mTOR signaling to inhibit caspase stimulation and apoptotic cell death. Throughout hypoxia-reoxygenation stress, EPO enhances mTOR signaling for the protection of neurons in the hippocampus region (Ryou et al. 2015). In the mTOR signaling pathway, AMPK also affects the physiological activity of EPO. EPO also requires a specific degree of AMPK activation to reduce the deleterious effects of oxidative stress (Wang et al. 2014a, b). However, the level and expression of EPO may affect the neuroprotective functions of mTOR and AMPK signaling pathways as excess levels of EPO may cause neuronal injury and knock down mTOR activity (Andreucci et al. 2009).

Neurotrophic actions under neuroprotective profile of erythropoietin

Studies from recent times have distinctly shown that EPO is a potent regulator of neuronal stability. In vitro administration of EPO in experimental models of AD produces

neuroprotection against cerebral ischemia (Brines et al. 2000), traumatic cerebral damage, and spinal cord injury (Celik et al. 2002). Exogenously administered EPO protects neurons against apoptosis in models of AD. Multiple mechanisms may be involved in the neuroprotective effects of EPO, namely, reduction in neuroinflammation (Villa et al. 2003), stimulation of survival kinase signaling pathways (Sirén et al. 2001), stimulation of nuclear anti-apoptotic genes. The discovery that exogenously administered EPO penetrates the BBB (Ehrenreich et al. 2004) and its efficacy in patients with stroke, has largely enhanced the utility of this cytokine as a potent therapeutic agent and gathered attention regarding the bio-molecular processes of its neuroprotective actions. The intracellular Ca^{2+} rise is an initial mechanism induced by EPO, which is considered as one of the mechanisms of neuroprotection. EPO catalyzes Ca^{2+} influx by activating voltage-sensitive Ca^{2+} channels, possibly as a result of neuronal membrane depolarization (Koshimura et al. 1999). Inhibition of voltage-sensitive calcium channels or complexation of extrinsic calcium by EGTA prevents the neuroprotective actions of EPO and thus causes neurodegeneration (Morishita et al. 1996) and Aß toxicity. Such results were obtained from the stimulation of NMDA (N-methyl-D-aspartate) or voltage-sensitive calcium receptors, which are in the role because of enhanced activation of survival genes. Therefore, EPO may lead to cell survival by increasing the transcription of these genes.

Brain-derived neurotrophic factor (BDNF) is a central target in the pathological framework of neurodegenerative diseases such as AD and the most widely dispersed neurotrophin in the CNS where it performs multiple important functions, for example, neuronal endurance, neuroplasticity, and immunity control (Chao and Lee 2004). AD is characterized by down-regulation in the neuronal signaling pathways that affect cerebral physiology and thus intellect, yet it is incompletely explained that peripheral BDNF possibly can represent a biosignature in AD (Goldstein and Young 2013). BDNF produces robust effects on the neural activity as well as endurance in several cells in the CNS, thus evolving as an applicant in the development of treatment for neurodegenerative disorders (Nagahara and Tuszynski 2011). Its potential function has been broadly examined in AD along with in other neurological diseases to a minor extent. In short, low levels of BDNF in serum have been studied in AD. Surprisingly, some inflammatory diseases like rheumatoid arthritis (Forsgren et al. 2011) and bronchial asthma have been linked with increased BDNF levels in chronic conditions. Eventually, increased plasma levels of BDNF seem to be related to the risk factors of cardiovascular disorders such as hypertension, hyperlipidemia, and increased body mass index (Golden et al. 2010). This proposes that how the plasma levels of BDNF increase or decrease is complex and may be regulated by interactions between various growth factors in the brain (Sargin et al. 2010). Like BDNF, EPO is also observed to be involved in several neurodegenerative diseases and can regulate various cellular signaling pathways to support neuronal endurance and to increase the differentiation of neurons. EPO slowly penetrates the BBB and is a strong cytokine that can protect neurons from apoptotic damage and increase their differentiation, thus being suggested as an effective agent in reversing the advancement of neurodegenerative mechanisms. Data from preclinical research and the latest clinical studies provide some evidence for cognitive improvement actions of EPO, which could be induced after binding with EPOR present in the CNS (Mengozzi et al. 2012). In preclinical trials, EPO appears to enhance BDNF generation (Viviani et al. 2005) and BDNF gene expression in the central nervous system, though the studies are insufficient. The effects of exogenously administered EPO on serum BDNF levels have not been scrutinized before. In recent studies, a theory was provided involving that delivery of EPO would be linked with enhanced BDNF levels in AD patients and partial remission with cognitive impairments. EPO-mediated BDNF generation has been lately associated with enhanced neurogenesis in response to neuronal injury. Some studies provide evidence for the association of this neurotrophin with the neuroprotective action of EPO, building the effectiveness of BDNF as a regulator of the neuroprotective effects of EPO (Lee et al. 2021).

Future challenges

Although EPO has a significant role as a "neuroprotective agent" in the central nervous system, the most crucial objectives that must be monitored before establishing EPO as a neurotherapeutic agent are blood-brain barrier permeability and negative erythropoietic effects. The discovery of EPOR in the cerebral vessels proposed that systemic EPO could penetrate the blood-brain barrier to exert its therapeutic actions on the nervous system. Based on such discoveries, it's been proposed that EPO crosses the blood-brain barrier through a specific receptor-dependent transport mechanism. The observation of exogenously delivered EPO in the cerebrospinal fluid has led to the belief that EPO enters the brain (CSF). The blood–CSF barrier was found to regulate EPO transfer from blood to CSF, and each particle in the circulation rushes the CSF to a degree that is proportional to its atomic mass. The belief is that EPO reaches the brain, mainly based on the evaluation of exogenously administered EPO in the cerebrospinal fluid (CSF). It was observed that the transfer of EPO from blood to CSF was regulated by the blood–CSF barrier and each particle present in the circulation rushes the CSF to an extent that is associated with their atomic mass. Following brain entry, studies

using radiolabeled EPO in experimental models reported that the degree of EPO uptake by the brain was slow and that EPO influx into the CNS was through a distinct mechanism. Although slightly more intrusive than the intracranial route, which bypasses the blood-brain barrier, the parenteral route requires a higher dose to permeate the BBB, which increases the risk of peripheral toxicity. Increased peripheral EPO levels, as an erythrocyte growth hormone, cause hematological side effects such as hypertension, hematocrit, seizures, iron deficiency, thromboembolism, and influenza-like symptoms. In cases of various illnesses, such as Alzheimer's disease, the above-mentioned difficulties connected to increasing peripheral dosage reduce the effectiveness of the parenteral route for EPO administration. EPO derivatives with less hematopoietic effects and related vascular difficulties are presently under clinical trials. One such analog is Asialo-EPO, obtained by removing the sialic acid residues from EPO to produce an agent with increased excretion, less adverse erythropoietic effects, and robust cytoprotective function in experimental models of neurodegenerative disorders.

Although the intranasal route is convenient over the parenteral route in respect of hematopoietic effects, the efficiency of the intranasal delivery system decreases as the mass and polarity of the molecule increase. Furthermore, intranasal administration depends upon universal drug absorption into the CNS from the olfactory CSF, and drug absorption reduces considerably with the amount. A greater extent of absorption in the human nervous system as compared to the brains of small animals may hinder the efficiency of the intranasal delivery of EPO in contrast to the trans vascular route. Therefore, altering EPO for straight and specified transport across the BBB through the trans vascular route and limited erythropoietic effects is required for the treatment of AD. EPO has progressed as a treatment regime for neurodegenerative conditions, providing its potential non-erythropoietic cytoprotective functions. Nevertheless, penetration across the BBB and negative erythropoietic effects are the two main challenges in the advancement of EPO for neurological diseases like Alzheimer's. Approaches for neuronal delivery of EPO, like MTH technology that utilizes in vitro RMT processes like the BBB TfR, guarantee effectively targeting EPO in the brain along with enhanced peripheral elimination to reduce erythropoietic effects.

Conclusion

The primary clinical use of EPO is to treat anemia in patients with disorders such as chronic renal failure. However, the increased activity of EPOR in the hippocampus, amygdala, and prefrontal cortex has been linked to an improvement in cognitive performance in such patients. Indeed, the EPO/EPOR axis has been widely studied in the neurodegenerative diseases field. Its potential therapeutic effects have been evaluated in multiple disorders, such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, spinal cord injury, as well as brain ischemia, hypoxia, and hyperoxia. EPO is showing great promise by counteracting secondary neuroinflammatory processes, reactive oxygen species imbalance, and cell death in these diseases. Multiple studies have been performed both in vitro and in vivo, characterizing the mechanisms through which EPO exerts its neurotrophic action. For this reason, the EPO/EPOR organization needs more research to fully comprehend its role as a neuroprotective molecule.

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