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Review Article

The therapeutic effects of erythropoietin and carbamylated erythropoietin derivatives in neurological and other disorders

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ABSTRACT

Erythropoietin (EPO) has been considered in several studies as a significant factor in the development of erythroid cells, the inhibition of neuronal cell death, and neurogenesis. Fortunately, a modified version of EPO called carbamylated erythropoietin (CEPO) possesses tissue-protective properties without eliciting erythropoietic effects. CEPO is a derivative of EPO that results in an alpha-amino derivative group with less biological hematopoiesis than EPO. In neurological diseases, CEPO and its carbamylated erythropoietin Fc fusion protein (CEPO-Fc) has been shown to play a better role than EPO. In this study, the effects of EPO and its derivatives on neurological diseases and their role in treatment have been reviewed.

Keywords:
EPO
CEPO
Neurological disorders
Alzheimer's disease

Introduction

Erythropoietin (EPO) is a well-known hormone manufactured in the the embryonic liver and mature kidney. It controls erythropoiesis in the lake of oxygen and the apoptosis of erythrocytes (Tsiftsoglou. 2021). This critical role of EPO makes it a potential factor in erythropoietin physiology research (Zhang et al., 2020).

EPO can play several roles in different tissues and cells (figure 1). Furthermore, it plays many non-hematopoietic biological activities like antioxidant, anti-apoptosis, and the prevention of neuronal cell death, induction of immunity, neurogenesis, and angiogenesis. Moreover, different sources have considered EPO for anemia treatment (Vittori et al., 2021).

EPO modulates molecular signaling such as cellular maintenance, growth, differentiation, and apoptosis pathways via binding to EPO receptors (EPORs). The cytoplasmic domain of this receptor is associated with Janus kinase (JAK) and stimulates the phosphorylation of eight tyrosine residues in the cytoplasmic section of EPOR, activating several molecular signaling pathways like MAPK/MEK/ERK 1/2, PI3K/AKT, STAT1, STAT3, and STAT5 pathways (Godoy et al., 2014, Vittori et al. 2021).

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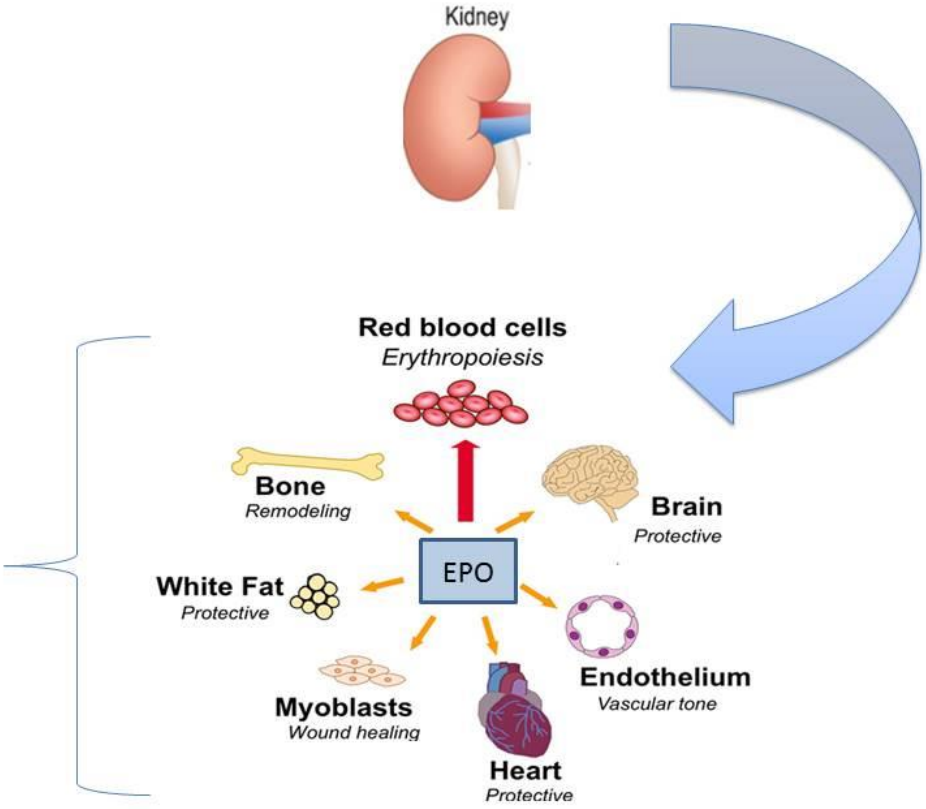

FIGURE 1. A pattern of the biological functions of erythropoietin in the human body.

PI3K is a signal transduction, an intracellular phosphatidylinositol kinase. Activated PI3K phosphorylates a critical transcription factor of hematopoietic-specific genes, GATA binding protein-1 (GATA-1). GATA-1 can stimulate the expression level of Bcl-xl and prolong the survival of hematopoietic progenitor cells (Zhang et al., 2020). EPO modifies the expression level of its receptor by binding to its receptor on the erythroid progenitor and induces cellular proliferation in mature red blood cells.

Other than erythroid progenitor cells, EPOR is additionally present in the central nervous system (CNS) and affects the normal development of the brain (McCook et al., 2012, Ma et al., 2022).

The Therapeutic Effects of EPO in the Brain and the Other Tissues

EPO and EPOR Functions

Human erythropoietin is a 30.4 KDa glycoprotein hormone composed of a single chain of 165 amino acid residues to which four glycans are attached. The kidneys serve as the primary source of EPO, with its synthesis regulated by hypoxia-inducible transcription factors (HIFs). Currently, EPO analogs devoid of erythropoietic activity but retaining neuroprotective abilities are under investigation. Derivatives such as Carbamylated erythropoietin (CEPO), EPO fusion proteins, and partial EPO peptides seem promising in this regard.

Upregulated expression of EPORs has been observed in the growing and adult mammal brain, particularly in neural progenitor cells compared to adult neurons. EPO is considered a novel therapeutic agent for treating CNS disorders, given its presence in neural cells and the observed EPOR expression in neurons, astrocytes, and microglia. Addressing two major challenges, blood-brain barrier penetration and adverse hematopoietic effects, remains crucial (Vittori et al., 2021, Rahmani et al., 2022).

EPO stimulates PI3K/Akt and RAS/ERK1/2 signaling pathways, contributing to cellular survival and growth. Also, EPO induces transcriptional factors such as STAT and NF-κB, which mediate anti-apoptotic and trophic action in the nervous system (Gadhave et al., 2021).

Role of EPO in Neurological Disorders

EPO has shown promise in reducing Aβ (β-amyloid) load in Alzheimer's Disease (AD) (Sun et al., 2019). In

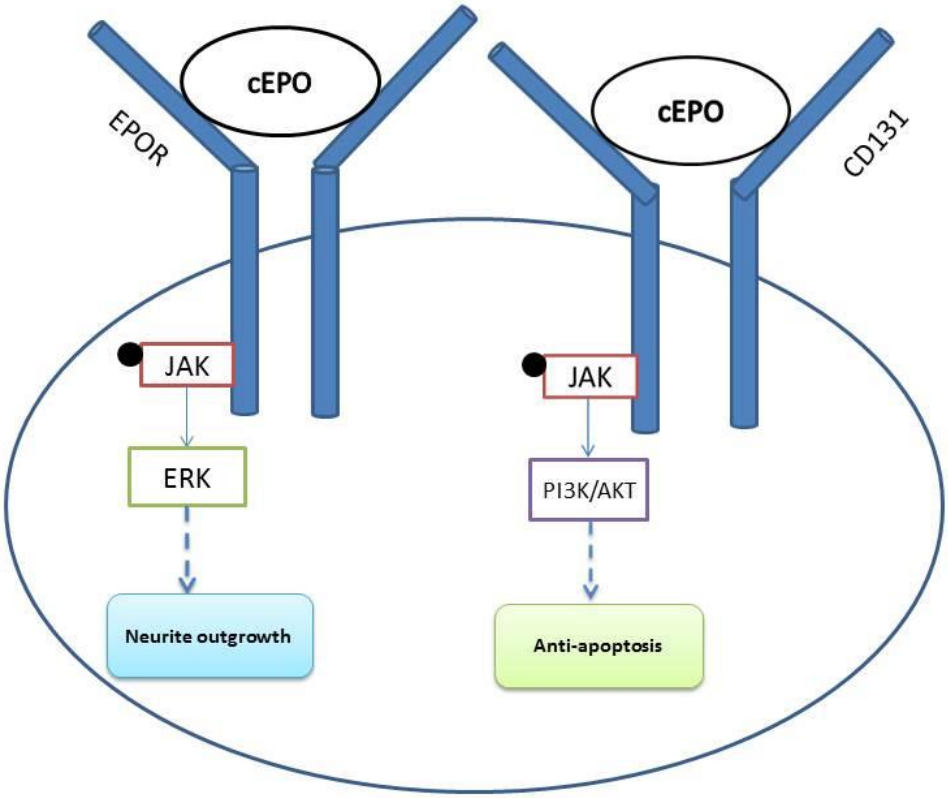

FIGURE 2. Schematic cEPO and EPO receptors. EPO joins two classic receptors including EPOR which is expressed in hematopoietic cells, while CD131 is expressed in cells in non-hematopoietic tissues. EPO modulates many intracellular pathways through EPORs, directing to anti-apoptotic gene expression and the suppression of pro-apoptotic genes. These functions lead to cell survival, growth, and differentiation. Induction of the homodimeric EPOR/EPOR leads to erythropoiesis, but induction of the heterodimeric EPOR/βcR (CD131) makes tissue repair.

AD brains, Aβ40 and Aβ42 are two forms of Aβ, with the latter being more abundant and presenting higher CNS toxicity. In mice, treatment with EPO resulted in a 20% decrease in total Aβ load and a 59% decrease in Aβ40 protein expression levels. Additionally, EPO counteracts oxidative stress associated with mitochondrial deficiency in AD by stopping lipid peroxidation accumulation in the hippocampus of mice (Cevik et al., 2017).

Also, EPO has an essential role in neurotrophin and neurogenesis. It promotes the manufacturing of brain-derived neurotrophic factor (BDNF) and tyrosine receptor kinase B, exerting protective effects, especially in safeguarding dopaminergic neurons. Since reduced dopamine neurotransmission contributes to attention deficit hyperactivity disorder, EPO might hold promise as a treatment. Recent concerns regarding early EPO administration exacerbating pathologic neovascularization associated with retinopathy of prematurity have been addressed. Notably, intra-cerebroventricular administration of EPO significantly increases BDNFs in the brain (Sun et al., 2019).

In a study by Cevik et al., EPO demonstrated a positive influence on sporadic AD in an intracerebroventricular (ICV)-streptozotocin (STZ) animal model. According to the results, EPO treatment meaningfully stopped ICV-STZ-induced primary deficiets by reducing the hippocampal neuronal loss, underscoring the therapeutic potential of EPO for AD (Cevik et al., 2017).

Recent studies have highlighted the positive effects of EPO in cerebral ischemia, preventing ischemic neuronal injury or stroke. EPO's neuroprotective effects on non-hematopoietic tissues can be controlled by both circulating and topically produced EPO. Furthermore, EPO helps in preserving oxygen deprivation in astrocytes and oligodendrocyte precursor cells in human ischemic brains (Ma et al., 2022).

Furthermore, it has been demonstrated that EPO induces the expression of Synapsin1 and PSD95, reducing axonal damage, and promoting axonal regeneration,

thereby enhancing the electrophysiological characteristics of synapses and spatial memory function following neonatal rat oxygen deprivation. EPO activates neuroprotective effects by simulating Akt, mTOR, Wnt, AMPK, and silent mating-type information regulation two homolog 1 (SIRT1) pathways (Simon et al., 2019).

Previous research indicates that exogenous administration of EPO decreases blood-brain barrier disruption post-cerebral ischemia, lowers vascular endothelial growth factor receptor-2 (VEGFR-2) expression in the penumbra section, and augments expression and function of matrix metalloproteinases 1 and 2 (MMP-1 and MMP-2) in ischemic micro-vessels (Wang et al., 2006, Kimáková et al., 2017, Pezeshkian et al., 2021).

EPO's potential to promote neurogenesis and angiogenesis after cerebral ischemia involves suppressing astrocytes and reducing their numbers. Moreover, EPO mitigates neuroinflammation in pathological conditions by decreasing microglial activation and modulating microglia polarization (Pavenski et al., 2011).

The Therapeutic Effects of Carbamylated Erythropoietin in the Brain and the Other Tissues

CEPO Functions

A modified form of EPO called CEPO exhibits tissue-protective properties without causing erythropoietic effects. CEPO results in a derivative α-amino group with reduced hematopoietic effects compared to EPO (Fantacci et al., 2006, Mahmood et al., 2007). Studies have indicated that CEPO may hold significant neuroprotective influences in animal models of neurological disorders, with various reports supporting these protective effects. These effects encompass the protection of central nerves across different models. Additionally, investigations using neurospheres aimed to understand how CEPO enhances neurogenesis and selectively encourages the differentiation of neuronal progenitor cells into neurons (King et al., 2007). Recently, numerous studies have highlighted CEPO's role as a neuroprotective agent in several CNS diseases, such as AD.

Role of CEPO in Non-Neurological or Peripheral (kidney, heart, etc.) Disorders

Wang et al., showed that EPORs may regulate certain kidney physiological functions. Other studies showed that CEPO had a more pronounced effect on kidney function. Imamura et al., demonstrated, similar to EPO, that CEPO exhibited superior protective effects on mouse kidneys in an ischemia-reperfusion (I/R) injury model. In this study, CEPO reduced tubular apoptosis, decreased α-SMA (alpha-smooth muscle actin) express sion, and enhanced the proliferation of tubular epithelial cells in nephrons (Wang et al., 2007).

In another experimental model, high doses of CEPO reduced obstructive tubular epithelial apoptosis induced by unilateral ureteral obstruction-induced renal injury. Furthermore, CEPO decreased α-SMA expression in the lack of polycythemia. In the I/R injury model after kidney transplantation, CEPO notably decreased serum creatinine levels at both 16 and 24 hours after transplantation. CEPO improved serum creatinine levels in the long-term compared to salt-treated kidney model mice at weeks 4 and 8 (Tögel et al., 2016).

The therapeutic efficacy of CEPO was evaluated using the endothelial formation method in the Henle tube in vitro and an I/R injury model in rats in vivo. In another study, the effect of CEPO on a rat model of I/R was investigated. Kidneys treated with CEPO exhibited inhibited tubular apoptosis and α-SMA expression in the interstitium compared to those treated with regular saline. These findings suggest that CEPO could be a beneficial therapeutic approach in protecting kidneys from I/R injury (Ding et al., 2017). One study looked at the effect of oxidative stress on diabetic rats and the impact of erythropoietin CEPO on renal oxidative stress in these rats. The investigation assessed kidney and blood samples to evaluate oxidative stress and kidney function. Results showed that diabetic rats represented increased oxidative stress in the kidney and associated physiological changes. Subsequent treatment with CEPO reduced oxidative stress and renal dysfunction in these models. These results suggests that CEPO may protect against oxidative stress damage and inhibit kidney damage (He et al., 2013).

Erythropoietin is the primary regulator of erythropoiesis and promotes the survival, proliferation, and differentiation of erythroid progenitor cells. The EPOR belongs to the same family of receptors as growth hormone, granulocyte-colony stimulating factor, granulocyte macrophage-colony stimulating factor, and some interleukins. In the erythropoietic process, EPO induces the homodimerization of the EPOR found on the surface of erythroid progenitor cells. EPO has demonstrated the ability to prevent apoptosis in adult rat cells from in vitro

and decrease cardiac cell loss in an ischemic rat model. This suggests that EPO could directly preserve cardiac cells against increased hemoglobin (Hb) concentrations. These results were confirmed through screening EPORs expressed in adult rat hearts (Liu et al., 2011). Like EPO, CEPO can reduce staurosporine-induced apoptosis in adult rat cardiomyocytes and reduce cardiac cell loss after rat myocardial infarction. These results extend the supportive influences of CEPO to other tissues. In one investigation, CEPO improved left ventricular (LV) output and reduced systolic pressure, similar to previous studies (Erbayraktar et al., 2006). EPO binds to EPORs, while CEPO activates heteromeric receptors containing an EPO receptor monomer and βcR (β-common receptor). In addition, these differences may be attributed to their various dependencies on the two EPOR subgroups. The bottom line is that different results from these experiments might be due to different sample contrasts, timing of CEPO administration, method of administration, and CEPO dosage. Nevertheless, the effects of CEPO extend to other tissues (Xu et al., 2009).

A study on lung tissue showed that CEPO could not reduce monocrotaline-induced pulmonary hypertension in mice, showing no positive effect. Conversely, in another study, CEPO demonstrated significant effectiveness in wound healing in a mouse model. CEPO operates through various mechanisms in body tissues. CEPO does not enable JAK-2 and connects to AKT from other signaling pathways without stimulating JAK-2. CEPO has shown neurotrophic and neurogenesis functions in some mouse models through stimulating neurotrophic factors like nerve growth factor (NGF) and BDNF. While CEPO fails to activate cellular proliferation signals, its anti-apoptotic effect is mediated by Akt activation (Adembri et al., 2008).

Role of CEPO in Neurological or Central (brain) Disorders

Researchers have demonstrated that CEPO could induce neuronal differentiation of human neural stem cells (hNSCs), correlating with Stat3, Stat5, and Akt activation. In an experimental autoimmune encephalomyelitis (EAE) model, CEPO reduced the production of inflammatory factors like TNF-α, IL-1H (Interleukin-1H and interleukin-1 receptor antagonist protein), and IL-1Ra in the spinal cord similar to EPO-treated models.

In addition, CEPO showed its activity by phosphorylating histone deacetylase 5 (HDAC5), inducing the PKC pathway, and promoting angiogenesis. However, reliable evidence supporting the clinical use of CEPO remains inconclusive (Nijboer et al., 2010). Therefore, more trials are needed across various clinical settings to determine the appropriate dosage, timing, and underlying mechanisms of CEPO responsible for its beneficial effects. Moreover, ensuring the safety profile of CEPO is imperative before its clinical application. Additional studies are needed to find the exact pathways affected by CEPO (Xiong et al., 2011). A recent clinical trial in patients with Friedrich's ataxia showed that recombinant human erythropoietin substantially increased frataxin expression, which plays a vital role in understanding the effect of this compound on the body's cellular signals. An in vitro investigation of EPOR function and increased recombinant human erythropoietin (rhEPO) frataxin demonstrated that non-erythropoietic carbamylated EPO, which cannot bind to classical EPOR, increased frataxin expression. It was observed that rhEPO increased frataxin expression in K562 cells (with EPO-R expression) as well as in THP-1 cells (without EPO-R expression). These findings proved that CEPO could not bind EPOR, increasing frataxin expression levels similar to rhEPO. Also, they showed that both EPO derivatives notably increased frataxin expression in vitro in controls and patients with Friedrich's ataxia. However, more studies are required to validate these results (Armand-Ugón et al., 2015).

In another examination, the influence of EPO on neurogenesis in the rat hippocampus and the capacity of both EPO and CEPO to stimulate the dendritic length of neurons in cultured hippocampal neurons were compared. Male mice were injected daily with EPO and sacrificed 12 hours after the last injection. CEPO treatment meaningfully increased neuron length and improved neuronal function in the spine. So, CEPO may be suitable for treating neurological problems, for example, hippocampal-related depression and anxiety in the limbic nervous system. Given the hippocampus's role in memory, these positive effects of CEPO on memory function are noteworthy (Tayra et al., 2013).

Additionally, beyond its prognosis associated with cerebral infarction, CEPO also improves microcirculation. A study indicated that CEPO could improve vascular endothelial cell proliferation in an animal model of cerebral infarction by increasing VEGF expression,

consequently improving blood flow to the affected area through increased angiogenesis (Choi et al., 2014). this enhancement in blood vessel levels reduces ischemic damage after brain injury. CEPO holds significant potential as a pharmacological agent for treating several diseases related to both the central and peripheral nervous systems. It is a promising candidate for therapeutic advancement due to its beneficial effects on cell proliferation and excellent performance improvement. CEPO's role in treating conditions like stroke, traumatic brain, and spinal cord injuries, which affect millions annually or lead to death, is crucial. However, clinical studies on CEPO for stroke patients have not yet reached an appropriate stage. Also, preclinical studies need to delve deeper into the cellular and molecular signals associated with CEPO treatment (Liu et al., 2015).

Moreover, Dang et al., presented that EPO and CEPO induced different protein expressions in different regions of the brain. For instance, CEPO stimulated synaptic plasticity-related proteins in the molecular layer, whereas EPO stimulated pleiotrophin in the increased vascular layer in the rat brain (Na et al., 2020).

Overall, CEPO has several effects on patients, demonstrating its potential for clinical studies. Its impact on renal angiogenesis and improvement, along with its effect on the nervous system and related patients, is particularly noteworthy (Na et al., 2020).

The Therapeutic Effects of CEPO-Fc in the Brain and the Other Tissues

CEPO-Fc Functions

Fusion proteins have been engineered to improve the pharmacological properties of EPO, aiming to alter its half-life or progressing its delivery to the brain via the blood-brain barrier (Li et al., 2017).

In this erythropoietin-Fc-fusion protein, two Recombinant Human EPO molecules are fused to the Fc domain of an IgG1 antibody. This fusion is then carbamylated, losing its ability to induce red cells, while retaining cell-preserving properties. Fc-fusion CEPO, obtained through carbamylation of EPO-Fc, exhibits an extended half-life, allowing EPO to interact with its receptors for an extended duration (Kontermann, 2011).

Non-erythropoietic EPO derivatives have demonstrated similar neuroprotective effects without common EPO side effects such as hypertension. CEPO-Fc activates the βcR without stimulating erythropoiesis. (figure2)

A Recombinant protein developed by Polymun Scientific meets these criteria. This new pharmaceutical construction, known as the EPO-Fc fusion protein (EPO-Fc), incorporates the persistent domain of immunoglobulin, prolonging its half-life (Schriebl et al., 2006). The carbamylation process, a chemical modification using the cyanate ion and the free Epsilon NH2 group of lysines, changes the shape of protein, rendering CEPO-Fc incapable of binding to the typical EPO receptor, hence lacking erythropoietic function. Also, CEPO can pass the blood-brain barrier in both rats and humans, similar to EPO (Moosavi et al., 2020).

Role of CEPO-Fc in Neurological Disorders

Following removing erythropoietic effects from carbamylated EPO, several investigations evaluated its neuroprotective influence in various animal models of neurotoxicity, such as spinal cord depression and sciatic nerve compression, highlighting the neuroprotective effects of this EPO derivative (Zhang et al., 2020).

Furthermore, some severe unfavorable effects and even seizures have been observed when a higher dosage of erythropoietin was used. CEPO-Fc is typically utilized in various neurodegenerative injuries like Alzheimer's, Parkinson's, and periventricular leukomalacia. (Chen et al., 2016).

In a study by Maghsoudi et al., CEPO-Fc was tested in an in-vivo rat model for its anti-neurotoxic effects induced by Aβ25-35. In their research, mature male Wistar rats were operated through the hippocampus, and Aβ25-35 was injected for four days. During the next six consecutive days, CEPO-Fc was administered. Additionally, immunoblotting was used to measure the levels of critical proteins involved in apoptosis (Bax, Bcl-2, and caspase-3), necroptosis phosphorylated receptor-interacting protein kinase 3 (p-RIP3), autophagy p-Beclin-1, and phosphorylated-1A/1B-light chain 3 (p-LC3-II). Their results showed that the efficacy of CEPO-Fc at 75 IU was better than 112 IU. Behavioral analysis, CEPO-Fc treatment significantly improved learning and memory. Additionally, molecular analysis indicated that CEPO-Fc enhanced p-Beclin-1 and p-LC3-II expression, while decreasing caspase-3, Bax/Bcl2 ratio, and p-RIP3 expression. Activation of autophagy and suppression of apoptosis and necroptosis processes were found to control CEPO-Fc's neuroprotective characteristics in an AD animal model. Moreover,

this study showed CEPO-Fc to be a beneficial protective compound against AD and other neurodegenerative diseases (Maghsoudi et al., 2021).

In a study of the hippocampus, immunoblotting was used to measure the molecular levels of apoptosis (Bax, Bcl-2, and caspase-3), necroptosis (phosphorylated receptor-interacting protein kinase 3 (p-RIP3)), autophagy (phosphorylated Beclin-1 p-Beclin-1), and phosphorylated 1A/1B-light chain 3 (p-LC3-II). These results suggest that an i.p. dose of CEPO-Fc may protect against the neurotoxicity associated with AD. Also, intraperitoneally injections of CEPO-Fc have neuroprotective influences on the AD rat model that can be controlled in part by stimulation and inhibition of the apoptosis and necroptosis pathways as well as it may be utilized as a viable therapeutic option for AD (Maghsoudi et al., 2021).

Pro-inflammatory cytokines generated by stimulated microglia, astrocytes, and neurons are associated with pain process and memory function. Although many investigations presented useful influences of IL-1β in natural memory processing, peripheral changes enhance hippocampal IL-1β levels and many functional side effects in hippocampus-dependent memory pathways. Also, CEPO-Fc was found to suppress learning and memory problems by reducing hippocampal cell death (Rahmani et al., 2022). Additionally, CEPO demonstrated efficacy in mitigating CFA-induced thermal hyperalgesia and memory problems by decreasing hippocampal microglial expression and IL-1β levels associated with inflammatory pain. These findings suggest the neuroprotective effects of CEPO-Fc in addressing pain-related recognition memory problems (Rahmani et al., 2022).

Moosavi et al., assessed the neuroprotective effects of CEPO-Fc against intracerebroventricular STZ-induced memory problems and apoptosis in the hippocampus. CEPO-Fc demonstrated preservation against STZ-induced water maze learning and memory deficits and hippocampal caspase-3 induction, showcasing its neuroprotective potential (Moosavi et al., 2020).

Hooshmandi et al., investigated CEPO-Fc's protective role in primary hippocampal cell cultures against Aβ25–35 toxicity. CEPO-Fc significantly diminished cell death due to Aβ25−35, possibly through its effect on caspase induction and Akt/GSK-3β, ERK, and MMP-2 signaling, emphasizing its protective impact in an AD model (Hooshmandi et al., 2020).

Erythropoietin and CEPO-Fc, acting as antioxidative and antiapoptotic agents, have shown neurological improvements in animal models following spinal cord ischemia. Simon et al., indicated the impact of EPO and CEPO-Fc on clinicopathological features, observing increased expression of ER stress-relevant proteins GRP78 and Caspase 12, potentially leading to improved clinicopathological features in mice (Simon et al., 2018).

The Fc part of EPO-Fc incorporates the hinge region along with the CH2 and CH3 domains of IgG. In IgG, covalent disulfide bonds exist between its hinge parts. Typically, amino acid residues ranging between 2 and 16 link the EPO molecules and the Fc portion of IgG. Compared to native or fused human EPO, rhEPO fused to the IgG Fc domain demonstrated an extended half-life and potential for anemia treatment. Clinical trials, such as a phase I trial in Korea, have commenced for EPO-Fc, although detailed information on successful clinical outcomes with EPO-Fc-based agents remains limited (Gattinger et al., 2021).

While some data, such as complementary determining regions and variable domain sequences, are available for EPO-Fc proteins, complete amino acid sequences remain undisclosed. The physicochemical properties and function of commercial versions of EPO-Fc may differ depending on the manufacturer's bioproduction processes. Currently, the European Union has not approved any EPO-Fc versions. Liquid chromatography-mass spectrometry (LC−MS) emerges as a valuable technique for protein drug analysis, offering selectivity, sensitivity, specificity, and relatively cost-effective method development, particularly in pharmacokinetics and toxicokinetic investigations of protein therapeutics. In addition, LC-MS can analyze intact and proteolyzed proteins (Mesonzhnik et al., 2018).

In a study by Mesonzhnik et al., the primary structure of industry-available EPO-Fc was determined using comprehensive LC-MS. The analysis identified EPO-Fc as a chimera with two EPOs linked to the Fc portion of IgG2 through a mono-dipeptide glycyl-seryl linker. Additionally, synthetic standards were used to verify peptides covering unknown fusion breakpoints. The insights provided by Mesonzhnik et al. in 2018 were instrumental in characterizing EPO-Fc sequences. Their study utilized intact MW measurements of deglycosylated EPO-Fc and its fragments, employing high-resolution mass spectrometry to accurately assess

TABLE 1: Summary of some investigations about CEPO-Fc in neurological disorders.

References	Samples	Method and Materials	Results
Maghsoudi et al. 2021	Adult male Wistar rats	Aβ25-35 (5 μg/2.5 μL) was injected into the dorsal hippocampus over four consecutive days. CEPO-Fc (75 or 112 IU) was administered intranasally for the subsequent six consecutive days.	The neuroprotective effects of CEPO-Fc in an animal model of AD may be attributed to its ability to activate autophagy while suppressing apoptosis and necroptosis pathways.
Maghsoudi et al. 2021	Adult male Wistar rats	Aβ25-35 (5 μg/2.5 μL) was injected into the dorsal hippocampus over four consecutive days. CEPO-Fc was injected intraperitoneally in two doses of 500 and 5000 IU during the next six days.	The neuroprotective effects of CEPO-Fc in the AD rats may be attributed to its ability to activate autophagy while regulating apoptosis and necroptosis processes
Rahmani et al. 2021	Adult male Wistar rats	Carbamylated erythropoietin was administrated intraperitoneally on the day of CFA (Complete Freund's Adjuvant) injection and continued for 21 days	The neuroprotective effects of CEPO-Fc in treating pain-related recognition memory impairment might be managed by diminishing hippocampal microglial expression and IL-1β production.
Moosavi et al. 2020	Adult male Wistar rats	Streptozotocin (STZ)- an inducer of sporadic AD was administered on days 1 and 3 (3 mg/kg in divided doses/icv), and CEPO-Fc was administered at a dose of 5000 IU/i.p/daily from days 4 to 14.	CEPO-Fc treatment at a dose of 5000 IU/kg/ip helped preserved the learning and memory deficits induced by icv-STZ
Hooshmandi et al. 2020	Primary hippocampal cultures from embryonic rat brains	The cells were exposed to Aβ25−35 (20 μM) in the absence or presence of CEPO-Fc (1 or 5 IU) along with PI3k and ERK inhibitors	The protective effects of CEPO-Fc against Aβ-induced cell toxicity might be primarily mediated through the PI3K/Akt pathway rather than ERK signaling.
Hooshmandi et al. 2018	Adult male Wistar rats	Aβ25-35 was administered intrahippocampally for 4 consecutive days (5μg/2.5μL/each side/day), followed by intraperitoneal injections of CEPO-Fc (500 or 5000 IU) from days 4 to 9.	CEPO-Fc preserved against Aβ-induced learning and memory deterioration while modulating hippocampal MAPKs, Akt/GSK-3β, and MMP-2 activity.
Simon et al. 2018	Male mice (C57BL/6J)	Three study groups (Epo, CEPO-Fc, and control) were observed for 96 hours. The clinical and neurological outcomes of the mice were investigated by the Basso-Mouse-Scale (BMS).	The significant positive effect of Epo and CEPO-Fc (increased expression of Caspase12) on the clinical, neurological, and histological outcomes of the mice was observed.
Mesonzhnik et al. 2017	Characterize EPO fusion proteins with unknown structures	LC−MS analysis	Peptides covering unknown fusion breakpoints (spacer peptides) were identified by multiple proteases. The "spacer peptides" can be utilized in the identification of EPO-Fc fusion proteins in biological samples via conventional LC−tandem MS methods.
Simon et al. 2016	New Zealand White rabbits	CEPO-FC (50 mg/kg; n = 8), rhEPO (5000 IU/kg; n = 10), or vehicle (control; n = 10) were administrated to an anesthetized but spontaneously breathing 30 minutes before and after infrarenal aortic clamping	Preconditioning with rhEPO attenuates spinal cord ischemia or reperfusion injury, whereas CEPO-FC indicated no significant influence on spinal cord function.

References	Samples	Method and Materials	Results
Matejkova et al. 2013	Twenty pigs of either sex (age 13–20 months)	Anesthetized and mechanically ventilated animals received CEPO-FC (50 μg kg^{-1}), rhEPO (5,000 IU kg^{-1}), or vehicle (n = 9 per group) before 120 minutes of aortic occlusion and over 4 hours of reperfusion	In swine with atherosclerosis, rhEPO and CEPO-FC failed to decrease prolonged ischemia-induced kidney injury within an 8-hour reperfusion period. This lack of effect might be attributed to decreased EPOR expression resulting from pre-existing oxidative stress and/or diminished NO release.
Tayra et al. 2013	Adult female Sprague-Dawley rats	EPO-Fc and CEPO-Fc were administrated intraperitoneally. Behavioral evaluations included rota-rod, cylinder, and amphetamine-induced rotation tests	The CEPO-Fc may provide neuroprotective and neurorescue benefits in a rat model of Parkinson's disease without the side effects associated with polycythemia, particularly evident in the amphetamine-induced rotation test
Simon et al. 2011	Adult pigs	CEPO-FC (50 μg kg^{-1}), rhEPO (5,000 IU kg-1), or vehicle (n = 9 per group) were administered to anesthetized and mechanically ventilated subjects 30 minutes before a 30-minute period of aortic occlusion and continued over the 4-hour reperfusion	In a porcine model of aortic balloon occlusion-induced spinal cord I/R injury, CEPO-FC, and rhEPO comparably preserved against ischemic spinal cord dysfunction and neuronal injury.

the Fc/2 fragment and its modifications. Spike-in experiments indicated the potential use of "spacer" peptides for identifying EPO-Fc fusion proteins in human matrices. They also suggested that analyzing individual fragments of EPO-Fc by LC−MS/MS could serve as an alternative method to traditional ligand-binding assays, offering advantages in assessing specific types of EPO-Fc fragments (Mesonzhnik et al., 2018).

Although the hematopoietic effects of rhEPO stem from inducing a homodimeric EPO receptor complex (EPO-R/EPO-R), its organ-preservative characteristics are associated with stimulating a heterodimeric receptor complex consisting of EPO-R and the common-receptor (EPO-R/ βcR). CEPOs do not bind to the EPO-R/ EPO-R homodimer but demonstrate cytoprotective effects as rhEPO. CEPO has shown efficacy in reducing I/R-induced inflammation in brain-dead rats, while the CEPO-Fc, a carbamylated EPO-Fc fusion protein combining EPO molecules with the Fc part of IgG1, demonstrated preservation against spinal cord I/R deficits (Chamorro et al., 2013).

Matejkova et al., examined the theory that rhEPO and CEPO-Fc can preserve against kidney I/R injury in swine with ubiquitous atherosclerosis. They found that both CEPO-Fc and rhEPO were unsuccessful in reducing I/R injury-induced organ deficiency and histological injury, with no notable alterations observed in inflammation, oxidative, and nitrosative stress markers (Matějková et al., 2013).

Moreover, in an experiment, the administration of EPO and CEPO-Fc shortly before and after ischemia exhibited promising results in diminishing the risk of spinal cord damage. A refined CEPO-Fc demonstrated the regeneration of motor evoked potentials (MEPs) in ischemic spinal cord defects and neuronal injuries in pig and rabbit models (Simon et al., 2011).

In the study conducted by Tayra et al., the neuroprotective and neurorescue effects of EPO-Fc and CEPO-Fc in a rat model of Parkinson's disease (induced by 6-OHDA) were explored. Carbamylated EPO exhibited neuroprotective functions by crossing the blood-brain barrier without demonstrating erythropoietic characteristics. The neuroprotection experiments iindicated significant advancements compared to the EPO-Fc category, but in neurorescue tests, CEPO-Fc treatment resulted in greater behavioral scores than the control group. (Tayra

et al., 2013).

Conclusion

The evidence supports EPO's potential as a therapeutic agent, showcasing its pivotal role in addressing neuronal injury and potentially heralding new paths for neuroprotective treatments in humans (Table 1). CEPO demonstrates significant neuroprotective effects, particularly in preserving primary hippocampal cells against Aβ25−35 toxicity through the modulation of Akt, GSK-3β, and ERK signaling pathways. These compelling findings emphasize CEPO's potential as a neuroprotective compound, holding promise for the treatment of AD and other neurodegenerative disorders (Table 1).

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Conflict of interest

The authors declare that they have no competing interests.

Ethics approval

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