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Positive biofeedback of erythropoietin by enhancing nerve regeneration and functional recovery

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Abstract
Erythropoietin also known as EPO, hematopoietin, or hemopoietin, is a glycoprotein hormone that controls erythropoiesis, or red blood cell production. It is a cytokine (protein signaling molecule) for erythrocyte (red blood cell) precursors in the bone marrow. Human EPO has a molecular weight of 34 kDa. Erythropoietin is produced by interstitial fibroblasts in the kidney in close association with peritubular capillary and proximal convoluted tubule. It is also produced in perisinusoidal cells in the liver. While liver production predominates in the fetal and perinatal period, renal production is predominant during adulthood. Erythropoietin in neuroprotection is the use of the glycoprotein erythropoietin (Epo) for neuroprotection. Epo controls erythropoiesis, or red blood cell production. Erythropoietin and its receptor are both present in the central nervous system with erythropoietin alpha capable of crossing the blood brain barrier via active transport. The presence of Epo within the spinal fluid of infants and the expression of Epo-R in the spinal cord, suggesting a role by Epo within the CNS. Epo and Epo-R is expressed in the mammalian retina, and therefore Epo represents a potential therapy to protect photoreceptors damaged from hypoxic pretreatment. Erythropoietin has been shown to protect nerve cells from hypoxia-induced glutamate toxicity. Acute hypoxia inducement in the adult mouse retina stimulates expression of Epo in addition to other growth factors. Epo response is stimulated by hypoxia and is capable of protecting against apoptosis of erythroid progenitors via a mechanism that is described in the Mechanism of Action section. Epo-R is present in cultured hippocampal and cerebral cortical neurons isolated from rat embryos. Epo was capable of protecting the cultured neurons from glutamate neurotoxicity after only a short exposure. It was concluded that Epo-mediated increase in intracellular calcium concentration is indicative of Epo's neuroprotective role after CNS-related hypoxia or ischemia.

Keywords: erythropoietin, hypoxia, erythrogenesis, neurogenesis, Neuroregeneration

Introduction

Exogenous erythropoietin is produced by recombinant DNA technology in cell culture. Several different pharmaceutical agents are available with a variety of glycosylation patterns, and are collectively called erythropoiesis-stimulating agents (ESA). The specific details for labeled use vary between the package inserts, but ESAs have been used in the treatment of anemia in chronic kidney disease, anemia in myelodysplasia, and in anemia from cancer chemotherapy. Boxed warnings include a risk of death, myocardial infarction, stroke, venous thromboembolism, and tumor recurrence. Exogenous erythropoietin has been used illicitly as a performance-enhancing drug; it can often be detected in blood, due to slight differences from the endogenous protein, for example, in features of posttranslational modification. While, on mutation, EpoR has been found in high levels in the embryonic brain, its role in brain development is unclear. Epo stimulates neural progenitor cells and prevents apoptosis in the embryonic brain in mice [6]. Mice without EpoR demonstrated severe anemia, defective heart development, and eventually death around embryonic day 13.5 from apoptosis in the liver, endocardium, myocardium, and fetal brain. As early as embryonic day 10.5 the lack of EpoR can affect brain development by increasing fetal brain apoptosis and decreasing the number of neural progenitor cells. By exposing cultures of EpoR positive embryonic cortical neurons to stimulation by Epo administration, the cells decreased apoptosis, as opposed to the decrease in neuron generation in EpoR negative cells. The neuroprotective activity of Epo can be observed as early as embryonic day 10.5 in the developing brain and contributes to selective cell survival in the developing brain.

However it has been questioned whether EpoR may or may not be a determining factor for the nervous system function [7]. The contribution of Epo and EpoR to neuroprotection and development are not as clearly understood as its role in erythropoiesis in hematopoietic tissue. In a line of mice that expressed EpoR exclusively in hematopoietic cells, the mice developed normally and were fertile, despite the lack of EpoR in nonhematopoietic tissue. Differential expression of EpoR between erythroid cells. Most notably, plasma Epo concentration is regulated by nonhematopoietic EpoR expression when the peak of plasma concentrations for induced anemia in mutant and wild-type mice. The expression of EpoR in nonhematopoietic tissue is dispensable in normal

mouse development, but that the sensitivity of erythroid progenitors to Epo is regulated by the expression of EpoR.
Erythropoietin mutants R103-E and S100-E (though S100 in Epo doesn't exist) has been reportedto be non-erythropoietin but retain the neuroprotective function. Epo with R103 mutation is a potent inhibitor of wild type Epo from binding to its receptor. Though, the viral vector expressed R103-E Epo mutant was shown to be inhibitory to the progression / development of nervous tissue damage in many models, it is not shown to recover the nervous tissue post damage. Given the associated risks, it would be foolish to administer / express Mutant as a preventive measure from neuronal injury. Hence, from a medical or commercial point of view, safe and feasible neuro-protective Epo mutants are not possible.
It should also be noted that quite a bit of research emphesis is on non erythropoietic but, neuroprotective Peptides of Erythropoietin. Peptide of Epo with amino acids 92-111 is neuroprotective while its erythropoietic potency is 10 fold less than the wild type.
A short peptide sequence from the erythropoietin molecule called JM4, has been found to be non-erythropoietic yet neuroprotective and is being readied for Stage 1 and 2 clinical studies.[8, 9]

Production and localization in Peripheral nervous system
Erythropoietin and its receptor are also present in the peripheral nervous system, specifically in the bodies and axons of ganglions in the dorsal root, and at increased levels in Schwann cells after peripheral nerve injury.[10] The distribution of EpoR is different from Epo, specifically in some neuronal cell bodies in the dorsal root ganglion, endothelial cells, and Schwann cells of normal nerves. Most importantly, experiments with immunostaining revealed that the distribution and concentration of EpoR on Schwann cells doesn’t change after peripheral nerve injury. This is in agreement with research that showed Epo is up-regulated according to mRNA expression in astrocytes and hypoxia-induced neurons, while EpoR is not.[11] A correlation between the expression of Epo-R in ganglion cells and binding to sensory receptors in the periphery like Pacini bodies and neuromuscular spindles suggests that Epo-R is related to touch regulation.[12]

Peripheral nerve injury
After nerve injury, the increased production of Epo may induce activation of certain cellular pathways, while the concentration of EpoR doesn’t change. In Schwann cells, increased erythropoietin levels may stimulate Schwann cell proliferation via JAK2 and ERK/MAP kinase activation to be explained later. Similar to stimulation of red blood cell precursor cells (erythrogenesis), erythropoietin stimulates non-differentiated Schwann cells to proliferate. [12]
Although the mechanism is unclear, it is apparent that erythropoietin has anti-apoptotic action after central and peripheral nerve injury. Cross-talk between JAK2 and NF-κB signaling cascades has been demonstrated to be a possible factor in central nerve injury. Erythropoietin has also been shown to prevent axonal degeneration when produced by neighboring Schwann cells with nitrous oxide as the axonal injury signal. [13]

Direct and indirect effects
Erythropoietin exerts its neuroprotective role directly by activating transmitter molecules that play a role in erythrogenesis and indirectly by restoring blood flow. [14] Subcutaneous administration of RhEpo on cerebral blood flow autoregulation after experimental subarachnoid hemorrhage was studied. In different groups of male Sprague-Dawley Rats, the injection of Epo after induction of hemorrhage normalized the autoregulation of cerebral blood flow, while those treated with a vehicle showed no autoregulation.

Cerebral damage and inflammation
Additionally to the anti-apoptotic effect, Epo reduces inflammatory response during different types of cerebral injury via the NF-κB pathway.[17] The NF-κB pathway activated by Epo/EpoR phosphorylation plays a role in regulating inflammatory and immune response, in addition to preventing apoptosis due to cellular stress.[18] NF-κB proteins regulate immune response through B-lymphocyte control and T-lymphocyte proliferation. These proteins are all important for the expression of genes specific to immune and inflammatory response regulation.

Neuroprotective effects
As a neuroprotective agent erythropoietin has many functions: antagonizing glutamate cytotoxic action, enhancing antioxidant enzyme expression, reducing free radical production rate, and affecting neurotransmitter release. It exerts its neuroprotective effect indirectly through restoration of blood flow or directly by activating transmitter molecules in neurons that also play a role in erythrogenesis. Although apoptosis is not reversible, early intervention with neuroprotective therapeutic procedures such as erythropoietin administration may reduce the number of neurons that undergo apoptosis. [12]

Neonatal brain injury
In infants with poor neurodevelopment, prematurity and asphyxia are typical problems. These conditions can lead to cerebral palsy, mental retardation, and sensory impairment. Hypothermia therapy for neonatal encephalopathy is a proven therapy for neonatal brain injury. However, recent research has demonstrated that high doses of recombinant erythropoietin can reduce or prevent this type of neonatal brain injury if administered early.[20] A high rate of neuronal apoptosis is evident in the developing brain due to initial overproduction. Neurons that are electrically active and make synaptic connections survive, while those that do not undergo apoptosis. While this is a normal phenomenon, it is also known that neurons in the developing brain are at an increased risk to undergo apoptosis in response to injury. A small amount of the RhEpo can cross the blood–brain barrier and protect against hypoxic-ischemia injury. Epo treatment has also shown to preserve hemispheric brain volume 6 weeks after neonatal stroke.[21] It demonstrated both neuroprotective effects and a direction towards neurogenesis in neonatal stroke without associated long-term difficulties.

Dopaminergic neurons
Epo was shown to specifically protect dopaminergic neurons, which are closely tied in to attention deficit hyperactivity disorder.[20] Specifically in mice, Epo demonstrated protective

effects on nigral dopaminergic neurons in a mouse model of Parkinson's Disease.[23] This recent experiment tested the hypothesis that RhEpo could protect dopaminergic neurons and improve the neurobehavioral outcome in a rat model of Parkinson's Disease. The intrastriatal administration of RhEpo significantly reduced the degree of rotational asymmetry, and the RhEpo-treated rats demonstrated improvement in skilled forearm use. These experiments demonstrated that intrastriatal administration of RhEpo can protect nigral dopaminergic neurons from 6-OHDA induced cell death and improve neurobehavioral outcome in a rat model of Parkinson's Disease.

Role in neurogenesis

Erythropoietin and its receptor have an essential role in neurogenesis, specifically in post-stroke neurogenesis and in the migration of neuroblasts to areas of neural injury.[24] Severe embryonic neurogenesis defects in animals that were null for Epo or EpoR genes are found. In EpoR knock-down animals, deletion of EpoR genes specific to the brain lead to a reduction in cell growth in the subventricular zone and impaired neurogenesis after stroke. This post-stroke neurogenesis was characterized by an impaired migration of neuroblasts in the peri-infarct cortex. This results is in agreement with the classical approach to Epo/EpoR contributions in development in that it demonstrated an Epo/EpoR requirement for embryonic neural development, adult neurogenesis, and neuron regeneration after injury. High doses of exogenous erythropoietin could demonstrate a neuroprotective role by binding to a receptor that contains the common beta receptor but lacks EpoR. These types of studies into Epo and EpoR null animals have seen and are further elucidating the neuroprotective role of Epo/EpoR in genetics and development.

Neuroregeneration

While the neuroprotective effects of Epo administration in models of brain injury and disease have been well described, the effects of Epo on Neuroregeneration are currently being investigated. Epo administration during optic nerve transaction was used to assess the neuroprotective properties in vivo as well as demonstrate the neuroregenerative capabilities.[25] The intravitreal injection of Epo increased retinal ganglion cell somata and axon survival after transaction. A small amount of axons penetrated the transaction site and regenerated up to 1 mm into the distal nerve. In a second experiment, Epo doubled the number of retinal ganglion cell axons regenerating along a length of nerve grafted onto the retrobulbar optic nerve. This evidence of Epo as a neuroprotective and neuroregenerative agent is extremely promising for Epo as therapy in central nerve injury and repair.

Conclusion

Erythropoietin has shown to have a neuroprotective role in both the central and peripheral nervous system through pathways that inhibit apoptosis. It has been successful in demonstrating neuroprotective effects in many models of brain injury and in some experiments. It is also capable of influencing neuron stimulation and promoting peripheral nerve regeneration. Epo has a lot of potential uses and could provide a therapeutic answer for nervous system injury. However, more studies need to be conducted to determine the optimal time and dosage for Epo treatment.

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Erythropioetin is a very potent molecule (One Unit ~ 10 ng)and is known to be extremely toxic in higher doses. Since, such a tight control of expression is impossible, genetherapy with viral vectors is not an option