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Review

Erythropoietin in ophthalmology: A literature review

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Abstract

***Purpose*:** To review the current literature on ocular application of erythropoietin (EPO).
***Methods*:** A comprehensive search was performed on Pubmed and Scopus databases. All selected articles were reviewed thoroughly by the authors to review current applications of the EPO in ocular diseases.
***Results*:** Various aspects of administration of EPO for different ischemic, traumatic, vascular, and degenerative disorders have been explained. The articles are generally preclinical with few small studies reporting clinical outcomes.
***Conclusion*:** EPO has been used for the treatment of different ophthalmic conditions with promising results. Further studies are needed to elaborate the role of EPO in management of ocular diseases.

Keywords: Erythropoietin; Neuroprotection; Apoptosis

Introduction

Erythropoietin (EPO) is a 30.4 kDa hematopoietic glycoprotein produced in the fetal liver and adult kidney. EPO is also produced in low levels in central nervous system (CNS) tissue, and the EPO receptor (EPOR) homodimer is expressed on most CNS cell types, including neurons, astrocytes, and microglia.[1] In fact, about 10% of EPO found in the bloodstream is of non-renal origin.[2] Historically, EPO has been known to promote hematopoiesis and used routinely for the treatment of anemia in clinical practice.[3] In addition, angiogenic, anti-inflammatory and endothelial cell stabilization effects of systemic EPO have been previously described.[4] In recent years, several studies have shown neuroprotective and neurotrophic activities for the EPO.[5–8] Although the exact mechanism is not clear, EPO has been shown to decrease the apoptosis, reactive oxygen species, excitotoxicity, and inflammation, and increase the progenitor cell proliferation.[4] It affects the regulators of apoptosis Bax, Bad, and Bcl-2/Bcl-xL by inhibiting formation of the Bax/Bcl complex and reducing activation of effector caspases.[9,10] EPO is an interesting candidate not only for stroke treatment, but also for delayed degenerative neurological conditions, such as amyotrophic lateral sclerosis, Parkinson's, and Alzheimer's disease.[11,12] There is emerging evidence that EPO has an important role in neuroprotection in the retina in addition to its effect in the central nervous system.[13] Expression of EPO and EPOR has also been found in the retina.[14]

Recently, promising results have been reported using systemic and intravitreal EPO for different ocular conditions. This review presents evidence derived from experimental studies in animals as well as human about the therapeutic effects of EPO on ocular tissue.

Methods

A Pubmed and Scopus search was performed in October 2015 using each of the following key words: “Erythropoietin”, “EPO”, “Eye”, and “Ocular”. All article types including original articles, reviews, and case reports that described the

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application of EPO in the eye were identified. Abstracts and non-English articles were excluded. All selected articles were reviewed thoroughly by the authors to review current applications of the EPO in ocular diseases.

Results

Preclinical studies

Glutamate- and Nitric oxide-induced toxicity

Glutamate- and Nitric oxide-induced toxicity is involved in glaucoma and ocular diseases caused by hypoxia and ischemia, such as diabetic retinopathy. Retinal ganglion cells (RGC) were isolated to test the neuroprotective effect of EPO, and brain-derived neurotrophic factor (BDNF), which is a potent neuroprotective agent, was used as a positive control. While EPO could not substitute for BDNF in improving RGC survival in serum free medium, it was useful in protecting RGCs from glutamate- and Nitric oxide-induced toxicity.[15] In a cultured medium for neurocytes, EPO induced a stable improvement of neurite outgrowth of retinal neurocytes. EPO was effective in promoting the survival and decreasing the apoptosis rates of the neurocytes suffering from glutamate-induced cytotoxicity.[16]

Diabetic retinopathy

In early stages of the disease in the streptozotocin (STZ)-induced diabetic rats, an increase in ganglion cells with swollen mitochondria, retinal glutamate, and EPOR in the retinas occurs. These changes can all be improved by intraperitoneal administered recombinant human erythropoietin (rhEPO).[17] Exogenous EPO administration by intravitreal injection in early diabetes prevented retinal cell death and protected the blood-retinal barrier function.[18] A single intravitreal injection of EPO (50 ng/eye) resulted in down regulation of EPOR, vascular endothelial growth factor (VEGF), and its receptor (VEGFR) in the diabetic rats. This effect persisted for at least 4 weeks.[19] A single intravitreal injection of EPO also produced therapeutic effects on blood-retinal barrier (BRB) function and neuronal survival at different time courses of retinopathy. The inhibition of exogenous EPO on hypoxia induced factor (HIF) -1 alpha-induced VEGF production in early diabetic retina may explain in part the protective function of EPO toward reestablishment of blood-retinal barrier integrity.[20] These findings suggest the EPO may be a useful agent for protection against progression of the diabetic retinopathy at the very early stage by reducing blood vessel degeneration.

Oxygen induced retinopathy (OIR)

EPO deficiency in heterozygous EPO-Tag transgenic mice resulted in more profound retinal dysfunction due to OIR. The dysfunction was detected by suppression in electroretinography (ERG) amplitudes; however, the extent of retinal ischemia, preretinal neovascularization, or neuroretinal degeneration in OIR, was not affected. Systemic administration of recombinant EPO protected EPO-deficient mice against this additional suppression.[21]

Glaucoma

In a rat model of glaucoma induced by cautery of the episcleral vessels, a single intravitreal 200 ng dose of EPO was effective to prevent at least some of the elevated pressure-induced loss of RGCs.[22] Systemic administration of rhEPO before or immediately after retinal ischemia induced by an increase in intraocular pressure (IOP) reduced histopathological retinal damage and promoted functional recovery as assessed by electroretinography. Exogenous EPO also significantly diminished terminal apoptotic events in the ischemic retina, implying an anti-apoptotic mechanism of action.[23]

Retinal detachment

Intravitreal injection of EPO in a rat model of retinal detachment suppressed caspase-3 activation and enhanced Bcl-XL expression, resulting in inhibition of apoptosis and protection of photoreceptor cells.[24]

Axotomy

Genetic engineering was used to develop a transgenic mouse line tg21 model that expresses human EPO preferentially in neuronal cells without inducing polycythemia. In this model, retina expresses human EPO and RGCs carry the EPO receptor. Upon axotomy, the RGCs of EPO transgenic tg21 mice were protected against degeneration, as compared with wild-type control animals. This protective effect was not only against acute, but also against slowly developing neuronal injury. EPO-induced neuroprotection in vivo is mediated by ERK-1/-2 signaling.[25] After optic nerve transaction in adult rats, intravitreal EPO was both neuroprotective and neuroregenerative for axotomized RGCs. Moreover, a small proportion of axons regenerated up to 1 mm into the distal nerve.[26]

Retinopathy of prematurity (ROP)

In the rat model of ROP, anti-VEGF treatment led to increased angiogenic signaling and recurrent intravitreal neovascular growth. Local EPO signaling in the retina may play a role in the formation of recurrent plaque-like intravitreal neovascularization (IVNV) following bevacizumab.[27] In addition to VEGF, EPO was found to be a key factor in the pathogenesis of ROP, especially in the development of neovascularization, suggesting a therapeutic possibility of EPO inhibitors in the ROP.[28] In a rat model of ROP, rhEPO penetrated into the eye in a dose- and time-dependent manner. Although the 30,000 U/kg dose of rhEPO increased, the ROP clock hour scores in ADPase-stained tissues, 5000 U/kg rhEPO did not change the incidence or severity of ROP by any measure. Considering the beneficial effect of high-dose rhEPO against preterm brain injury, the treatment may be instituted with minimal impact on ROP.[29]

Photoreceptor protection

The direct neuroprotective effect of EPO on photoreceptors in the mouse model of retinal degeneration has been

shown. This effect was not limited to the area of injection and represented the ability of EPO to diffuse through and across the retina. Photoreceptor survival could prevent Müller cell hypertrophy through decreased reactivity of glial fibrillary acidic protein (GFAP) in the Müller cells.[30] The protective effects of systemic EPO on photoreceptors and the retinal vasculature in rats could be due to multiple mechanisms including regulation of retinal gliosis, increased infiltration of microglia into the retina, and inhibition of p75NTR-pro-NT3 signaling in conjunction with stimulation of production and mobilization of bone marrow-derived cells.[31] The point is that apoptotic mechanisms during acute, light-induced photoreceptor cell death are different from inherited retinal degeneration. Therefore, EPO alone is not sufficient for a successful therapeutic intervention with cell death in hereditary retinal degenerations.[32]

Age-related macular degeneration (ARMD)

Oxidative damage of the retinal pigment epithelium (RPE) may play a role in the development and progression of ARMD. RPE cells express the EPO receptor. Administration of EPO either before radical exposure or shortly after radical exposure of the cultured RPE cells remarkably reduced apoptosis rates. Consequently, EPO should be evaluated as a potential candidate for therapy and prevention of dry ARMD.[33]

Light-induced retinal apoptosis

Retinal expression of EPO and its receptor, protected RPE cultured cells against daily light-induced, oxidatively mediated retinal apoptosis and these stimuli increase EPO and EPOR expression in cultured cells.[34] Modulation of Akt1 phosphorylation, mitochondrial membrane potential, and cysteine protease activity are the suggested mechanisms for cytoprotection.[35] A single intravitreal rhEPO injection with the optimal dose of 5 U could postpone photoreceptor apoptosis 24 h prior to light exposure to 5 h after.[36]

Optic neuritis

In rats suffering from optic neuritis, systemic application of EPO significantly increased survival and function of RGCs.[37] Assessment of retinal ganglion cells and optic nerves in a rat model of multiple sclerosis revealed that neuron and axon protection was most effective when EPO treatment that started at immunization was combined with high-dose methylprednisolone therapy given from days 1–3 of encephalomyelitis. However, isolated neuronal or axonal protection without clinical benefit was achieved under monotherapy with EPO or methylprednisolone, respectively.[38]

Corneal epithelial erosions

EPO had no beneficial effect on the rate of healing of corneal epithelial erosions in rabbit eyes, and the process may be complicated by corneal stromal neovascularization.[39]

Safety of local injections

Intravitreal administration of EPO (at doses up to 625 ng) in a rodent animal model did not affect retinal function as assessed by electroretinography (ERG). Moreover, single intravitreal dosing did not elicit retinal neovascularization.[40] Clinical examination, ERG, and pathologic evaluation demonstrated that intravitreal delivery of EPO is safe, well tolerated, and nontoxic to the intraocular structures at doses from 0.6 U to 1000 U.[41] Another study showed that intravitreal injection of rhEPO in rabbit eyes was not associated with adverse toxic effects up to 5000 IU doses.[42] After subconjunctival injection, EPO reached all the neuroretinal cell layers in the animal rat model after 24 h and was still present 60 h after the administration.[43]

Human studies

Diabetic retinopathy

The role of the EPO in diabetic retinopathy and diabetic macular edema is not completely understood. Several studies have reported higher levels of EPO in eyes of patients with diabetic retinopathy. EPO and VEGF levels were measured in the vitreous fluid of 73 patients with proliferative diabetic retinopathy (PDR) and 71 patients without diabetes with the use of radioimmunoassay and enzyme-linked immunosorbent assay. They were reported to be independently associated with proliferative diabetic retinopathy, and EPO was more strongly associated with the presence of PDR than was VEGF.[44]

In another study, undiluted vitreous samples were obtained from 24 patients with PDR and 20 patients with retinal detachment, preretinal macular membranes, and macular holes. The concentration of EPO was significantly higher in patients with PDR than in the control group.[45] In a comparative study of aqueous samples of 28 patients with diabetic macular edema, 59 patients with exudative ARMD, and 49 patients with cataract, eyes with diabetic retinopathy in contrast to exudative age-related macular degeneration had markedly increased aqueous humor levels of EPO.[46] Similarly, EPO concentrations were markedly higher in vitreous fluid samples of 59 PDR patients compared to 16 macular holes. When vitreous samples from 12 type 2 diabetic patients with diabetic macular edema without significant retinal ischemia and 12 PDR patients were compared with 10 nondiabetic patients with macular holes, the EPO concentrations were found to be strikingly higher in diabetic maculae edema. Also, the EPO levels were found to be upregulated in diabetic patients even without retinopathy. These findings suggested that other factors apart from ischemia are involved in the overexpression of EPO in various stages of diabetic retinopathy[47]. Although EPO blockade is likely to be beneficial for the treatment of PDR, it may be hazardous for retinal diseases that involve apoptosis of retinal photoreceptors.[48] The possibility that EPO might have a beneficial neuroprotective effect on neuronal damage may be counterbalanced by the risk of increased or the development of neovascularization for patients who also have retinal vasoproliferative diseases.[45] Moreover, intravitreal injection of EPO may have other clinical applications in diabetic retinopathy. To determine the effects of intravitreal injections of EPO in eyes with severe, unresponsive diabetic macular edema, 5 eyes of 5 patients

underwent injections of rhEPO, visual acuity of all patients was subjectively improved by 3 or more lines in 3 eyes, and 1 line in 2 eyes was not highly correlated with anatomical improvement.[49] Another study reported elevated aqueous level of EPO in 11 eyes of 11 patients with diabetic macular edema in comparison with 10 eyes of 10 patients with cataract surgery as controls. The EPO level was correlated with the level of VEGF. After intravitreal EPO injection in diabetic eyes, aqueous EPO levels were significantly elevated, whereas aqueous VEGF levels were varied according to the time interval since injection. The authors concluded that the effect of intravitreal EPO in eyes with diabetic macular edema needs to be further defined.[50]

In conclusion, clinical application and regulation of the EPO/EPOR system will require careful dosing and better understanding of EPO/EPOR role in the pathogenesis of diabetic retinopathy, so that vessel proliferation is inhibited without impairment of neuronal survival.[51]

Glaucoma

In patients with chronic renal failure (CRF) undergoing peritoneal dialysis (PD), retinal nerve fiber layer thickness (RNFL) was found to be significantly reduced. In those patients who were treated with systemic EPO, RNFL parameters were statistically significantly different from other patients in the temporal quadrant. Therefore, EPO might be able to preserve RNFL in patients with CRF undergoing PD in addition to its hematopoietic properties.[52] EPO is increased in the aqueous humor of patients with primary acute angle-closure glaucoma, primary chronic angle-closure glaucoma, primary open-angle glaucoma (POAG), and neovascular glaucoma (NVG).[53,54] Analysis of the aqueous samples of 92 glaucomatous eyes (POAG, 40 eyes; pseudoexfoliative glaucoma, 26 eyes; NVG, 26 eyes) and 26 control eyes, showed higher concentrations of EPO in glaucomatous eyes both with and without controlled IOP. The aqueous level of EPO was reported to be more proportionate to the level of IOP in eyes with pseudoexfoliative glaucoma compared to eyes with POAG and NVG.[55] Another study, however, could not find any difference between levels of EPO in aqueous humor and serum in patients with pseudoexfoliation syndrome and pseudoexfoliative glaucoma, and controls.[56] One study evaluated the levels of EPO and soluble CD44 (sCD44) in the aqueous and plasma of human eyes with POAG. The levels of both biomarkers were significantly higher in patients compared to the controls and a high positive correlation was found between EPO and sCD44 in aqueous of POAG patients. A significant correlation was also found between EPO or sCD44 levels and severity of visual field loss. Although sCD44 had been considered as a cytotoxic protein before, the authors proposed that EPO and sCD44 may be useful proteins, with increased levels in aqueous of POAG patients being a result of glaucoma damage, not a cause.[57]

Methanol optic neuropathy

Two patients with methanol optic neuropathy were treated with a combination of intravenous EPO, methylprednisolone, vitamin B12, vitamin B6, and folic acid. Both responded dramatically to the treatment with a rapid recovery of vision.[58]

Optic neuritis

In a double-blind, placebo-controlled, phase 2 study, 20 patients with a first episode of optic neuritis were treated with systemic EPO and compared with 17 placebo-treated patients. RNFL thinning was less apparent after EPO treatment, and the retrobulbar diameter of the optic nerve was higher in the EPO group. Visual evoked potential (VEP) latencies at week 16 were shorter in EPO-treated patients, and visual functions improved after EPO treatment.[59] Contrary to this study, another clinical trial showed that adding rhEPO to the intravenous methylprednisolone for the treatment of acute unilateral optic neuritis of unknown or demyelinative origin had no significant effect on visual acuity, perimetric, and OCT variables; however, an improvement in the perimetric mean deviation was noted.[60]

Pterygium

In an immunohistochemistry study on 9 surgically-excised pterygia and 2 normal bulbar conjunctivas, the number of EPOR-expressing epithelial cells was much higher in the pterygium compared to the normal conjunctiva; however, immunoreactivity for EPO was not noted in pterygium epithelium and stroma, or in normal conjunctiva. Consequently, EPO-independent EPOR-signaling pathway may play a potential role in cell proliferation and angiogenesis in human pterygium.[61]

Merkel cell carcinoma (MCC) of eyelid

In pathologic examination of 3 surgically-excised MCCs of the eyelid, in spite of undetectable immunoreactivity of EPO, an increased expression of EPOR was noted in the carcinoma cells. These findings indicate that the EPO-EPOR pathway plays an important role in the formation of MCC.[62]

Retinopathy of prematurity

In a retrospective chart review of infants with a birth weight <1000 g, threshold retinopathy of prematurity occurred in 26.9% infants who received EPO, as compared with 13.5% of those who did not receive it (OR 2.35). The authors proposed that EPO is independently and significantly associated with the development of the most severe stages of ROP in extremely low birth weight neonates.[63] Similarly, other studies reported an increased risk of development and worsening of ROP with EPO therapy.[64–67] A systematic review on neonates receiving rhEPO concluded that EPO initiated at less than 8 days of (postnatal) age may lead to a significant increase in the risk of ROP.[68]

Retinal detachment

In a case report, the vitreous concentrations of EPO and VEGF of an adolescent with a history of mild retinopathy of prematurity who presented with a chronic retinal detachment associated with neovascularization were measured by enzyme-linked immunosorbent assays and compared with control

levels. The EPO level was higher than those of patients with PDR; however, the VEGF level was not as high as those of patients with PDR. The authors speculated that EPO may have roles in the prevention of apoptosis as well as neovascularization in chronic retinal detachment.[69]

Another study reported the vitreous EPO level in 64 patients with rhegmatogenous retinal detachment (RRD) in whom 13 had proliferative vitreoretinopathy (PVR) and compared the results with patients with macular holes. The mean level of EPO in the RRD group was significantly higher than that in the idiopathic macular hole control group, and the mean EPO level in PVR was higher than that in RRD, but the difference was not significant.[70]

Retinal vein occlusion (RVO)

Similar to those found in diabetic retinopathy, the intraocular levels of EPO are increased in patients with RVO and correlate with VEGF and the extent of macular edema. When serum and vitreous concentration of EPO were measured from 6 patients with branch RVO, 6 patients with central RVO, and 12 control subjects, serum EPO levels did not differ between the RVO and control groups; however, vitreal EPO was elevated both in branch RVO and central RVO and correlated well with vitreal VEGF and the level of central macular edema.[71] Similarly, higher aqueous EPO levels were found in 27 patients with macular edema secondary to recent onset RVO compared to control subjects, and patients with central retinal vein occlusion have higher EPO levels than those with branch retinal artery occlusion. Moreover, a positive correlation was found between the level of EPO and central macular thickness and non-perfusion area.[72] In contrast, in another study on 12 patients with mild non-PDR and diabetic macular edema (DME), 12 patients with RVO, 9 with central RVO, 3 with branch RVO, and 20 controls, EPO levels were found higher in patients with DME and not elevated in patients with macular edema secondary to RVO.[73]

Because of its role both in neuroprotection and angiogenesis, and similar to diabetic retinopathy, EPO might represent an interesting target to investigate in patients with RVO.

Traumatic optic neuropathy

Intravenous injections of EPO resulted in a significant improvement in visual acuity in 7 eyes with indirect traumatic optic neuropathy compared to 8 patients with indirect traumatic optic neuropathy who had received no specific treatment. Therefore, intravenous EPO might be evaluated as an alternative treatment for indirect traumatic optic neuropathy.[74]

Non-arteritic anterior ischemic optic neuropathy (NAION)

In a prospective interventional case series, 31 eyes of 31 patients with NAION received intravitreal injection of EPO. Six months after injections, visual acuity improved in 87%, and 54.8% had $\geq$3 lines of visual improvement. A biphasic pattern of response was observed with a decline in visual acuity after initial response. The authors concluded that intravitreal injection of EPO may be considered effective in NAION.[75]

Discussion

Ischemic, traumatic, or degenerative destruction of the neurons is mediated by similar pathophysiological mechanisms including apoptosis (programmed cell death), increased release of excitotoxic amino acids (particularly glutamate), intracellular accumulation of calcium, oxidative stress by radical oxygen species, and inflammatory reactions accompanied by infiltration of cells and production of cytokines. Neuroprotection aims to "halt or possibly reverse the common pathway leading to neuronal cell injury or death" instead of classical treatment modalities directed toward cure or alleviation of specific disease etiologies.[76] Consequently, neuroprotection may be beneficial in glaucoma, vascular occlusions, traumatic, ischemic, or degenerative retinal disorders.

Understanding the mechanism of action of EPO could give rise to novel therapeutic strategies for the treatment of neurodegenerative diseases and injuries. This is especially important in the anti-VEGF era, since the treatment with available anti-VEGFs is not always successful. Moreover, application of anti-VEGF medications may change the balance of other growth factors and cytokines. Systemic complications of EPO including increased level of red blood cells and blood pressure, and the risk of thrombotic and cardiac complications should be remembered.[4] The systemic administration of EPO-derived peptides without capacity to raise hematocrit or to worsen neovascularization but maintain tissue-protective properties could be a promising approach. Because of the excellent safety profile of intravitreal EPO, local administration may be a safe alternative to avoid possible systemic side effects.

All available clinical experiences with EPO are from small nonrandomized studies with low quality of evidence. Despite this, ocular applications of EPO are encouraging. Large, randomized, multi-center clinical trials with adequate sample size and long follow-up times are required to clarify the potential benefits of EPO in different ocular conditions.

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