The Protective Effect of Erythropoietin on the Retina

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The Expression of Epo in the Retina

As mentioned above, Epo can be expressed by retinal tissue. This is proven by the highly elevated concentration of Epo in the vitreous fluid, compared with the plasma, of patients with diabetic macular edema and proliferative diabetic retinopathy (PDR) [5]. A lower level of Epo catabolism also contributes to this elevation via the glycosylation of Epo that reduces its ability to bind Epo-R, which is the only way known to degrade Epo.

Retinal Epo and Epo mRNA can be detected both in the retinal pigment epithelial (RPE) cells and in the neuroretina, once again indicating autocrine or paracrine action rather than telecrine. Moreover, compared with the
neuroretina, the expression behavior is stronger in RPE cells, which are known to support and act as barriers of the neuroretina.

The Role of Epo in Anti-Inflammatory Action in the Retina

Epo is found to have a protective effect in the brain by attenuating inflammatory response [8], and a similar action occurs in the retina. A study conducted by Chang et al. [9] found that Epo does benefit retinal ganglion cells (RGCs) by facilitating their resistance to injury induced by tumor necrosis factor-α (TNF-α). It is known that Epo can activate NF-κB by facilitating their resistance to injury induced by tumor necrosis factor-α (TNF-α). It is known that Epo can activate NF-κB [10], which acts as a survival signal of RGCs [11], thereby forming an antagonizing action on TNF-α-induced injury.

Apart from antagonizing action, Epo also plays a positive role in reducing the expression quantity of TNF-α, as well as two other proinflammatory cytokines: interleukin (IL)-6 and IL-1β. According to the research of McVicar et al. [12], application of a peptide based on the Epo helix-B domain (pHBSP), which is nonerythrogenic but retains tissue-protective properties, can decrease mRNAs for TNF-α and IL-6 in diabetic rats, and can also reduce IL-1β levels. In contrast, the transcripts for the anti-inflammatory cytokine IL-10 are significantly increased. This anti-inflammatory effect is confirmed by Wang et al. [13], who observed an attenuation of TNF-α and IL-1β by 1 IU/ml of Epo after oxidative damage to RPE cells. The decline of proinflammatory cytokines may be related to the JAK/STAT pathway, which can be activated by Epo and which down-regulates the expression of inflammatory factors [14]. The pathway is also mentioned in the research conducted by Xie et al. [15], in which a raised level of phosphorylated JAK2 is observed after an intravitreal injection of Epo in a rat with retinal detachment. In addition, Müller cells are identified as the main source of IL-1β production in the retina of rats with streptozotocin-induced diabetes, as indicated by colocalization. Additionally, Epo can hinder the Müller cell expression of IL-1β via modulation of the activity of AP-1 but not JAK or NF-κB [16].

There is now a general consensus that inflammation is one of the basal pathogenetic factors in diabetic retinopathy (DR) [17]. Epo is up-regulated in DR [7], in both the RPE cells and the neuroretina of diabetic patients’ eyes [5], implying a mechanism of protection against inflammatory cytokines like TNF-α, IL-6, and IL-1β.

Epo Facilitates the Survival of Cells in Hyperoxia and Hypoxia

Oxidative injury, which can lead to RPE cell dysfunction and death, is thought to be one of the key pathophysiology processes in age-related macular degeneration. As previously mentioned, Epo has the capacity to reduce oxidative damage to RPE cells by down-regulating inflammatory cytokines. However, other factors are induced by oxidative stress and harm the retina, in addition to inflammatory cytokines [13]. The experiment demonstrates that oxidative stress has destructive power in disrupting the barrier integrity of the RPE cells, causing cell DNA fragmentation, giving rise to intracellular reactive oxide species, mediating mitochondrial membrane depolarization, activating caspase-3, and leading cells to apoptosis, which can be detected by membrane phosphatidylserine exposure to the outer cell membrane. These changes fully reflect the influence of oxidative damage on RPE cells, and combined with the increase of inflammatory cytokines, the negative impact will be offset after exposure to Epo. However, the protection will be interrupted by the blocking of p-Akt1, which is significantly increased after Epo application, shedding light on the fact that the antioxidant effect of Epo is partly dependent on the activation of Akt1. There is also evidence indicating that hydrogen peroxide brings about an overexpression of Epo [18], demonstrating the oxidation resistance effect of Epo from a different perspective.

Interestingly, it has been reported that the expression of Epo can also be induced by hypoxia, and subsequently provides protection against cerebral ischemia [19]. A transgenic mice line (tg21) constitutively overexpressing human Epo shows improved adaptation to both acute and chronic hypoxic exposure [20]. In fact, hypoxia is a major stimulus for both systemic and intraocular Epo production [21], which is mainly controlled by hypoxia-inducible transcription factors (HIF), a transcription factor that acts as a cellular oxygen sensor and can be activated by decreased oxygen tension [22]. HIF-2, rather than HIF-1, plays the protagonist in inducing Epo expression during hypoxia [23]. An in vivo study by Junk et al. [24] illustrates that the expression of Epo-R in the retina is up-regulated by ischemia, and even the content of Epo presents a downward trend due to extensive cell loss. Soluble Epo-R, which can neutralize the endogenous Epo, exacerbates ischemic injury, sufficing the importance of the endogenous Epo/Epo-R system. After the systemic administration of exogenous Epo, the histopathological

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damage is mitigated and retinal function is recovered with a diminished apoptosis level of neurons. These results support the premise that Epo is a neuroprotective agent in acute neuronal ischemic injury. Epo protects the brain from cerebral ischemia by activating the ERK-1/-2 and Akt pathways [25], and a similar mechanism is predicted in signal transduction in the retina. Moreover, Epo is an independent angiogenic factor from vascular endothelial growth factor (VEGF), contributing to its antihypoxic effect in another way [26].

**Epo Helps Protect the Retina from Degeneration**

Degeneration occurs in several chronic retinopathies including glaucoma, DR, age-related macular degeneration, and retinitis pigmentosa [27, 28]. However, there is currently no cure for degenerative retinal diseases. Recent studies [29] have presented a feasible treatment in an animal model of retinal degeneration induced by sodium iodate, by subretinal transplantation of Epo gene-modified mesenchymal stem cells, which compared to normal rat mesenchymal stem cells can significantly enhance the survival, distribution and differentiation of retinal cells. Moreover, an intravitreal injection of soluble Epo-R, which can neutralize endogenous Epo, will exacerbate photoreceptor cell apoptosis in a rat model of retinal detachment [30]. Recently, researchers have shown that Epo can relieve retinal degeneration, including the degeneration of RGCs [9, 31–34] (table 1) and photoreceptors [35–38] (table 2).

Knowing that retinal degeneration is a chronic disease, these studies have focused on solving clinical problems such as choosing the drug-delivery method, avoiding increasing hematocrit levels, and whether exogenous Epo would affect the intraocular pressure (it does not) [32, 34]. The conclusion can be made that Epo performs better in withstanding photoreceptor death through systemic delivery rather than local administration [35, 36], suggesting a systemic mechanism in the rescue of retinal degeneration. Another phenomenon is that molecules derived from Epo maintain a retinal protective characteristic without increasing hematocrit, implicating an independent relationship between these two effects [31, 32, 36]. Epo retards degeneration by antiapoptosis, including down-regulation of caspase-3 through the PI3K/Akt pathway, the up-regulation of Bcl-XL [31], and the suppression of the mitochondrial release of cytochrome C and the regulation of intracellular Ca levels [38]. Moreover, Epo can provide protection to RGCs and photoreceptors by stimulating neurotrophin expression in Müller cells. Hu et al. [39] found that Epo up-regulated the expression of ciliary neurotrophic factor (CNTF) and brain-derived neurotrophic factor (BDNF) via the ERK-1/-2 and Akt pathways. CNTF and BDNF helped the RGCs and photoreceptors. Additionally, Epo can reduce the content of glial fibrillary acidic protein, a protein expressed in reactive Müller cells, indicating mitigation of the reactive gliosis in degenerative diseases [39].

In addition to the mechanism described above, Epo protects RGCs and photoreceptors in other ways. Epo-R is mainly expressed in RGCs, allowing Epo to interact with the somata or axons of the RCGs to directly maintain metabolic activity [32]. During axotomy-induced degeneration of RGCs, Epo activates ERK-1/-2 signaling [33]. It has been shown that increased oxygen levels can slow down the death of photoreceptors, while hypoxia aggravates photoreceptor death [40]. Systemic overexpression of Epo can raise arterial oxygen content, suggesting the use of Epo to rescue photoreceptors [37]. Finally, Epo is able to promote neuroregeneration, evidenced by the regeneration of RGC axons in optic nerve transactions [41–43]. Additionally, this promotion can be inhibited in the presence of ethosuximide, a T-type Ca channel blocker [42].

**Epo Contributes to the Maintenance of Osmotic Pressure in RPE**

Multiple studies demonstrate that Epo provides protection against the permeability of the blood–brain barrier [44, 45]. As the structure of the blood-retinal barrier (BRB) is similar to that of the blood-brain barrier, Epo seems to potentially maintain the osmotic pressure in the BRB. Actually, the research performed by Garcia-Ramirez et al. [46] demonstrated that Epo does benefit RPE cells by preventing the increase of permeability induced by diabetic conditions. APRE-19 cells are cultured in D-glucose together with IL-1β, which is known to breaking down the monolayer. Compared to the controls, a subsequent administration of Epo during the last 2 days of the experiment results in a more moderate increase in dextran permeability, with the subsequent prevention of the disruption of the ARPE-19 monolayer.

Epo administration induces a rapid and transient recruitment of cytosolic Ca from extracellular spaces into the RPE cells. The source of calcium was demonstrated to be extracellular by the maintaining of the Ca peak in spite of the presence of BAPTA-AM, which inhibits Ca mobi-
lization from intracellular stores. A lack of Ca will result in an opening of the tight junctions followed by a disruption of the BRB, and the influx of Ca induced by Epo can help RPE cells handle the Ca shortage, thereby resisting permeability.

Treatment with Epo goes along with the activation of JAK2 and PI3K/Akt signaling. Additionally, blocking of the JAK2 pathway more significantly reduces the Epo effect in maintaining the barrier function of RPE cells, though inhibition of two signaling processes can also work. The phosphorylation of JAK2 and PI3K/Akt lead to the activation of STAT5, Ras/MAP and NF-κB; however, the whole pathway remains to be studied further.

Hyperosmotic stress is involved in DR. Hyperglycemia not only causes damage to the vascular endothelium but also disrupts the BRB and contributes to the development of diabetic macular edema. Epo can assist RPE cells against permeability and therefore slow down the subsequent pathological changes.

It is known that barium chloride can block potassium channels, which are implicated in the regulation of glial cell volume [47]. Research conducted by Krügel et al.

### Table 1. Studies on the protection of Epo against degeneration in RGCs

<table>
<thead>
<tr>
<th>Method</th>
<th>Epo form</th>
<th>Model</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exogenous systemic mutant Epo</td>
<td>Epo-R76E [32]</td>
<td>Optic nerve crush</td>
<td>A protection effect on RGCs in a dose-dependent manner</td>
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<tr>
<td>administration</td>
<td></td>
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<td>Epo increased in a dose-dependent manner</td>
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<td></td>
<td>Epo-R76E [33]</td>
<td>DBA/2J mouse (pigmentary glaucoma)</td>
<td>Hematocrit increased in a dose-dependent manner, weaker than Epo</td>
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<td></td>
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<td></td>
<td>No protective effect in axons</td>
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<td></td>
<td></td>
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<td>Decreases the loss of healthy axons</td>
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<td>Protects NeuN-positive cells</td>
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<td>An ameliorated visual function detected by F-VEP</td>
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<td>No effect on intraocular pressure</td>
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<td></td>
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<td>Little change in hematocrit</td>
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<tr>
<td>Transgenic</td>
<td>Epo (tg21 mice) [34]</td>
<td>Optic nerve transection</td>
<td>A robust expression of Epo-R on RGCs without difference between wild-type and tg21 mice</td>
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<td></td>
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<td>Epo increases the density of surviving RGCs</td>
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<td>A higher level of ERK-/-2, Akt and STAT5 in tg21 mice before surgery</td>
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<td></td>
<td>ERK-/-2 and Akt signaling are increased after RGC axotomy in tg21 mice, while JNK and caspase-3 are decreased, no change in wild type</td>
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<td></td>
<td>JAK-2, STAT5, Bcl-Xl are attenuated after RGC axotomy in tg21 mice, no change in wild type except an increase of STAT5</td>
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<td></td>
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<td></td>
<td>PI3K/Akt inhibitor did not influence RGC survival while inhibition of ERK-/-2 did</td>
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<tr>
<td>Intraperitoneal injection</td>
<td>Epo [35]</td>
<td>DBA/2J mouse</td>
<td>Epo prevents age-dependent death of RGCs</td>
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<td></td>
<td></td>
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<td>Epo does not affect intraocular pressure</td>
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<td>Epo-R expression detected in the RGC layer, with a few in astrocytes, inner nuclear layer and photoreceptor layer</td>
</tr>
<tr>
<td>In vitro experiment</td>
<td>Epo [9]</td>
<td>Trophic factor withdrawal (TFW, glaucoma)</td>
<td>RGCs survived in a dose-dependent manner of Epo under NMDA-induced toxicity, large RGCs excluded</td>
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<td>In comparison to GDNF, Epo is more efficacious in protecting large RGCs from TFW-induced toxicity</td>
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<td>Under TNF-α-induced toxicity, large RGCs survived at all doses of Epo, while total and small RGCs can only be improved by 100 ng/ml Epo</td>
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<td>Effect of Epo against NMDA is gone with inhibitors; also the effect against TFW and TNF-α is decreased or abolished</td>
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showed that the swelling of retinal glial cells induced by barium chloride under hypotonic conditions can be inhibited by Epo. A release of VEGF evoked by Epo via the activation of JAK2 and ERK-1/-2 pathways induces the subsequent activation of glutamatergic-purinergic signaling, which will eventually result in the opening of potassium and chloride channels and stop cells from swelling.

**Epo and Neovascularization**

As the vitreous Epo level is significantly higher in patients with DR than in nondiabetic patients, and the association between DR and Epo is stronger than that with VEGF, Epo was first identified as a retinal angio-

**Table 2. Studies on the protection of Epo against degeneration in photoreceptors**

<table>
<thead>
<tr>
<th>Epo form</th>
<th>Drug administration</th>
<th>Model</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exogenous mutant Epo expression [36]</td>
<td>Vectors injected: intramuscularly subretinally intravitreally</td>
<td>Light-damaged rat rds rat rd10 rat</td>
<td>Circulating Epo and hematocrit only increase in the group with intramuscular delivery Subretinal and intravitreal delivery increases AC Epo Intramuscular administration, not intraocular rescue photoreceptors in light-damaged and rds mice; no histological rescue in rd10 Same conclusion supported by ERG results, protective effect is independent of the intraocular concentration of Epo</td>
</tr>
<tr>
<td>Epo derivatives [37]: S100E helix A-derived peptides (NP1) helix B-derived peptides (NP2)</td>
<td>Vectors injected: subretinally intramuscularly</td>
<td>Light-induced retinal degeneration mice rds mice (RP) Aipl1 mice (LCA)</td>
<td>S100E vectors systemic injection result in an increase in hematocrit, while no hematocrit variations occurred in the delivery of subretinal vectors According to the ERG result, both systemic and subretinal S100E administration can improve the retinal function in light- or genetic-induced retinal degeneration, and the protection is lower after local application Increased photoreceptor survival is consistent with the functional preservation N11 and NP2 only preserve photoreceptors when given locally</td>
</tr>
<tr>
<td>Transgenic Epo expression [38]</td>
<td>Transgenic Intravitreal injection</td>
<td>Light damage to: tg6 bred with rd1 mouse tg6 bred with VPP mice tg6 mice</td>
<td>Light-induced photoreceptor lesions are distinctly less severe in the tg6 mouse compared to the wild type huEpo transgene expression and intravitreal injection cannot rescue photoreceptor cells in the VPP mouse as well as the rd1 mouse Rhodopsin levels are not detectable in the rd1/tg6 mouse Rhodopsin levels do not differ from VPP/tg6 and VPP mice</td>
</tr>
<tr>
<td>Epo [39]</td>
<td>Intravitreal injection</td>
<td>Rat model of retinal detachment (RD)</td>
<td>No impact of Epo on retinal function, morphology or structure was detected Epo significantly reduced the photoreceptor apoptosis induced by RD A thicker outer nuclear layer preserved by Epo against RD Decreased caspase-3 activation and increased Bcl-X expression</td>
</tr>
</tbody>
</table>
next pathological process of further ischemia and neovascularization. Another study involving progenitor cells conducted by Hu et al. [53] demonstrates that the content of Epo-positive endothelial progenitor cells, which are able to repair the denuded or dysfunctional endothelium, is higher in PDR patients than normal people as a result of bone marrow mobilization induced by ischemia. In patients with nonproliferative diabetic retinopathy (NPDR), the number is reduced, probably due to the shortened peripheral survival or weak bone marrow mobilization. These results show changes of Epo-positive endothelial progenitor cells through the compensatory to the decompensatory stage, reflecting the vascular protective effect of Epo by attracting vessel repair by endothelial progenitor cells and thus preventing neovascularization in NPDR, in addition to the angiogenic role it plays in PDR.

Conclusions

Epo is a systemic erythropoietic regulation hormone and has many other effects. Epo has revealed its tissue-protective properties in the brain, heart and inner ear. Additionally, Epo exhibits its capabilities in the retina, including its resistance to inflammation, oxide-induced
damage, ischemia, degeneration and permeability (fig. 1). Moreover, Epo is viewed as an endothelial protective factor as well as an angiogenic factor, from NPDR to PDR, ameliorating or aggravating the severity of disease. Epo is now regarded as a neuroprotective agent apart from ‘erythropoietin’ as its name declares. An increase in hematocrit also increases the risk of thrombosis, and therefore the systemic administration of Epo-derived peptides that are nonerythropoietic but retain tissue-protective properties has been tested. Apart from intravitreal injections, intraperitoneal injections and vector deliveries, the subconjunctival application of Epo is affirmed to reach the RGC layers [54], providing multiple routes for ocular Epo administration. However, the use of eyedrops, the most convenient route of administration, has not been examined. In addition, the mechanism involved in tissue protection is yet to be fully defined.

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Disclosure Statement

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