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Erythropoietin in diabetic retinopathy

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ABSTRACT

Over the past years, knowledge has expanded with regards to the multiple roles played by erythropoietin (EPO) in the body. Once believed to be a hormone synthesised in the kidney and involved only in the modulation of erythrocyte production, it is recognised now that EPO can be produced in many tissues, including the retina, and by many cells. In these tissues EPO is released in response to "tissue injury" and appears to have protective functions. Despite the extensive research conducted to date, the cues leading to release of EPO and its effects in the normal and diseased retina have not been fully elucidated.

In vitro and *in vivo* experimental studies as well as small interventional clinical studies suggest a potential beneficial effect of externally administered EPO in early diabetic retinopathy and diabetic macular oedema. In contrast, controversy exists with regards to the possible use of EPO in proliferative diabetic retinopathy. Non-erythropoietic EPO-derived peptides, produced with the aim of increasing effectiveness and reducing side effects of EPO, are currently under investigation in early phase clinical trials.

1. Introduction

Although initially thought that erythropoietin's (EPO) only role was the regulation of erythrocyte production in the bone marrow, it is now evident that this glycoprotein hormone plays other important functions in the body. EPO consists of 165 amino-acids (molecular mass of 30.4 kDa) which are arranged in four connected alpha helices (Davis, Arakawa, Strickland, & Yphantis, 1987; Jacobs et al., 1985; Lin et al., 1985; Miyake, Kung, & Goldwasser, 1977) (Fig. 1). After birth, the main site of circulating EPO production is the kidney, where it is synthesised by peritubular interstitial cells in response to hypoxia (Bachmann, Le Hir, & Eckardt, 2017; Maxwell et al., 1993). Expression of the EPO gene is stimulated by a mechanism dependent upon the hypoxia inducible factor (HIF) pathway, the chief signalling pathway involved in sensing tissue oxygen levels and adaptive responses to hypoxia, tightly regulated by the ubiquitin system [Reviewed by Jelkmann (Jelkmann, 2011)] and Günter et al. (Günter, Ruiz-Serrano, Pickel, Wenger, & Scholz, 2017)]. In normoxia, oxygen is available and the HIF-α subunits are hydroxylated by hydroxylases [prolyl-4-hydroxylase domain (PHD) proteins 1–3 and asparagine hydroxylase factor inhibiting HIF (FIH)]. Hydroxylated HIF-α binds the von Hippel Lindau (VHL) protein which, on its turn, recruits the E3 ubiquitin ligase which catalyzes the polyubiquitination of HIF-α leading to its proteosomal degradation. In hypoxia, however, hydroxylation of HIF-α is compromised by the reduced levels of oxygen; HIF-α then combines with HIF-1β/ARNT to form the active heterodimer transcription factor HIF which stimulates the expression of the EPO gene, among others. To fulfill its role in erythropoiesis [Reviewed by Brines (Brines, 2010)]. Expanded knowledge indicates, however, that EPO can be produced also in many tissues in response to tissue injury where it appears to exert a protective action through a different receptor, the so called innate repair receptor (IRR) [also named tissue protective receptor (TPR)] (Brines & Cerami, 2005; Brines et al., 2004). The TPR is composed of an EPOR monomer and the β common receptor (EPOR/βCR) (Brines & Cerami, 2012) (Fig. 1). It is expressed in the kidney, heart, muscle, endothelium, the nervous system and retina as well as in immune cells such as macrophages, among others [reviewed by Brines and Cerami (Brines & Cerami, 2012)]. Activation of the TPR by the binding of EPO leads to phosphorylation of janus tyrosine kinase 2 (JAK-2) and, subsequently, an intracellular cascade of events including activation of STATs, PI3k/Akt, MAPKs and GSK3β which lead to reduced apoptosis, inflammation and edema (Collino, Thiemermann, Cerami, & Brines, 2015).

Whereas EPO has high affinity for the EPOR homodimer, its affinity for the EPOR/βCR complex is low; thus, a much greater

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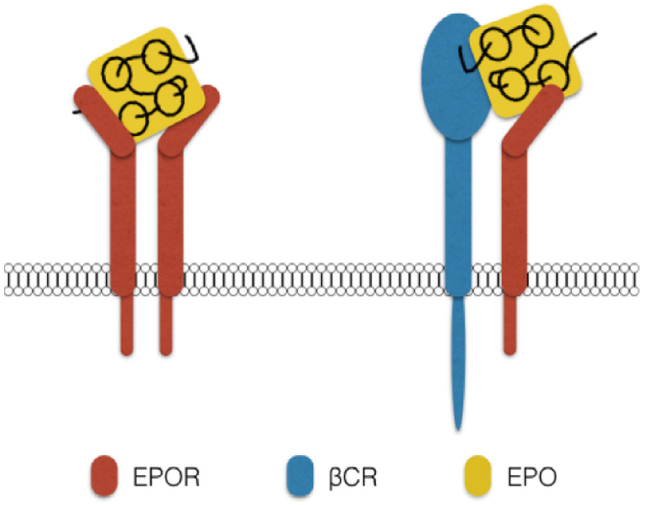

Fig. 1. Cartoon depicting the erythropoietin receptor homodimer (EPOR) (left) and the innate repair receptor (tissue protective receptor) (EPOR/βCR) (right) binding erythropoietin. Adapted from Brines & Cerami, 2012

concentration of EPO is required to elicit a tissue protective response than to stimulate erythropoiesis (Brines & Cerami, 2008).

Extensive research has been conducted in an attempt to better understand the regulatory mechanisms of EPO production and the non-haematopoietic effects of EPO. This article aims to summarise current knowledge on the effects of EPO in the retina and its potential therapeutic use, and that of EPO-derived molecules, in diabetic retinopathy.

2. Expression of EPO and EPOR in healthy and diabetic retina

During the embryological stages of human eye development the concentration of EPO mRNA in the retina and EPO protein in the vitreous increases with increasing gestational age. Throughout this period the vitreous EPO concentration is greater than that of the serum (Patel, Rowe, Winters, & Ohls, 2008). These findings suggest a role of EPO in the development of the retina; research conducted on the oxygen-induced retinopathy (OIR) model appears to support this concept.

Post-mortem studies have demonstrated expression of EPO and its receptor in the adult human retina (Hernández et al., 2006; Shah, Tsang, & Mahajan, 2009). Shah and colleagues found expression of EPOR in the neuroretina, predominantly in the retinal ganglion cell layer, with minimal expression in outer and inner nuclear layers (Shah et al., 2009). Hernandez and associates detected expression of EPO in the neural retina and retinal pigment epithelium (RPE), with higher levels in the latter (Hernández et al., 2006).

Expression of EPO and EPOR appears to be altered in diabetic retinopathy. In post-mortem human diabetic donor eyes without overt diabetic retinopathy, expression of EPO in the neuroretina and RPE was found to be higher than in non-diabetic eyes (Hernández et al., 2006). In a post-mortem human eye with proliferative diabetic retinopathy which had been treated with panretinal photocoagulation, EPOR was found to be highly expressed in photoreceptor cells (Shah et al., 2009).

In experimental animal studies, primary cultures and cell lines, expression of EPO-R has been shown in endothelial cells, pericytes, smooth muscle cells and glial cells, including Muller cells, as well as in ganglion cells (Anagnostou et al., 1994; Wang et al., 2011; Weishaupt et al., 2004). Some of these studies (Wang et al., 2011; Weishaupt et al., 2004), however, used anti-EPOR antibodies which may not be sufficiently specific to the EPOR (Elliott, Sinclair, Collins, Rice, & Jelkmann, 2014; Elliott et al., 2006) and, thus, require to be interpreted with caution.

Although incompletely elucidated, it appears that EPO may have neuroprotective, anti-apoptotic, anti-inflammatory, anti-oxidative, anti-permeability and angiogenic functions in the retina (Becerra & Amaral, 2002; García-Ramírez et al., 2011; Shirley Ding et al., 2016; Wang et al., 2012).

3. EPO and diabetic retinopathy

Many experimental studies have been conducted in an attempt to elucidate the effect of EPO in the retina in patients with diabetic retinopathy. Most were undertaken with the view of using EPO as a potential treatment strategy for this disease and have concluded that EPO could potentially be beneficial for the treatment of early diabetic retinopathy and for one of its complications, namely diabetic macular oedema (DMO). Some, however, have raised concerns with regards to the possible detrimental effect that EPO could have on proliferative diabetic retinopathy (PDR) and controversy exists in this regard (see *Potential effects of EPO for the treatment of PDR,* below*).*

EPO, administered intraperitoneally or intravitreally, was noted to reduce VEGF expression in the streptozotocin-induced diabetic rat model (Mitsuhashi et al., 2013; Wang et al., 2011). The EPO-mediated VEGF inhibition has also been demonstrated in other studies (Krügel et al., 2010; Zhang et al., 2010). Furthermore, treatment with EPO reduced diabetic induced pericyte loss and the formation of acellular capillaries (Wang et al., 2011; Mitsuhashi et al., 2013) and prevented diabetic-induced inner and outer retinal layer thinning as well as ganglion cell layer loss when administered either intraperitoneally (Wang et al., 2011) or intravitreally (Zhang et al., 2008). In streptozotocin-induced diabetic rats treatment with EPO (5000 IU/kg intraperitoneally three times a week), two weeks after the induction of diabetes, prevented the reduction of b-wave and oscillatory potential amplitudes as well as degeneration of the retinal ganglion cells, including vacuolation and swelling of the mitochondria (Zhu, Wang, Gu, & Xu, 2008). It also prevented the increased content of glutamate in the retina, which was observed in diabetic animals with no treatment but not in those receiving recombinant human EPO (Zhu et al., 2008) and glutamate-mediated neurotoxicity (Gu et al., 2014).

EPO was found to downregulate HIF-1α in the diabetic rat model (Xu et al., 2015; Zhang et al., 2010). EPO helped maintaining the homeostasis of intracellular zinc in retinal cells, altered as a result of diabetes, by restoring Zinc transporter 8 (ZnT8) expression (Xu et al., 2015).

It is widely accepted that inflammation plays a critical role in the pathogenesis of diabetic retinopathy (Joussen et al., 2004). Altered levels of inflammatory cytokines have been detected in this retinal disease (Reviewed by Stitt et al., 2016). It has been demonstrated that, in the streptozotocin diabetic rat model, intravitreal injection of EPO significantly reduced TNF-alpha and Muller cell-IL-1β production and attenuated levels of IL-6 and VEGF in the retina (Lei et al., 2011).

4. Potential effects of EPO in DMO

Several lines of evidence suggest that EPO could be beneficial for the treatment of DMO. Thus, an improvement of DMO was observed following EPO administration to patients with azotemia-induced anaemia (Friedman, L'Esperance, Brown, & Berman, 2003). Similarly, intravitreal administration of EPO (5U/50 μl) every 6 weeks (three doses) to a small group (n = 5) of patients with refractory DMO appeared to confer functional benefit

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