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Flipping the molecular switch for innate protection and repair of tissues: Long-lasting effects of a non-erythropoietic small peptide engineered from erythropoietin

Massimo Collino [a,*,1], Christoph Thiemermann [b,1], Anthony Cerami [c], Michael Brines [c]

[a] Department of Drug Science and Technology, University of Turin, Turin, Italy
[b] Queen Mary University of London, Centre for Translational Medicine and Therapeutics, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, London, UK
[c] Araim Pharmaceuticals, Tarrytown, NY, USA

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ABSTRACT

Many disease processes activate a cellular stress response that initiates a cascade of inflammation and damage. However, this process also triggers a tissue protection and repair system mediated by locally-produced hyposialated erythropoietin (hsEPO). Although recombinant EPO is used widely for treating anemia, potential use of recombinant EPO for tissue-protection is limited by rises in hematocrit, platelet activation, and selectin expression resulting in a high risk of thrombosis. Importantly, the erythropoietic and tissue-protective effects of EPO are mediated by different receptors. Whereas EPO stimulates red cell progenitors by binding to an EPO receptor (EPOR) homodimer, a heterodimer receptor complex composed of EPOR and β common receptor (βcR) subunits, termed the innate repair receptor (IRR), activates tissue protection and repair. The IRR is typically not expressed by normal tissues, but instead is rapidly induced by injury or inflammation. Based on this understanding, EPO derivatives have been developed which selectively activate the IRR without interacting with the EPOR homodimer. The latest generation of specific ligands of the IRR includes an 11 amino acid peptide modeled from the three dimensional structure of the EPO in the region of helix B called pyroglutamate helix B surface peptide (pHBSP; ARA-290). Despite a short plasma half-life (~2 min), pHBSP activates a molecular switch that triggers sustained biological effects that have been observed in a number of experimental animal models of disease and in clinical trials. This review summarizes pharmacokinetic and pharmacodynamic data and discusses the molecular mechanisms underlying the long-lasting effects of this short-lived peptide.

Contents

Abbreviations: ACTH, adrenocorticotropic hormone; AMPK, AMP-activated protein kinase; eNOS, endothelial nitric oxide·synthase; EPO, erythropoietin; EPOR, EPO receptor; FGF, fibroblast growth factor; GLP-1, glucagon-like peptide-1; HIF, inducible factor transcription factors (HIF); hsEPO, hyposialated erythropoietin; I/R, ischemia/reperfusion; IRR, innate repair receptor; JAK2, Janus kinase 2; MI, myocardial infarction; pHBSP, pyroglutamate helix B surface protein; STAT-5, signal transducers and activators of transcription-5; β cR, beta common receptor (CD 131).

* Corresponding author at: Department of Drug Science and Technology, University of Turin, via P. Giuria 9, 10125 Torino, Italy. Tel.: +39 011 6706861; fax: +39 011 2367955.
E-mail address: massimo.collino@unito.it (M. Collino).

[1] Both authors contributed equally to this work.

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1. Introduction

Tissue damage or stress generally activates an evolutionarily ancient inflammatory reaction that has been termed the innate immune response. Central to this process is a self-amplifying production of pro-inflammatory cytokines that cause further tissue damage through necrosis and apoptosis via a positive feedback loop. Additionally, the microenvironment becomes relatively hypoxic due to a disrupted microcirculation coupled with increased metabolic activity of both resident cells and infiltrating immune cells. This process is a prominent component in a range of inflammatory disorders, such as chronic infection, or inflammatory bowel disease (Taylor & Colgan, 2007; Werth et al., 2010). Within this system, hypoxia- and inflammation-responsive molecular pathways are closely inter-related and co-regulated (Palazon et al., 2014). One critical molecular component of this closely choreographed biological response is the family of hypoxia inducible factor transcription factors (HIF) that activate a host of metabolic pathways to restore cellular integrity (Semenza, 2012). Tissue injury has been observed to cause an increase in HIF expression (Bernaudin et al., 2002; Shein et al., 2005). Prominent among HIF-regulated gene transcription is erythropoietin (EPO).

EPO is an evolutionarily ancient protein (Nogawa-Kosaka et al., 2010) that can be synthesized by many cells. A major form of EPO is as a hormone, a 31 kDa glycoprotein predominantly produced in the adult kidney and primarily known for its role in promoting proliferation, differentiation and survival of erythroid progenitor cells (Watowich, 2011). It is clinically used for the treatment of patients with anemia secondary to chronic kidney disease or myelodysplasia following chemotherapy or radiotherapy.

When anemia develops, hypoxia is detected within the kidney which stimulates the production and secretion of EPO which travels to the bone marrow. There, EPO increases the production of erythrocytes and therefore the circulating red cell mass, and in this manner, reduces hypoxia. Within the setting of immune activation or tissue damage throughout the body, however, the production of EPO acts as a master regulator of apoptosis and pro-inflammatory cytokine production, as well as of the repair of tissue damage.

2. Erythropoietin and its receptor isoforms

EPO produced by the kidney possesses 4 oligosaccharide chains terminated by sialic acids that provide for a plasma half-life of 4–6 h. In contrast, EPO that is produced locally as the result of innate immune system activation is poorly sialated (Masuda et al., 1994), as would be expected for a molecule operating in a paracrine/autocrine mode, and therefore has a short half-life (Imai et al., 1990). Notably, completely desialated EPO has a plasma half-life of approximately 1.4 min (Erbayraktar et al., 2003). Hyposialated EPO (hsEPO) is believed to be the ligand activating the innate repair system.

The EPO-receptor that mediates erythropoiesis is comprised of two identical EPO receptor monomers (EPOR) which become localized rapidly within the microdomains of the membrane rafts of hematopoietic cells following stimulation (within several minutes) (McGraw et al., 2012). Here, the EPOR subunits spontaneously dimerize when in close proximity, forming EPOR-EPOR [$(EPOR)_2$] and assemble with Janus kinase 2 (JAK2) and other members of the molecular signaling machinery (McGraw et al., 2012). Subsequently, when EPO binds to and bridges across the receptor subunits, a conformation change occurs, leading to activation of JAK2, which in turn phosphorylates tyrosine residues within the EPOR cytosolic domain. The phosphorylation of $(EPOR)_2$ results in the activation of several signal transduction proteins, including the mitogen-activated protein kinase extracellular-regulated kinase (ERK)-1/2, phosphatidyl inositol 3 kinase (PI3K)/Akt, and signal transducers and activators of transcription (STAT)-5. STAT-5 that stimulates mitochondrial anti-apoptotic proteins, such as Bcl-XL, and consecutively inhibits cytochrome c-dependent caspases, thus, suppressing erythroid progenitor cell apoptosis (Chong et al., 2002). Additionally, $(EPOR)_2$ also mediates platelet and endothelial cell activation, including up-regulation of E-selectin (Heinisch et al., 2012). These actions serve to limit blood loss following vascular trauma by activating physiologically relevant thrombosis, but also serve to initiate and maintain inflammation. One prominent characteristic of $(EPOR)_2$ signaling pathways is that activation consists of both binary (switching on) as well as graded responses, for example, as in STAT-5 signaling (Porpiglia et al., 2012).

EPO has a high affinity (~200 pmol/L) for $(EPOR)_2$, and as only a few percent occupancy is needed for adequate signaling (Krzyzanski & Wyska, 2008), the normal human serum EPO concentration is in the 1–10 pmol/L range. The endothelial cell was the first non-hematopoietic cell found to respond to EPO for which activation of the receptor causes endothelial cell mitosis and migration. However, the effective concentrations of EPO needed for this activity (1–2 nmol/L) were significantly higher than those required for hematopoiesis (Anagnostou et al., 1990). Subsequently, the nervous system (Konishi et al., 1993), the kidney (Westenfelder et al., 1999), and the heart (Calvillo et al., 2003) were also shown to respond to EPO, but similar to endothelial cells, required higher EPO concentrations than that needed for hematopoiesis. The substantial difference between the affinity of the $(EPOR)_2$ expressed by erythrocyte precursors and the one on non-hematopoietic cells suggested that a different receptor might transduce tissue protection. Additionally, the EPO receptor isolated from neuronal-like PC-12 cells was characterized as having a different molecular weight and with distinctive accessory proteins (Masuda et al., 1993).

Using a derivative of EPO with the binding sites to $(EPOR)_2$ blocked, Leist and colleagues demonstrated that the erythropoietic and cytoprotective effects of EPO could be separated (Leist et al., 2004). Subsequent experimental data have provided good evidence that the extra-hematopoietic effects of EPO are mediated, at least in part, by an alternative receptor that is proposed to consist of a hetero-complex between the EPOR and the β-common receptor (βcR; known also as CD131) (Brines et al., 2004). βcR is a subunit also used for the receptors of other type 1 cytokines, i.e., interleukin (IL)-3, IL-5 and granulocyte-macrophage colony stimulating factor (Murphy & Young, 2006). We have named this alternative EPO receptor as the innate repair receptor (IRR) to differentiate it from hematopoietic effects and to emphasize its protective role in inflammation and tissue injury to reduce damage, while also initiating healing and repair.

A distinct temporal-spatial relationship exists between components of the innate immune response and initiation of tissue protection and repair via IRR activation (Fig. 1). In quiescent cells, EPOR and βcR are typically localized within the intracellular compartment and not present on the cell surface. Stress, e.g. by hypoxia or inflammation, induces a rapid (several minutes) movement to the cell surface (Bohr et al., 2015) with a likely coalescence into membrane rafts, as has been described for other heteroreceptors that utilize βcR (Saulle et al., 2009), and as has been described above for the EPOR dimer (McGraw et al., 2012). Assembly of the mature receptor would then allow for stimulation if hsEPO is available in the surrounding locale. In this way, the innate repair system acts as a complex lock and key system that requires a specific time and spatial resolution to be present before being activated.

As in the case of the dimeric erythropoietic receptor $(EPOR)_2$ complex, binding of EPO to the EPOR-βcR complex causes phosphorylation of JAK2. This activation of JAK2 then switches on three principle signaling cascades that depend upon the specific tissue examined: STAT-5, PI3K/Akt, and mitogen-activated protein kinases (see below). These signaling pathways induce regeneration, inhibit apoptosis and inhibit inflammation (Brines & Cerami, 2008).

The binding affinity of the classic $(EPOR)_2$ complex is significantly greater than the binding affinity of the tissue-protective EPOR-βcR complex (Brines & Cerami, 2008). Thus, to induce local tissue protection, considerably higher systemic doses of recombinant EPO (rEPO) are