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COMMENTARY

EPO tecting the Endothelium

TE Peterson and ZS Katusic

Departments of Anesthesiology and Molecular Pharmacology, and Experimental Therapeutics, Mayo Clinic College of Medicine, Rochester, MN, USA

Erythropoietin is a 30.4 kDa protein that is produced and secreted from the kidney in response to anemia and hypobaric hypoxia. Binding of EPO to its receptor (EPO-R) on bone marrow-derived erythroid progenitor cells results in the stimulation of red blood cell production. Evidence is accumulating however, that the biological effects of recombinant EPO therapy extend beyond the stimulation of erythropoiesis. The discovery that the EPO-R is expressed on vascular endothelial cells suggests that the vasculature may be a biological target of EPO. Indeed, several studies have now demonstrated that the protective effect of EPO administration involves the activation of the protein kinase B/Akt pathway which can protect cells from apoptosis. Future work is likely to provide further insight into the mechanisms by which EPO protects vascular endothelial cells from injury and give us a better understanding of the pharmacological doses that are required to achieve this protection.
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Over the past decade, erythropoietin (EPO) has been successfully used to stimulate erythropoiesis in patients suffering from anaemia. It has become apparent, however, that the beneficial effects of EPO have extended well beyond the stimulation of red blood cell production in these patients (Brines and Cerami, 2005). Indeed, previous studies by Chong *et al*. (2002) established that EPO protects vascular endothelium against ischemic injury. More recently, several *in vivo* studies have demonstrated that EPO administration has a protective effect on endothelial cells in diverse models of vascular disease (Santhanam *et al*., 2005; Satoh *et al*., 2006; Urao *et al*., 2006). The present study by Chong and Maiese (2007), in the current issue of the *British Journal of Pharmacology*, sheds new light on the molecular mechanisms by which EPO transduces anti-apoptotic signals that may help to divert 'death signals' from entering the cell nucleus during oxygen and glucose deprivation (OGD) in endothelial cells *in vitro*.

EPO is a 30.4 kDa protein that is synthesized and secreted mainly by the kidney. Early studies have demonstrated a direct correlation between tissue oxygen levels and the production of EPO as well as expression of its receptor. Subsequently it has been shown that hypoxia triggers the activation of the transcription factor, hypoxia-inducible factor-1, which binds to the hypoxia response element on the EPO gene resulting in robust EPO production. Recent studies, however, have demonstrated that both EPO and its receptor (EPO-R) are expressed in a number of tissues including vascular endothelial and smooth muscle cells (Anagnostou *et al*., 1994; Ammarguellat *et al*., 1996; Brines and Cerami, 2005), further emphasizing that the vasculature may be an important target organ for the biological effects of EPO.

Binding of EPO to its receptor results in the phosphorylation and activation of Janus kinase 2, which can initiate multiple signal transduction pathways associated with cell survival (Figure 1). Of particular interest is the activation of the protein kinase B (PKB)/AKT pathway, which has been shown to stimulate anti-apoptotic signals that facilitate the inhibition of mitochondrial cytochrome *c* release and help to maintain mitochondrial membrane potential. Additionally, AKT activation prevents the translocation of the pro-apoptotic protein, Bad, to mitochondria and translocation of the transcription factor, FOXO3A, to the nucleus by facilitating the binding of the protein 14-3-3 to these molecules. 14-3-3 proteins are involved in the inhibition of apoptosis by binding to pro-apoptotic signalling molecules and diverting these signals away from their target. In the present study by Chong and Maiese, this appears to be the major mechanism by which EPO protects the endothelium during OGD. The authors were able to demonstrate that stress imposed by OGD *in vitro* significantly reduced survival of microvascular endothelial cells. Although the stress-activated pathways leading to endothelial apoptosis were not investigated, it was shown that EPO treatment either 1 h before OGD or up to 4 h after OGD was able to rescue endothelial cells from apoptosis. By selectively knocking down AKT levels using small interfering RNAs, the authors found that EPO could no longer protect endothelial cells from OGD, suggesting that the protective effects of EPO were dependent on AKT activity.

Correspondence: Dr ZS Katusic, Departments of Anesthesiology and Molecular Pharmacology, and Experimental Therapeutics, Mayo Clinic College of Medicine, 200 First Street SW, Rochester, MN 55905, USA.
E-mail: Katusic.zvonimir@mayo.edu
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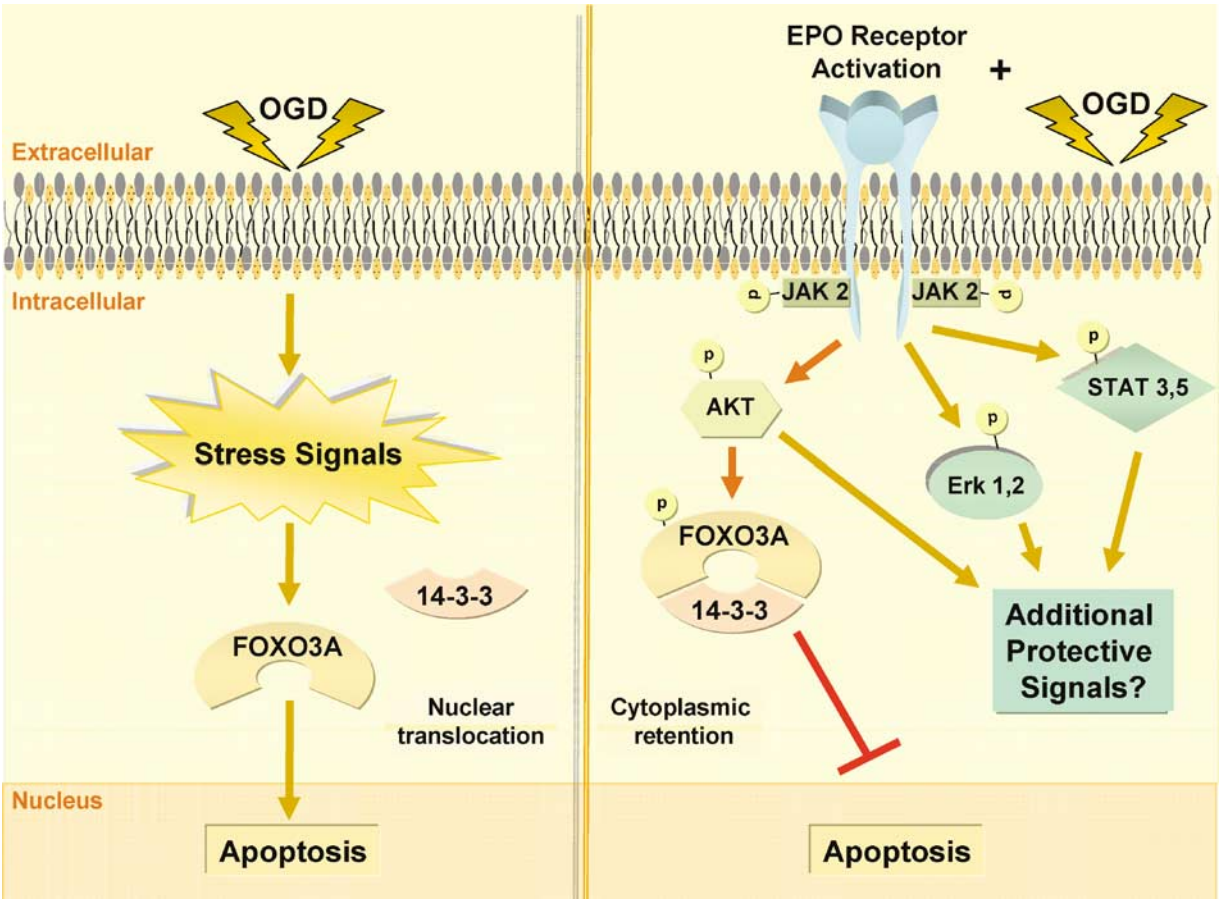

Figure 1 Activation of the EPO signaling cascade confers vascular protection through multiple signaling pathways.

Additionally, it was demonstrated that EPO promoted the binding of FOXO3A to 14-3-3 in an AKT-dependent manner as AKT downregulation inhibited this protein–protein interaction.

Existing evidence supports the concept that circulating EPO may have protective effects on vascular endothelium. However, the importance of this effect for cardiovascular function in patients treated with EPO is not well understood. Under physiological conditions, circulating levels of EPO are in the range of 1–8 pM, whereas anaemic patients have circulating levels of just below 100 pM. In addition, hypobaric hypoxia is also known to cause significant increase in circulating levels of EPO (Eckardt *et al*., 1989). However, the reported levels of EPO are still substantially lower than the reported affinities of the EPO receptors in endothelial cells, which would require EPO concentrations of up to 1–10 nM. Therapeutic administration of EPO may elevate circulating levels of EPO in the nM range, thus activating endothelial receptors. In addition, the proposed existence of alternative receptor complexes for EPO signalling may help to explain tissue protective effects of EPO. Brines *et al*. (2004) have suggested that the β-common receptor, which belongs to the same class of type 1 cytokine receptor family as EPO-R, may in fact play a role in EPO signalling by hetero-trimerizing with the EPO-R to elicit protective signals. Although it is still unclear if this signalling system exists in endothelial cells, it emphasizes the fact that our understandings of the signalling processes of EPO are still incomplete. Future studies should provide new insights into the mechanisms of protection in endothelial cells by EPO and help to translate this knowledge into the clinical arena.

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