Cell Death and Differentiation (2004) 11, S37–S44

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Review

Erythropoietin as an antiapoptotic, tissue-protective cytokine

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Received 16.2.04; revised 29.3.04; accepted 02.4.04
Edited by Dr G Melino

Abstract

Erythropoietin (EPO) increases the number of circulating erythrocytes primarily by preventing apoptosis of erythroid progenitors. In addition to this proerythroid action, results of recent studies show that systemically administered EPO is protective *in vivo*, in several animal models of neuronal injury. *In vitro*, EPO prevents neuronal apoptosis induced by a variety of stimuli. This review summarizes the neuroprotective actions of EPO and discusses the underlying mechanisms in terms of signal transduction pathways involved. The understanding of these mechanisms will help differentiate the neuroprotective actions of EPO from its role in the bone marrow.
Cell Death and Differentiation (2004) **11,** S37–S44.
doi:10.1038/sj.cdd.4401450

Keywords: erythropoietin; neuron; neurodegeneration

Abbreviations: AMPA, amino-3-hydroxy-5-methylisoxazole-4-propionic acid; CNS, central nervous system; EPO, erythropoietin; EPOR, EPO receptor; GFAP, glial fibrillary acidic protein; HIF, hypoxia-inducible factor; IGFBP, insulin-like growth factor binding protein; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; VEGF, vascular endothelial growth factor

Introduction

Erythropoietin (EPO) was named for its long-appreciated hormonal effect of maintaining the circulating erythrocyte mass. In recent years, however, it has been recognized that EPO is a member of the cytokine type I superfamily. Typical for cytokines, EPO has multiple functions outside of the bone marrow. Many of these effects parallel its action in hematopoiesis, where EPO functions to promote proerythroblast survival and maturation. Over the last 10 years, a prominent role for EPO has been defined in the nervous system and there is a growing interest in the potential therapeutic use of EPO for neuroprotection. In this review, we will outline the mechanism of action of EPO in erythropoiesis with a focus on inhibition of apoptosis. This concept will then be extended to examine the neuroprotective activities of EPO, which in addition to a critical role in antiapoptosis, also embody other beneficial actions.

Erythropoietin is a cytoprotective cytokine induced by the hypoxia inducible factor family

EPO is the hematopoietic factor responsible for the production of red blood cells and for this function is produced mainly by the adult kidney. EPO is induced by hypoxia via the hypoxia-inducible factor (HIF) family of transcription factors. This growing family mediates physiological adaptation to low tissue oxygen concentration (such as that encountered by high altitude or anemia) ultimately resulting in an increased number of erythrocytes within the circulation and therefore improved tissue oxygenation. Along the same line, HIF also regulates genes for neoangiogenesis (e.g., vascular endothelial growth factor, VEGF) as well as for vascular tone (e.g., nitric oxide (NO) synthase).

While erythropoiesis and angiogenesis represent adaptive responses to improve tissue oxygenation that require several days to develop (for instance, the time required for EPO-mediated appearance of mature erythrocytes in the circulation is about a week), HIF also mediates short-term adaptive responses to hypoxia, and several glucose transporters and glycolytic enzymes are transcriptionally regulated by HIF, as well as genes involved in cell growth and survival including insulin-like growth factor 2 and IGFBP (reviewed in: Semenza,[1] Wiesener and Maxwell[2] and Wenger[3]). The induction of glycolytic enzymes demonstrates a role for HIF in the metabolic, cell-autonomous adaptation to hypoxia represented by the switch of ATP generation from oxidative phosphorylation to glycolysis, the Pasteur effect.[4] It has also been suggested that, because HIF is often constitutively expressed in tumors, it may mediate the high constitutive expression of glycolytic enzymes that is the basis of the Warburg effect.[3,5]

The metabolic changes induced by hypoxia clearly play a role in ischemic preconditioning, a well-known phenomenon, reported for various organs including the heart, brain, kidney and liver, whereby pre-exposure to hypoxia (such as a minor ischemic event) protects from subsequent, severe ischemia.[1] Activation of HIF and/or pre-exposure to hypoxia also protects neuronal cells from apoptosis induced by oxidative stress.[6]

However, HIF-mediated protection from apoptosis may also involve other hypoxia-inducible cytokines implicated in the physiological responses to hypoxia, such as EPO and VEGF. In fact, there is much evidence showing that EPO protects neuronal cells *in vitro*, an action independent of its erythropoietic activity. In particular, EPO not only protects neurons from cell death induced by hypoxia, but also from a variety of other agents including excitotoxins and glucose deprivation (Table 1). More recently, it was reported that

Table 1 Neuroprotective effects of EPO *in vitro* (selected references)

Type of neurons	Type of toxicity	Effect of EPO	Ref.
Motor neurons	Kainate	Protection	39
Hippocampal neurons	Hypoxia	Protection	39
PC12 cell line	Serum/NGF deprivation	Protection	82
P19 cell line	Serum deprivation	Decreased apoptosis	39
Hippocampal neurons	Anoxia	Decreased apoptosis, Caspase activation	41
Cortical neurons	NMDA, NO	Decreased apoptosis	60
Hippocampal and cortical neurons	Glutamate	Protection	83
Primary cortical	Oxygen/glucose deprivation	Decreased apoptosis	61
Hippocampal neurons	NO	Protection	84
Cortical neurons	Oxygen/glucose deprivation, AMPA	Protection	35
Hippocampal neurons	Chemical hypoxia	Decreased apoptosis	62

VEGF is also a neuroprotective and trophic factor for neurons.[7–9]

The finding that systemically administered EPO, a widely used drug with a well-known safety profile, crosses the blood–brain barrier in rats and in humans[10,11] and has protective effects in various models of nervous system injury (Table 2), has greatly focused attention on the neuroprotective actions of EPO and the underlying mechanisms. The protective effects of EPO are not restricted to the CNS as it was recently shown that EPO has also antiapoptotic effects on cardiomyocytes *in vitro* and *in vivo* in a rat model of myocardial infarction, where it normalizes hemodynamic functions.[12] All these data stress the importance of EPO as an antiapoptotic and cytoprotective, not only neuroprotective, cytokine.

Inhibition of apoptosis in the hematopoietic activity of EPO

Erythrocyte development proceeds from progenitor cells that require multiple growth factors to initiate cellular differentiation. Cells entering into development express receptors for EPO (EPOR) during specific phases in differentiation and unless EPO is present during the critical period, the progenitors will undergo apoptosis. EPO specifically augments the number of circulating erythrocytes by promoting the survival and therefore enabling the proliferation and differentiation of erythroid progenitor cells.[13,14] Although EPO, through the EPOR, can effectively support the proliferation of murine erythroid progenitor cells *ex vivo*[15] and induce the entry of erythroid progenitors into cell cycle if dormant,[16] promotion of survival due to prevention of apoptosis of late erythroid progenitors is thought to be a major mechanism in EPO action.[17,18]

Using the EPO-dependent human erythroid progenitor cell line HCD-57, Silva *et al*.[19] showed that EPO maintained their viability via repressing apoptosis by upregulating Bcl-xL, an antiapoptotic gene of the Bcl-2 family. When these cells are cultured in the absence of EPO, Bcl-2 and Bcl-xL are downregulated and the cells undergo apoptosis.[19] To further support the importance of this pathway, Bcl-xL knockout mice exhibit fetal liver hematopoietic defects and severe anemia during embryogenesis.[20]

EPO exerts its erythrodifferentiating effect via the EPOR, which belongs to the hematopoietic growth factor receptor superfamily. Other members include receptors for IL-2 beta chain, IL-3, IL-4, IL-5, G-CSF, GM-CSF, thrombopoietin, growth hormone, prolactin and the receptors for several cytokines of the IL-6 family (IL-6, leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF) and oncostatin M).[21,22]

One possible signal transduction pathway involved in the antiapoptotic action of EPO in erythroid cells is outlined in Figure 1, based on the known signal transduction pathways activated by EPO (excellently reviewed in Wojchowski *et al*.[23]). Binding of EPO to the extracellular domain of preformed EPOR dimers[24] initiates signaling that in turn results in recruitment and activation of Janus kinase 2 (JAK2) by the intracellular domain of EPOR. JAK2 then phosphorylates several proteins including STAT5. There is evidence in favor of a role of STAT5 in the antiapoptotic action of EPO on erythroid precursors. In particular, STAT5 appears to mediate the induction of Bcl-xL by EPO,[25] and anemia and high levels of apoptosis in erythroid progenitors are observed in STAT5 knockout mice, possibly due to a disruption of the Bcl-xL response pathway.[26] Further, EPOR activation activates voltage-sensitive Ca^{2+} channels, which in turn modulates neurotransmitter.[27]

Another pathway involves activation of phosphatidylinositol-3 kinase (PI3 K). In fact, PI(3)K is activated by EPO in the EPO-dependent UT-7 leukemia cell line, where it recruits Akt.[28] This PI3K–Akt pathway also leads to upregulation of Bcl-xL and inhibition of apoptosis in Baf-3 cells.[29] A further mechanism could be represented by NF-kB that is also a target of the PI3K-Akt pathway and mediates antiapoptotic signaling by platelet-derived growth factor.[30]

EPO as a neuroprotective agent

Study of experimental brain ischemia has shown that EPO and EPOR function as components of an innate response to metabolic stress. Multiple studies have shown that whereas normal brain expresses both EPO and EPOR in a highly restricted and limited manner, following ischemic and other stressors a marked induction of both proteins occurs in animal models[31] as well as in human disease.[32,33] Presumably, this regulation is accomplished by specific proinflammatory cytokines, for example, TNF-alpha, IL-1 and IL-6.[34] Temporally, EPOR upregulation occurs first by 12 h, primarily in neurons and endothelial cells of the microcirculation, followed hours later by an increase in EPO by both astrocytes and neurons.[31] These effects are especially prominent in the

Table 2 Nonexhaustive listing of *in vivo* models of tissue injury showing nonclassical activities of recombinant human EPO

Tissue	Model	Species	Method/dosing	Effect of EPO	Ref.
Brain	Ischemia–reperfusion	Rat	Three-vessel occlusion. Single i.p. dose	Reduction of stroke volume by ~75%; partial efficacy as late as 6 h	10
Brain	Phase II trial stroke in the territory of the MCA	Human	Enrollment up to 8 h; mean 5.5 h 3 daily i.v. doses	Significant improvement in clinical scores and outcome	80
Brain	Blunt trauma	Mouse	Closed impact. Single i.p. dose	Reduced cavitation volume and inflammation 7-10 days later	10
Brain	Experimental autoimmune encephalitis	Rat	Female Lewis rat. Multiple dosing i.p. beginning at day 3 following antigen injection	Marked reduction in clinical score and reduced inflammatory cytokine levels	10
Brain	Subarrachnoid hemorrhage	Rabbit	Autologous blood injection into cisterna magna. Single i.p. dose	Virtually complete protection	85
Brain	Neonatal hypoxia–ischemia	Rat	Carotid occlusion and brief hypoxia. Single i.p. dose	Markedly reduced infarct volume, reduced apoptosis, reduced NF-kB and caspase-3 in neurons	86
Brain	Ischemia	Rat	Three-vessel occlusion. Single i.p. dose	Decreased inflammation	40
Brain	Ischemia	Gerbil	Carotid artery occlusion.	Reduced apptosis in CA induced $BclX_L$	62
Brain	Neonatal hypoxia–ischemia	Mouse	Carotid artery occlusion and brief hypoxia. Single i.p. dose	Markedly reduced infarct volume, reduced apoptosis, reduced NF-kB and caspase-3 in neurons	68
Brain	Hypothermic circulatory arrest	Pig	Hypothermia. Single i.v. dose	Reduced apoptosis and glutamate concentrations.	87
Brain	Parkinson	Mice	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine	Survival of nigral dopaminergic neurons	88
Brain	Kainate toxicity	Mouse	Seizures caused by kainic acid. Single i.p. dose	Pretreatment markedly reduces seizure severity	10
Brain	MK801 toxicity	Rat	Newborns given MK801 with a single dose of EPO	Reduction of neuronal apoptosis	65
Retina	Ischemia/reperfusion	Rat	Increased intraocular pressure above aortic. Single ip dose	ERG and histology nearly normal for 45 minutes ischemia	89
Spinal cord	Ischemia/reperfusion	Rabbit	Transient occlusion of the abdominal aorta. Single i.p. dose	Normal neurological function; decreased motoneuron apoptosis	90
Spinal cord	Compression	Rat	Application of aneurysm clip for 1 min. Single and multiple i.v. doses	Single, multiple doses lead to marked neurological recovery	91
Sciatic nerve	Compression	Rat	Aneurysm clip. Multiple iv doses	Attenuated injury and faster recovery of function	81
Peripheral nerve	Compression	Rat	Crush; daily s.c. doses	Decreased pain; less DRG apoptosis	66
Peripheral nerve	Diabetic neuropathy	Rat	Streptozotocin; chronic i.p. dosing	Decreased nociceptive alterations; restored NCV	92
Heart	Ischemia/reperfusion	Rat	Occlusion of main coronary artery for 45 min. Multiple i.p. doses	Less cardiomyocyte loss and preserved ventricular function	12
Heart	Preservation of function isolated heart	Mouse	Single i.p. dose	Pretreatment (24 h) protected from post ischemic injury	72
Kidney	Cisplatinum injury	Rat	Cisplatinum-induced acute renal failure. Multiple i.p. doses	Enhanced rate of recovery	75
Kidney	Ischemia/reperfusion	Rat	Single pretreatment i.p. dose	Reduced tubular apoptosis and increased proximal tubular mitosis	73
Skin	Wound healing; ischemia	Rat	Random skin flap revascularization. Multiple s.c. doses	Improved wound healing and reduced inflammatory response	76,77
Intestine	Hypoxia/reoxygenation	Rat	Necrotizing enterocolitis. Multiple i.p. doses	Decreased lipid peroxidation	93,94

penumbral (at risk) region surrounding injury. Study has shown that following the induction, this region is characterized by an increased resistance to subsequent stressors.

These activities *in vivo* appear to depend upon several processes. In many of these models, diverse pathogenic components play a role, including neuronal death, inflammation and, in the recovery phase, neurorepair. In some cases, the primary mechanism of action of EPO is represented by neuroprotection, while in other models (e.g., experimental autoimmune encephalitis) it could be due to reduced neuroinflammation. We have listed in Table 1 some of the *in vitro* models where EPO shows neuroprotective effects. It can be seen that, in most models, inhibition of cellular apoptosis was documented. In contrast to its antiapoptotic effect, EPO has not shown any activity in preventing necrosis in either the nervous system (Sinor and Greenberg[35] and Mennini *et al*., unpublished) or the heart.[12]

The EPO/EPOR is highly prominent during fetal development,[36] with the very high levels of expression found in many tissues diminishing rapidly after birth to the generally low levels found in the adult. Gene knockout experiments[37] have suggested the importance of this system in development, as

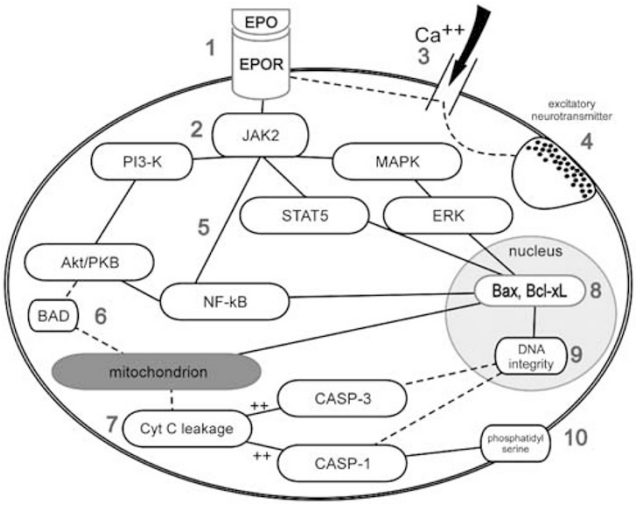

Figure 1 Summary of demonstrated EPO-signaling pathways in neuronal protection, binding of EPO to its receptor (1) leads to phosphorylation of janus kinase 2 (2). This subsequently activates multiple cascades recruiting PI3-K, Stat5 and MAPKinase. Further, flux of Ca^{2+} through voltage sensitive channels is modulated (generally inhibited (3)), affecting neurotransmitter release and therefore neuronal excitability (4). NF-kB is reported to be dually activated by JAK-2 and by Akt (5). The net effect is a reduction in the proapoptotic protein BAD (6), and therefore the probability of mitochondrial leakage of cytochrome *C* (7), an increased production of antiapoptotic proteins of the Bcl-x family (8) and ultimate preservation of the DNA integrity (9). To the extent cytochrome *C* leakage is not prevented, caspase activation also occurs, inducing DNA degradation and the externalization of phosphatidyl serine on the cell membrane (10), promoting the activation of the inflammatory cells. Solid lines indicate activation; dashed indicate inhibition

the embryos of EPOR −/− animals have abnormal brain development characterized by decreased neuronal progenitor cells as well as grossly decreased neuronal densities associated with massive neuronal apoptosis. Additionally, the heart is underdeveloped. On the other hand, erythroid-specific expression of EPOR in EPOR knockout mice rescues them from lethality suggesting that nonhematopoietic EPOR is not essential for development.[38] It would be interesting to evaluate these mice in models of tissue injury.

EPO affects the interaction between apoptosis and inflammation

In many *in vivo* models of CNS disease for which EPO shows a protective effect, inflammation is also an important pathogenic component, induced by production of cytokines and chemokines followed by leukocyte infiltration and glial activation and proliferation. The complicated inter-related protective effects initiated by EPO administration have been examined in some detail in experimental stroke in the rat. Using a model of ischemia with reperfusion, it was shown that neurons within the penumbra would undergo apoptosis unless exposed to EPO within 3 h of injury.[39] In addition to this antiapoptotic effect, EPO administration is also associated with a marked decrease in proinflammatory cytokines within the hemisphere undergoing infarction.[40] This profoundly reduced the activation of astrocytes within the region (as indicated by increased glial fibrillary acidic protein (GFAP) expression) as well as grossly decreased influx of inflammatory microglia. The marked *in vivo* reduction of recruitment of microglia into the region of injury is likely explained by the reduced expression of cell surface markers of apoptosis, for example, phosphatidylserine.[41] It should be noted that this proinflammatory role of phosphatidylserine conflicts with other works showing that it stimulates phagocytosis but not inflammation – rather anti-inflammation (for instance: Chan *et al.*,[42] De Simone *et al.*[43] and Brouckaert *et al.*[44])).

Using an *in vitro* system of neurons cocultured with glia, Villa *et al.*[40] have shown that the anti-inflammatory effects of EPO do not arise from a direct antagonism or a reduction of proinflammatory cytokines, but rather by an antiapoptotic effect on neurons. In fact, neurons exposed to trimethyltin, a toxin that induces neuronal apoptosis,[45] release as yet unidentified factors that stimulate inflammatory TNF production by glial cells that, in turn, amplifies neurotoxicity.[46] In this context, EPO inhibits TNF production by TMT-exposed neuron-glia co-cultures but not by pure glial preparations directly exposed to neuronal products or LPS.[40] This pathway of 'anti-inflammation by neuroprotection' is outlined in Figure 2.

According to a well-accepted scheme, apoptosis is a way of dying that triggers much less inflammation than by necrosis, and this is certainly true in many experimental models.[47,48] However, this model of 'docking without shocking',[49] may not apply to all pathological conditions, and production of inflammatory cytokines by tissue macrophages during phagocytosis of apoptotic cells.[50,51] Similarly to what is observed in a model of cerebral ischemia with EPO,[40] inhibition of apoptosis by lysophosphatidic acid or caspase inhibitors decreases inflammation in models of ischemic injury in the kidney and the brain.[52,53]

It should be noted, however, that an anti-inflammatory effect of EPO was demonstrated in a model of experimental autoimmune encephalomyelitis, an inflammatory pathology of the CNS of autoimmune origin with marked inflammation in the almost complete absence of neuronal death, at least in the earliest times of the disease.[54] Thus, it cannot be ruled out that EPO exerts an anti-inflammatory action on the CNS by different mechanisms than those depicted in Figure 2, although it is presently unclear what these mechanisms may be.

EPO signaling in nervous system cells

EPO has been shown to have multiple effects on neurons. Activation of the EPOR modulates Ca^{2+} influx[55,56] *in vitro*. Inhibition of Ca^{2+} influx upon depolarization directly reduces synaptic vesicle release of glutamate, which acts to reduce the magnitude of neuronal injury (Figure 1). In addition, it could explain the effects of EPO in reducing seizure severity[10] as well as reported effects on learning and memory.[57] Other indirect effects of EPO that reduce neuronal injury have also been delineated. For example, EPO treatment increases astrocyte production of glutathione peroxidase and in this manner ameliorates neuronal damage caused by excitotoxins.[58] EPO also promotes the differentiation and maturation of astrocytes and oligodendrocytes and directly induces the proliferation of astrocytes,[59] actions that can modify potential

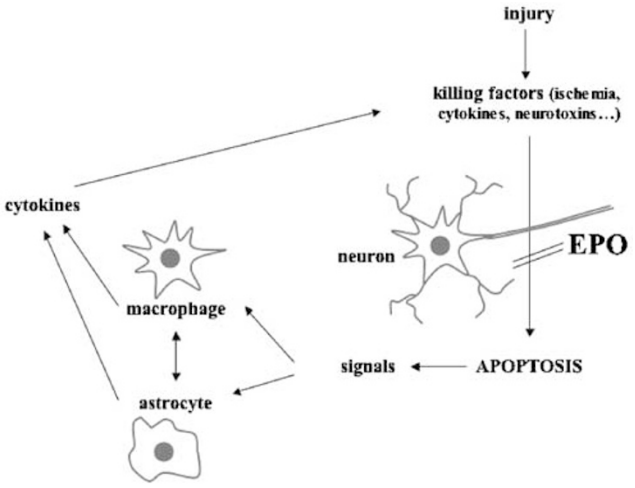

Figure 2 Interplay between the antiapoptotic action of EPO and neuroinflammation

injury. Undoubtedly, other indirect neuronal protection functions remain to be described.

Several antiapoptotic pathways regulated by EPO in erythroid precursors are also activated in neurons and may play a role in prevention of neuronal apoptosis. Triggering NF-kB via activated Jak2 has been reported, and the antiapoptotic action of EPO on nitric oxide-induced cortical neuron apoptosis is blocked by inhibitors of NF-kB translocation as well as by overexpression of an IkBalpha super-repressor or of a dominant interfering form of Jak2.[60] The antiapoptotic action of NF-kB in neurons also involves activation of Akt1 and Bad phosphorylation[41,61] as well as Bcl-xL upregulation.[62,63] Bcl-xL was also found induced by EPO in a study where DNA microarrays were used to identify modulated genes in PC12 neuronal-like cells[64] and *in vivo* in a gerbil model of cerebral ischemia.[62]

Of note, Ruscher *et al*.,[61] studying the protective effect of EPO against oxygen glucose deprivation-induced apoptosis of primary cortical neurons, confirmed that the protective effect requires Jak2, as shown by Digicaylioglu and Lipton,[60] and of PI(3)Kinase, as shown by us,[39] using specific inhibitors of these kinases. In particular, the MAPK inhibitor PD98059 and the PI(3)Kinase inhibitor LY984002 blocked the protective effect of EPO in hippocampal neurons exposed to hypoxia.[39] On the other hand, unlike others reported in erythroid cells, the other pathway downstream of Jak2, activation of STATs, was not activated by EPO in this study, and no induction of mRNA for Bcl2, Bcl-xL, Bag-1, Bax or Bad, was observed, as measured by PCR.[61]

In addition to directly activating survival kinases such as ERK, EPO, while not activating Akt under normal conditions, prevents the decrease in Akt phosphorylation induced by a 15-h exposure of neurons to hypoxia.[39] A similar pattern has been recently observed *in vivo* where administration of EPO, protects newborn rats from neuronal apoptosis induced by MK801 and prevents MK801-induced decrease in the phosphorylation level of ERK1/2 and Akt.[65]

EPO has profound effects in reducing injury and enhancing recovery in compression injuries of peripheral nerves.[66,67] Campana *et al*.[66,67] performed a crush injury of a spinal nerve root for which follow-up showed a robust effect by EPO to prevent apoptosis in dorsal root ganglion neurons which occurred by Jak2 signaling, as well as a corresponding improvement in pain behavior.

Clearly, the relevance of the pathways outlined above in the neuroprotection observed *in vivo* with EPO is far from being established. For instance, in a study of neonatal hypoxic ischemic injury in mice has reported that NF-kB-immunoreactivity in neurons in the injured areas was not decreased by EPO, neither was its subcellular distribution, despite a marked neuroprotective effect.[68]

Other tissue-protective activities of EPO

Capillary endothelial cells express EPOR on their intraluminal surfaces. EPO has been shown *in vitro* to antagonize the apoptosis of endothelial cells subjected to ischemic stressors.[69] EPO thus plays a role in maintaining the integrity of the microvasculature. Additionally, EPO has also been shown to stimulate mitogenesis and support angiogenesis, both functioning to improve tissue oxygenation. The endothelial cell is additionally important as a conduit for systemically administered EPO to pass into organs with a barrier to the circulation. Recent *in vitro* studies have shown that EPO present on the apical (luminal) side is transported in a unidirectional manner through the endothelial cell by transcytosis.[70] Further, EPO strengthens the tight junctions of endothelial cells and will antagonize the effects of VEGF, which is known to reduce tight junction proteins allowing leakage from the capillary around the endothelial cells into the brain parenchyma.[70]

EPO has been shown to be protective of myocardial ischemia either permanent or with reperfusion.[12,71] Further, cardiomyocytes losses were not completely prevented in EPO-treated animals, being reduced by $\sim$50%. In spite of tissue loss, maladaptive remodeling did not occur, resulting in a maintained normal cardiac function.[12] Further, studies performed on isolated hearts have shown that EPO mediates ischemic preconditioning.[72]

The kidney is protected by EPO in the setting of both ischemic and toxic injuries.[73–75] In ischemic injury with reperfusion, EPO prevents apoptosis of tubular epithelial cells as well as mediates intense mitotic activity of the surviving population of proximal tubular epithelial cells.[73]

The skin has also been shown to be protected by EPO in rodent flap models.[76,77] In these studies, the tissue-protective effects appear to depend strongly on the dose and treatment duration, suggesting that tissues may vary in their responses to EPO.

From the perspective of the evolving concept of EPO and EPOR as a endogenous local system of protection and the widespread expression of these molecules, it is likely that many other organs and tissues when examined for protective effects of EPO will demonstrate a positive role.

Therapeutic perspectives

Recombinant human EPO has been shown in general to be an exceedingly safe drug, as millions of patients have received it over the last decade for treatment of anemia. However, some

side effects, including increased blood pressure and risk of thrombosis have been noted and these are of special concern for patients who are not anemic. EPO has been shown to mediate angiogenesis in the microcirculation, with a potency similar to VEGF.[78] However, unlike VEGF, EPO does not reduce the tight junctions between endothelial cells, and therefore does not promote capillary leakage.[70]

The experimental models characterized by potential cellular barriers for the systemic administration of recombinant human EPO (e.g., brain, retina, spinal cord) have been found to require a minimum effective dose that is generally higher than the doses of EPO currently employed for treatment of anemia (but those used experimentally for bone marrow rescue have been much higher, as reviewed by Baron *et al*.[79]). Although acute (i.e. a few doses) administration of recombinant human EPO for treatment of tissue injury is not likely to be harmful, chronic dosing will at the least provoke increases in erythrocyte mass. This is undesirable from a rheologically perspective and could worsen injury. The recent Phase II trial of human stroke by EPO used three daily doses substantially higher than the clinical norm and, in these patients, EPO was shown to be safe and did not raise the hemoglobin concentration.[80] Thus, this might represent a valuable therapeutic approach to acute CNS injury, such as brain and spinal cord trauma. On the other hand, use of EPO in chronic diseases associated with neuronal apoptosis or neuroinflammation, such as Parkinson's disease, Alzheimer disease or multiple sclerosis would be possible only if the erythropoietic effect was blocked.

The studies on the signaling mechanisms of the cytoprotective *versus* the hemopoietic actions of EPO, and the receptors involved, will help identifying molecules that retain the cytoprotective actions of EPO but not its hemopoietic effects. It was recently reported that desialylated EPO is a neuroprotective cytokine that does not induce erythrodifferentiation,[81] indicating the feasibility of this approach.

In summary, EPO is a major modifier of apoptosis of multiple cell types in different tissues and organs in the setting of potential injury. However, EPO also exhibits many other actions that serve to protect cells either directly or indirectly. EPO should therefore be regarded also as a general tissue-protective cytokine.

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