bjh review

The non-haematopoietic biological effects of erythropoietin

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Summary

In the haematopoietic system, the principal function of erythropoietin (Epo) is the regulation of red blood cell production, mediated by its specific cell surface receptor (EpoR). Following the cloning of the Epo gene (*EPO*) and characterization of the selective haematopoietic action of Epo in erythroid lineage cells, recombinant Epo forms (epoetin-alfa, epoetin-beta and the long-acting analogue darbepoetin-alfa) have been widely used for treatment of anaemia in chronic kidney disease and chemotherapy-induced anaemia in cancer patients. Ubiquitous EpoR expression in non-erythroid cells has been associated with the discovery of diverse biological functions for Epo in non-haematopoietic tissues. During development, Epo–EpoR signalling is required not only for fetal liver erythropoiesis, but also for embryonic angiogenesis and brain development. A series of recent studies suggest that endogenous Epo–EpoR signalling contributes to wound healing responses, physiological and pathological angiogenesis, and the body's innate response to injury in the brain and heart. Epo and its novel derivatives have emerged as major tissue-protective cytokines that are being investigated in the first human studies involving neurological and cardiovascular diseases. This review focuses on the scientific evidence documenting the biological effects of Epo in non-haematopoietic tissues and discusses potential future applications of Epo and its derivatives in the clinic.

Keywords: erythropoietin, cytokine receptor, signal transduction, apoptosis.

The primary sites of Epo production reside in the fetal liver and adult kidney (Zanjani *et al*, 1977; Dame *et al*, 1998) where Epo gene (*EPO*) expression occurs mainly under the control of an oxygen-sensing, hypoxia-inducible factor-dependent mechanism (Gruber *et al*, 2007; Rankin *et al*, 2007). Hepatic Epo expression is modulated by other transcription factors including WT1 (Dame *et al*, 2006) and the GATA family of transcription factors (Imagawa *et al*, 1997; Dame *et al*, 2004). Epo expression has been found in many extra-renal tissues and cell types including astrocytes (Marti *et al*, 1996), neurons (Bernaudin *et al*, 1999), the female genital tract (Masuda *et al*, 2000), male reproductive organs (Magnanti *et al*, 2001; Kobayashi *et al*, 2002), mammary glands (Juul *et al*, 2000), placental trophoblasts (Conrad *et al*, 1996), bone marrow macrophages (Vogt *et al*, 1989) and erythroid progenitors (Stopka *et al*, 1998; Sato *et al*, 2000). The expression of *EPOR* in non-erythroid tissues such as the brain (Liu *et al*, 1997), retina (Grimm *et al*, 2002), heart (Wu *et al*, 1999), kidney (Westenfelder *et al*, 1999), smooth muscle cells (Ammarguellat *et al*, 1996), myoblasts (Ogilvie *et al*, 2000) and vascular endothelium (Anagnostou *et al*, 1994) has been associated with the discovery of novel biological functions of endogenous Epo signalling in non-haematopoietic tissues and the ability of exogenous Epo to modulate organ function and cellular responses to diverse types of injury. Thus, in addition to its essential role in the regulation of mammalian erythropoiesis, Epo signalling – activated either by exogenous Epo or by endogenous Epo in an autocrine or paracrine fashion – has emerged as a major tissue-protective survival factor in various non-haematopoietic organs. Characterization of the non-erythropoietic biological effects of Epo and understanding the mechanisms of Epo–EpoR signalling activation in non-haematopoietic organs and cell types are critical to the future development of novel applications for Epo and its derivatives as well as the optimization of the use of recombinant Epo for its current clinical indications including anaemia in chronic kidney disease and chemotherapy-induced anaemia in cancer patients.

Epo and EpoR function during development

Targeted disruption of either *Epo* or *Epor* in mice leads to in utero death between embryonic days E11·5 and E13·5 because of lack of definitive erythropoiesis in the fetal liver and severe anaemia indicating the vital role for Epo–EpoR signalling in the proliferation, survival and terminal differentiation of erythroid progenitors during development (Wu *et al*, 1995; Kieran *et al*, 1996; Lin *et al*, 1996). EpoR expression found in non-haematopoietic tissues such as the brain (Liu *et al*, 1997; Tsai *et al*, 2006) and heart (Wu *et al*, 1999) has suggested

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a role for Epo signalling in the embryonic development of specific non-haematopoietic organs. Detailed examination of *Epo*- or *Epor*-null mouse embryos revealed the presence of cardiac ventricular hypoplasia at day E11·5 (Wu *et al*, 1999). In the embryonic chick heart, Epo signalling was found to promote cardiac myocyte proliferation (Stuckmann *et al*, 2003). The discovery of increased apoptosis in the myocardium and brain of *Epor*-null mouse embryos further suggested an important role for *EPO–EPOR* in non-haematopoietic tissues during development (Yu *et al*, 2001, 2002). By intercrossing human *EPOR* transgene harbouring mice and heterozygous $EPOR^{+/-}$ mice, the expression of human *EPOR* rescued not only the erythropoietic defect but also ameliorated the apoptosis in the heart and brain of $EPOR^{-/-}$ mice (Yu *et al*, 2001). In a subsequent study, erythroid expression of EpoR under the control of the *Gata1* promoter that is active in haematopoietic cells was shown to rescue *Epor*-null mice from embryonic lethality by restoring normal erythropoiesis and demonstrated that EpoR expression in non-haematopoietic tissues was not critical for life (Suzuki *et al*, 2002). In *Gata1–Epor* transgene-rescued embryos which lack non-haematopoietic EpoR expression, no defects or abnormalities were observed in the myocardial walls suggesting that Epo signalling is not required for the initial development of the myocardial layer. Although the activity of the *Gata1* mini-cassette used in these studies appears to be haematopoietic-restricted, another GATA family member transcription factor GATA-3 has been shown to be involved in EpoR expression induction in neuronal cells (Yu *et al*, 2002). Thus, complete elucidation of the role of EpoR in normal heart development may require the development of a cardiomyocyte-specific EpoR knock-out model.

Endogenous Epo signalling is essential for early embryonic neural development and contributes to neuron survival and neurogenesis in adults. Epo has been reported to regulate neural progenitor cell (NPC) differentiation (Studer *et al*, 2000; Shingo *et al*, 2001) and EpoR expression levels were found to be downregulated as NPCs terminally differentiated into mature neurons (Chen *et al*, 2007). The temporal and spatial regulation of Epo and EpoR expression in the developing embryo is consistent with a role for Epo signalling in early neurogenesis, as *Epo*- or *Epor*-null mouse embryos exhibit incomplete neural tube closure at day E10·5 (Tsai *et al*, 2006). Because the severe anaemia observed in *Epo* or *Epor* knock-out embryos results in systemic hypoxia, transgenic approaches were used to develop models to investigate the consequences of selective *Epor* inactivation in the brain during development and in adult mice (Tsai *et al*, 2006; Chen *et al*, 2007). Conditional, brain-specific *Epor* deletion results in reduced cell proliferation in the subventricular zone where *in vivo* neurogenesis takes place in adult mice (Tsai *et al*, 2006). These animals exhibit impaired poststroke neurogenesis through defective migration of NPCs to the peri-infarct cortex. Interestingly, infarct volume after ischaemia-reperfusion (I/R) injury was similar between *Epor* conditional knock-down mice and control animals, suggesting a relatively limited role for endogenous neuronal EpoR activation in adult neuroprotection. It is also possible that endothelial EpoR expression and activation in the brain may contribute to the endogenous neuroprotective effect of Epo signalling (Wang *et al*, 2004, 2006). In a different animal model, *Epor*-null mouse embryos were rescued by conditional *Epor* activation by Cre recombinase expression under the direction of the Tie2 promoter/enhancer that is also active in embryonic endothelium and which gives rise to haematopoietic stem cells (Chen *et al*, 2007). In this model, increased apoptosis was observed in the *Epor*-null embryonic brain. The lack of Epo signalling in embryonic *Epor*-null neuronal cells resulted in an intrinsic defect leading to increased cell death in neural cultures in response to hypoxia. In the adult *Epor*-null brain, fewer proliferating NPCs were present and increased sensitivity to glutamate toxicity was observed consistent with a role for Epo signalling in the proliferation and survival of adult NPCs.

Epo- or *Epor*-null mice also exhibit defects in physiological angiogenesis that occurs actively during embryogenesis (Wu *et al*, 1999; Kertesz *et al*, 2004). In the developing mouse embryo, EpoR expression was observed in yolk sac vasculature and co-expression of Epo and EpoR was present in the intersomitic vessels consistent with the potential for generation of an autocrine signalling loop (Kertesz *et al*, 2004). Close examination of *Epo*- or *Epor*-null embryos revealed that while the process of vasculogenesis remained intact, abnormalities in the second wave of blood vessel formation were observed starting from day E10·5 and this angiogenesis defect was partially rescued by expressing human Epo during embryogenesis.

Erythropoietin biology and function in the nervous system

The expression of EpoR in cultured cells of neuronal origin (Masuda *et al*, 1993), brain capillary endothelial cells (Yamaji *et al*, 1996), and in the mouse brain (Liu *et al*, 1994) is associated with the presence of specific in situ Epo binding sites in the brain (Digicaylioglu *et al*, 1995). EpoR expression in the mouse brain is developmentally regulated, with the expression levels dropping by three orders of magnitude from day E10·5 to birth (Liu *et al*, 1997). In earlier studies investigating the functionality of neuronal EpoR, recombinant Epo was shown to exhibit neurotrophic effects on cultured embryonal neurons (Konishi *et al*, 1993) and EpoR expression found in hippocampal and cortical neurons was associated with the ability of Epo pretreatment of cultured cells to reduce cell death induced by glutamate neurotoxicity (Morishita *et al*, 1996, 1997). In an animal model of forebrain ischaemia, continuous infusion of Epo into the lateral ventricle reduced ischaemia-induced neuron death, an effect that was associated with attenuation of neurological dysfunction and a reduction in the number of degenerating synapses (Sakanaka *et al*, 1998). The neuroprotective effect of exogenous Epo as well as the presence of hypoxia-inducible Epo expression in the adult rat

brain, the primate brain and in primary cultured astrocytes (Tan *et al*, 1992; Masuda *et al*, 1994; Marti *et al*, 1996) suggested that endogenous brain Epo–EpoR may be important for neuronal survival. Targeting brain Epo–EpoR by a continuous infusion of the antagonist soluble EpoR (sEpoR) protein into cerebral ventricles of gerbils with forebrain ischaemia enhanced the severity of the neurological dysfunction that was associated with decreased hippocampal neuron density and increased apoptosis consistent with a role for endogenous brain Epo–EpoR signalling in the brain's innate response to injury (Sakanaka *et al*, 1998). In rodents subjected to middle cerebral artery (MCA) occlusion, increased expression of Epo and EpoR has been observed in the ischaemic penumbra (Sadamoto *et al*, 1998; Bernaudin *et al*, 1999). In cultured neuronal cells, hypoxia induces EpoR expression leading to increased sensitivity to Epo that may play a role in the activation of the endogenous neuroprotective pathways (Chin *et al*, 2000; Yu *et al*, 2002). Consistent with this mechanism of increased Epo sensitivity, recombinant Epo restored the hypoxia-induced reduction in the phosphorylation of STAT5 (signal transducer and activator of transcription-5) and AKT in cultured rat hippocampal neurons (Siren *et al*, 2001). In purified, cultured human neurons, EpoR expression may also be induced by alternative mechanisms through the action of inflammatory cytokines, such as tumour necrosis factor α (TNFα) (Nagai *et al*, 2001).

In animal models of stroke, systemic Epo administered before or up to 6 h postischaemia was reported to cross the blood–brain barrier and lead to the reduction of the volume of cerebral infarction and neuronal apoptosis (Brines *et al*, 2000; Siren *et al*, 2001; Matsushita *et al*, 2003; Villa *et al*, 2003). The mechanisms of increased Epo levels in the brain after injury or after systemic Epo administration and the role of blood–brain barrier integrity require further characterization. The absence of a correlation between endogenous plasma and cerebrospinal fluid (CSF) Epo levels in neonates with central nervous system injury (Juul *et al*, 1999) or in adults with subarachnoid haemorrhage (Springborg *et al*, 2003) suggested that Epo may not cross the blood–brain barrier. In other studies, CSF Epo concentrations correlated with the degree of blood–brain barrier dysfunction in patients with traumatic brain injury (Marti *et al*, 1997) and systemic administration of high doses of Epo in animals and humans resulted in elevated CSF Epo levels consistent with the ability of Epo to cross the blood–brain barrier (Juul *et al*, 2004; Xenocostas *et al*, 2005). The neuroprotective effects of Epo are not limited to ischaemic injury involving the brain and cortical neurons. Epo therapy exerts neuroprotective effects in animal models of blunt trauma (Brines *et al*, 2000) and subarachnoid haemorrhage (Grasso, 2001). In primary spinal cord motoneuron cultures, Epo was also protective against kainate-mediated injury (Siren *et al*, 2001). In a rat model of contusive spinal cord injury, a single dose of recombinant Epo significantly improved functional recovery (Gorio *et al*, 2002, 2005). The beneficial effect of Epo in spinal cord traumatic injury was associated with preservation of myelin in the ventral white matter and the generation of new myelinating oligodendrocytes (Vitellaro-Zuccarello *et al*, 2007). The optimal dose and concentration of Epo required for neuroprotection requires further characterization, especially in view of potential increase in neurotoxicity associated with very high doses of Epo under hypoxic conditions (Weber *et al*, 2005; Buhrer *et al*, 2007; Kellert *et al*, 2007).

In the retina, Epo and EpoR were shown to be expressed and Epo–EpoR blockade by pretreatment with sEpoR antagonist was associated with exacerbation of I/R injury (Junk *et al*, 2002). Furthermore, systemic administration of Epo was shown to cross the blood–brain barrier to reduce neuronal damage in the ischaemic eye (Junk *et al*, 2002). In other studies, elevation of endogenous Epo concentrations in the eye by hypoxic preconditioning or exogenous application of recombinant human Epo were found to protect the mouse retina against light-induced degeneration by inhibiting photoreceptor cell apoptosis (Grimm *et al*, 2002). Transgenic, constitutive overexpression of Epo in neural tissues also protects retinal photoreceptors against light-induced degeneration, however, elevated levels of endogenous Epo do not modulate the course or the extent of retinal degeneration in mouse models of retinal dystrophy with inherited susceptibility to degeneration (Grimm *et al*, 2004).

In addition to its activity as a neuronal survival factor, endogenous Epo signalling plays a role in the protection against axonal injury and degeneration, a process that is involved in the pathogenesis of peripheral neuropathies. In an animal model of distal axonopathy, Epo administration prevents axonal degeneration, an effect that was associated with reduction of limb weakness and neuropathic pain behaviour (Keswani *et al*, 2004). In other studies, recombinant Epo was reported to exhibit activity in the prevention and treatment of experimental diabetic neuropathy (Bianchi *et al*, 2004), peripheral nerve crush injury (Sekiguchi *et al*, 2003), distal sensory polyneuropathy induced by the chemotherapeutic agent paclitaxel (Melli *et al*, 2006) and cisplatin-induced neurotoxicity (Bianchi *et al*, 2006).

The mechanisms and intracellular signalling pathways involved in mediating the neuroprotective effects of Epo have been investigated in animal studies and in cultures of primary neurons. In an *in vivo* study, the neuroprotective effect of Epo was associated with upregulation of the anti-apoptotic protein BCL-X_L in the hippocampus of ischaemic gerbils (Wen *et al*, 2002). In cultured neurons, kinase inhibitors targeting the mitogen-activated protein (MAP) kinase and phosphoinositide-3 kinase (PI3K)-AKT pathways blocked the ability of Epo to reduce neuronal apoptosis because of hypoxia (Siren *et al*, 2001). During *N*-methyl-D-aspartate (NMDA)- or nitric oxide (NO)-mediated neuronal apoptosis, Epo induces the phosphorylation and degradation of IκB resulting in the release and nuclear translocation of nuclear factor-κB (NF-κB) and its binding to DNA in primary neurons in a Janus kinase 2 (JAK2)-dependent manner, counteracting apoptosis by

upregulating the X-linked inhibitor of apoptosis (XIAP) and c-IAP2 proteins (Digicaylioglu & Lipton, 2001). Epo can act synergistically with insulin-like growth factor I (IGF-I) to reduce cerebrocortical neuron apoptosis induced by NMDA exposure (Digicaylioglu *et al*, 2004). This co-operative effect of Epo and IGF-I was associated, at least in part, with synergistic activation and prolonged phosphorylation of AKT and by facilitation of the interaction between XIAP protein and activated caspase-3.

The discovery of the neuroprotective effects of Epo in preclinical studies have been followed by the first clinical trials investigating the potential beneficial effects of Epo in various neuro-psychiatric disorders. In a randomized clinical trial involving acute MCA territory ischaemic stroke patients, intravenous Epo given within 8 h after the onset of symptoms significantly improved neurological function at 1 month with a strong trend toward reduction in infarct size (Ehrenreich *et al*, 2002). The ability of Epo to improve synaptic transmission (Weber *et al*, 2002), prevent brain atrophy (Siren *et al*, 2006), improve cognitive functioning in mice (Ehrenreich *et al*, 2004), and the discovery of increased brain EpoR expression and recombinant Epo penetration into the brain of schizophrenic subjects (Ehrenreich *et al*, 2004) have suggested the potential for beneficial effects in neuro-psychiatric disorders. In healthy volunteer studies, Epo was reported to modulate cognitive function – independent of its haematopoietic effects – exerting beneficial effects on memory retrieval, emotional processing, mood and verbal fluency (Miskowiak *et al*, 2007a,b,c,d). In a randomized clinical trial of Epo therapy in patients with chronic schizophrenia in which patients received intravenous Epo 40 000 U weekly ($n = 20$) or placebo ($n = 19$) for 3 months (Ehrenreich *et al*, 2007), Epo therapy was associated with significant improvement of cognitive performance compared with placebo-treated controls, but without an effect on psychopathology or social functioning parameters. There were no significant adverse events and Epo therapy was associated with a significant decrease in serum S100B level, a glial damage marker. These early translational clinical trials of Epo in neurological and psychiatric disorders suggest the potential for beneficial therapeutic effects and feasibility of the development of novel clinical applications in the future.

Cardiovascular effects of erythropoietin

Erythropoietin as an angiogenic and vascular-protectant cytokine. Epo signalling modulates the regulation of angiogenesis – the formation of new blood vessels from existing vessels. Physiological angiogenesis is a tightly regulated and essential process that occurs actively in the developing embryo, in the female reproductive organs to support the cyclic remodelling of tissues and during wound healing response. EpoR expression in various types of vascular endothelial cells has been associated with the ability of Epo to promote the migration and proliferation of endothelial cells in different *in vitro* experimental models (Anagnostou *et al*, 1990, 1994; Carlini *et al*, 1995; Haller *et al*, 1996; Yamaji *et al*, 1996; Jaquet *et al*, 2002). *In vivo*, Epo stimulates the physiological angiogenesis that takes place in the developing mouse and chick embryos (Ribatti *et al*, 1999; Kertesz *et al*, 2004) and during wound healing (Buemi *et al*, 2002, 2004; Haroon *et al*, 2003; Galeano *et al*, 2004, 2006). In the female genital tract, an endogenous Epo–EpoR system is involved in the regulation of cyclic uterine angiogenesis (Yasuda *et al*, 1998). The oviduct and the uterus were found to produce Epo in an oestrogen- and hypoxia-dependent manner and the administration of sEpoR antagonist into the mouse uterus was shown to inhibit angiogenesis and oestrus cycle-dependent endometrial growth, suggesting that Epo signalling is an important factor for the regulation of oestrogen-dependent cyclical angiogenesis in the uterus (Yasuda *et al*, 1998; Masuda *et al*, 2000). In cultures of primary endothelial cells, Epo treatment induced the expression of its receptor EpoR, eNOS expression and the production of NO, particularly under hypoxic conditions (Beleslin-Cokic *et al*, 2004). In cultures of brain capillary endothelial cells, Epo was found to exert protective effects against anoxia, NO- or glucose deprivation-induced cellular injury and apoptosis via a signalling pathway dependent on activation of AKT, upregulation of BCL-X_L and inhibition of caspase activity (Chong *et al*, 2002, 2003; Chong & Maiese, 2007).

In certain pathological conditions, such as diabetic retinopathy and tumour growth, angiogenesis takes place in an unregulated manner and contributes to the development and progression of disease. Although Epo is a survival factor for retinal photoreceptors (Grimm *et al*, 2002; Junk *et al*, 2002), significant upregulation of endogenous Epo in the vitreous of patients with diabetes was found to be associated with proliferative diabetic retinopathy (Watanabe *et al*, 2005). Furthermore, Epo blockade was shown to inhibit retinal neovascularization in the ischaemic mouse retina leading to the proposal that Epo signalling may be a potential therapeutic target in the pathological angiogenesis of proliferative diabetic retinopathy (Watanabe *et al*, 2005). In a meta-analysis of Epo treatment of the anaemia of prematurity, early Epo administration was associated with a significantly increased risk for retinopathy of prematurity (Ohlsson & Aher, 2006), an effect that may be associated with EpoR activation on endothelial cells and neovascularization in the developing retinal vessels (Morita *et al*, 2003).

A proposed mechanism for Epo-mediated stimulation of tissue neovascularization is the mobilization of bone marrow-derived endothelial progenitor cells (EPCs) into the circulation (Heeschen *et al*, 2003; Bahlmann *et al*, 2004; George *et al*, 2005a). Bone marrow-derived EPCs are thought to promote cardiovascular reparative processes, however, recent data indicates the challenges involved in the accurate identification of EPCs capable of forming perfused blood vessels *in vivo* (Yoder *et al*, 2007). The cardioprotective effect of Epo in animal models of myocardial infarction (MI) has been

associated with increased myocardial neovascularization (Hirata *et al*, 2006; Prunier *et al*, 2007). In a recent study utilizing a rodent bone marrow transplant model, human placental alkaline phosphatase-labelled bone marrow cells were used to track the potential homing of bone marrow-derived EPCs into the myocardial microvasculature post-MI (Westenbrink *et al*, 2007). Darbepoetin-alfa was associated with a significantly increased percentage of endothelium consisting of bone marrow-derived cells with a threefold increased homing of EPCs into the cardiac microvasculature comprising 30% of newly formed capillaries. Epo was also associated with increased myocardial expression of vascular endothelial growth factor (VEGF), which correlated with neovascularization. An important role for Epo in VEGF regulation was demonstrated in a model of femoral artery ligation using erythroid-rescued *Epor*-null adult mice that lack non-haematopoietic EpoR expression (Nakano *et al*, 2007). In this model, blood flow recovery, activation of VEGF/VEGF receptor system, and mobilization of EPCs were all impaired in erythroid-rescued *Epor*-null mice as compared with wild-type mice. In another study using a mouse femoral artery injury model, systemic Epo administration was found to inhibit neointimal hyperplasia and resulted in enhanced re-endothelialization, associated in part with EPC mobilization, in an eNOS/NO-dependent manner (Urao *et al*, 2006). The contribution of bone marrow-derived EPCs into regenerated endothelium was *c.* 30%, tracked using green fluorescent protein-labelled bone marrow cells in a transplantation model, suggesting that Epo-mediated stimulatory effect on re-endothelialization consists both of differentiation of bone marrow-derived EPCs as well as the facilitated differentiation of resident endothelial cells. In a model of hypoxia-induced pulmonary hypertension, Epo signalling was found to play an important role in the recruitment of EPCs to promote neovascularization and protection from the development of chronic vascular disease (Satoh *et al*, 2006). In *Gata1–Epor* transgene-rescued *Epor*-null adult mice, which lack non-haematopoietic EpoR expression, the development of hypoxia-induced pulmonary hypertension and vascular remodeling was markedly accelerated. In this model, EpoR expression and signalling was required for the endothelial response to hypoxia exposure and the activation of endothelial NO synthase in the lungs. Taken together, these findings suggest an important role for Epo in vascular protection/repair through the mobilization and recruitement of EPCs to sites of vascular injury.

Cardiac effects of erythropoietin. A series of recent studies have provided evidence that Epo administration exerts significant cardioprotective effects during ischaemic injury in various preclinical experimental models including I/R injury in isolated, *ex vivo* perfused hearts and in permanent or transient coronary artery ligation models *in vivo* (Lipsic *et al*, 2006a). Epo administration prior to coronary artery ligation and reperfusion results in improved global cardiac function and decreased cardiomyocyte apoptosis associated with increased phosphorylation of AKT, glycogen synthase kinase-3β (GSK3β) and extracellular signal-regulated kinase (ERK) proteins in the myocardium (Calvillo *et al*, 2003; Parsa *et al*, 2003; Nishihara *et al*, 2006). In a rat model of permanent coronary artery ligation, systemic Epo administered up to 12 h following the induction of MI was protective, significantly reducing apoptotic cell death, infarct size at 4 weeks and functional decline (Moon *et al*, 2005). The cardioprotective effects of Epo are not limited to ischaemic cardiac injury, as Epo treatment has also been shown to ameliorate anthracycline-induced cardiomyopathy *in vivo* (Hamed *et al*, 2006; Li *et al*, 2006a). Based on the cardioprotective effects of Epo in preclinical studies, a pilot clinical trial has been performed to investigate the effects of recombinant Epo in patients with acute MI. In this trial, 22 patients with a first acute MI were randomized to darbepoetin-alfa treatment or no medication prior to primary coronary intervention (Lipsic *et al*, 2006b). Darbepoetin-alfa was found to be safe and well-tolerated although there was no significant difference in the left ventricular ejection fraction (LVEF) at 4 months in this small trial.

The isolated, Langendorff-perfused heart I/R models in which Epo preconditioning attenuates LV dysfunction in a dose-dependent manner have been useful for the characterization of the mechanisms involved in the cardioprotective effects of Epo. EpoR expression in the heart and in isolated primary cardiac myocytes is associated with Epo-mediated activation of specific signal transduction pathways, the reduction of ischaemia-induced cardiac myocyte apoptosis and improved recovery of LV function in the *ex vivo* perfused heart (Cai *et al*, 2003; Wright *et al*, 2004). Epo is cardioprotective also when administered at the time of ischaemia or even at reperfusion in different experimental models leading to significant reduction in infarct size and attenuation of LV dysfunction (Lipsic *et al*, 2004; Parsa *et al*, 2004; Bullard *et al*, 2005; Hanlon *et al*, 2005). Using ^{31}P nuclear magnetic resonance spectroscopy to measure high energy phosphate levels during ischaemia, Epo-mediated cardioprotection was shown to involve preservation of myocardial ATP levels (Wright *et al*, 2004). Direct Epo treatment of the isolated, perfused heart was found to promote the acute phosphorylation of AKT, ERK1/2, p38 MAP kinases and the translocation of protein kinase C (PKC)ϵ isoform to the membrane fraction (Cai & Semenza, 2004; van der Meer *et al*, 2004a; Shi *et al*, 2004; Hanlon *et al*, 2005). The cardioprotective effect of Epo preconditioning has been blocked by treatment with kinase inhibitors targeting the PI3K or PKC pathways (Cai & Semenza, 2004; Shi *et al*, 2004; Hanlon *et al*, 2005), whereas the cardioprotective effect of Epo administered postischaemia, at the time of reperfusion, appears to be dependent on the activity of the PI3K-AKT pathway and not the PKC pathway (Hanlon *et al*, 2005).

Cultures of isolated primary cardiac myocytes have also been used to characterize signal transduction pathways activated by Epo and to investigate the mechanisms of cardioprotection

in vitro. Epo treatment significantly attenuates apoptotic cardiomyocyte death induced by hypoxia, the kinase inhibitor staurosporine and the anthracycline chemotherapeutic agent doxorubicin in a dose-dependent manner (Calvillo *et al*, 2003; Tramontano *et al*, 2003; Fiordaliso *et al*, 2005; Fu & Arcasoy, 2007). Epo induces the phosphorylation and increased kinase activity of AKT and the p44/42 MAP kinases ERK1/2 in primary cardiac myocytes (Tramontano *et al*, 2003; Fu & Arcasoy, 2007). The cardioprotective effect of Epo during hypoxia or doxorubicin-induced apoptosis was dependent on PI3K-AKT pathway activity and associated with the increased phosphorylation and inhibition of GSK3β.

The potential role of endogenous Epo in the pathophysiology of cardiac dysfunction has been investigated in preclinical and clinical studies. In a model of cardiac dysfunction induced by LV pressure overload because of transverse aorta constriction in *Gata1–Epor* transgene erythroid-rescued *Epor*-null adult mice, enhanced susceptibility to LV dilatation, LV dysfunction and cardiac death were observed in animals lacking cardiac *Epor* expression. These findings in *Epor*-null adult mice were associated with impaired phosphorylation of STAT3 and p38 MAPK as well as decreased expression of VEGF and impaired capillary growth in the LV myocardium (Asaumi *et al*, 2007). Endogenous Epo signalling in the heart was also reported to play a role in the protection against myocardial I/R injury. Erythroid-rescued *Epor*-null transgenic adult mice subjected to coronary artery ligation and reperfusion were shown to exhibit larger myocardial infarct size that was associated with increased caspase-3 activity and apoptotic cardiac myocytes (Tada *et al*, 2006). These findings suggest that endogenous Epo signalling in the heart may activate protective mechanisms during acute ischaemic injury. Consistent with this possibility, in patients with first MI, elevated endogenous Epo levels were found to correlate with decreased infarct size measured by creatinine kinase release (Namiuchi *et al*, 2005), however, further clinical studies are required to confirm these findings.

Recent studies have also investigated the potential role of endogenous and exogenous Epo in chronic heart failure (CHF). Anaemia is prevalent in patients with CHF and is an independent factor associated with increased risk of hospitalization and all-cause mortality (Tang & Katz, 2006). Consistent with potential beneficial effects of Epo therapy in heart failure, in anaemic CHF patients, recombinant Epo therapy was associated with improved New York Heart Association (NYHA) function class, increased LVEF, reduced need for diuretics (Silverberg *et al*, 2000, 2001, 2003) and improved exercise capacity (Mancini *et al*, 2003). In a randomized, placebo-controlled trial of anaemic patients with CHF, preliminary results indicate beneficial effects of improved quality of life and exercise duration associated with darbepoetin-alfa therapy without severe adverse effects (Cleland *et al*, 2005). In anaemic CHF patients, the erythropoietic properties of Epo leading to anaemia correction are likely to contribute to the beneficial clinical effects although direct cardiac actions of Epo have not been ruled out. It is noteworthy that endogenous Epo levels have been reported to be elevated in CHF patients compared with controls, the severity of CHF correlates with more significantly elevated Epo levels, and higher endogenous plasma Epo level may be an independent factor predicting mortality and morbidity (van der Meer *et al*, 2004b; George *et al*, 2005b). Despite this correlation between high endogenous Epo levels and the severity of CHF, in preclinical studies investigating the potential beneficial effects of Epo therapy in heart failure, recombinant Epo treatment starting 3 or 6 weeks after acute MI in rats did not affect infarct size, but significantly improved cardiac function associated with increased myocardial neovascularization and reduced atrial natriuretic peptide levels (van der Meer *et al*, 2005). In another study, following Epo treatment of mice in a chronic post-MI heart failure model, cardiac function improvement was associated with increased angiogenesis as well as near normalization of the myocardial levels of inflammatory cytokines interleukin (IL)-1β, IL-6, TNFα and transforming growth factor β1 and significantly decreased fibrosis in the heart (Li *et al*, 2006b). Although these non-anaemic preclinical rodent models of CHF suggest that Epo may exert direct beneficial effects on cardiac function, these studies are limited by the development of significant polycythemia in Epo-treated animals and the possibility of secondary effects. Several ongoing and future clinical trials are anticipated to better characterize the potential benefits and risks of Epo therapy in patients with acute coronary syndromes and CHF.

Modulation of immunological, inflammatory and wound healing responses by Epo

Several studies have investigated the ability of Epo to affect immunological responses. In a mouse model of autoimmune encephalomyelitis, Epo treatment upon onset of paresis was reported to significantly improve neurological functional recovery associated with a significant reduction in inflammatory infiltrates and demyelination (Agnello *et al*, 2002; Zhang *et al*, 2005). In an MCA occlusion stroke model in rats, Epo was found to reduce astrocyte activation and the recruitment of leucocytes and microglia in the infarction associated with a reduction of levels of inflammatory cytokines including monocyte chemoattractant protein-1 (MCP-1), TNF and IL6 in the ischaemic brain (Villa *et al*, 2003). In this study, although Epo did not directly inhibit the cytokine release, neuronal injury-related inflammation was attenuated by an indirect mechanism involving Epo-mediated reduction of neuronal apoptosis and increased resistance to secondary inflammatory injury. In an experimental model of autoimmune myocarditis in which rats were immunized with cardiac myosin, systemic recombinant Epo therapy resulted in significant reduction in the myocarditis area and inflammation and led to the attenuation of cardiac dysfunction (Mitsuma *et al*, 2006). The beneficial effects of Epo were associated with reduction in the number of apoptotic cardiomyocytes as well as decreased expression of inflammatory cytokines TNFα and

IL-6 in the hearts. In a rodent model of multiple myeloma, Epo administration was associated with an anti-tumour effect that was dependent on a T-cell-mediated mechanism (Mittelman *et al*, 2001). More recently, in patients with multiple myeloma, Epo therapy has been associated with normalization of the CD4:CD8 ratio and Epo was found to exert immunomodulatory effects on T-cell function leading to enhanced phytohaemagglutinin-mediated activation and proliferation potential and co-stimulatory molecule (CD28) expression as well as decreased serum IL-6 levels (Prutchi-Sagiv *et al*, 2006). The mechanisms of the immunological and anti-inflammatory effects of Epo require further characterization.

A series of recent preclinical studies have suggested that Epo may contribute to the regulation of physiological wound healing responses. In a rat model of wound healing, exogenous Epo administration locally into subcutaneous fibrin chambers was found to promote the formation of wound granulation tissue – an effect that was associated with stimulation of physiological angiogenesis and upregulation of iNOS expression (Haroon *et al*, 2003). In this study, local administration of the antagonist sEpoR protein or a neutralizing anti-Epo antibody resulted in delayed wound healing consistent with a role for endogenous Epo signalling in the wound healing cascade. In other studies, systemic Epo administration was also found to promote wound healing, associated with increased angiogenesis and wound collagen and VEGF content (Buemi *et al*, 2002, 2004). Wound VEGF levels were also found to be upregulated during wound healing in a genetically diabetic mouse model where systemic Epo therapy alleviated the impaired healing response (Galeano *et al*, 2004). In an experimental model of burn wounds, recombinant Epo promoted wound re-epithelialization and shortened the time to final wound closure (Galeano *et al*, 2006). Epo therapy in burn wounds was associated with increased granulation tissue thickness, increased tissue collagen, VEGF, iNOS and eNOS content and NO production. More recently, in a murine closed femur fracture model, Epo has been reported to promote bone repair and fracture healing (Holstein *et al*, 2007). Further work is required to determine whether the beneficial effects of Epo on wound and fracture healing responses in preclinical studies can be translated to the clinical setting.

Renoprotective effects of erythropoietin

The expression of EpoR transcripts and protein in the kidney in mesangial, proximal tubular and medullary collecting duct cells has been associated with specific binding of ^{125}I-Epo ligand to reveal a single class of high- to intermediate-affinity EpoRs with kD range from 96 pm to 1·4 nm (Westenfelder *et al*, 1999). In the kidney, Epo treatment was shown to reduce the extent of renal dysfunction induced by I/R injury in animal models and this renoprotective effect was associated primarily with a reduction in apoptotic cell death (Yang *et al*, 2003; Gong *et al*, 2004; Patel *et al*, 2004; Sharples *et al*, 2004; Sharples & Yaqoob, 2006; Spandou *et al*, 2006). In cultures of human proximal tubular cells, Epo treatment significantly reduced apoptosis induced by hypoxia (Vesey *et al*, 2004). Preconditioning with Epo in rodent kidneys subjected to I/R injury was associated with decreased caspase-3 activity, upregulation of BCL-2 and heat shock protein-70 expression (Yang *et al*, 2003), and a reduction in renal tissue inflammation markers (Patel *et al*, 2004). Epo is also protective against medication-induced renal dysfunction, facilitating the recovery from cisplatin-induced acute renal failure (Vaziri *et al*, 1994; Bagnis *et al*, 2001) and attenuation of renal interstitial inflammation and fibrosis in chronic cyclosporine nephropathy (Lee *et al*, 2005). In contrast to the renoprotective effects of Epo during I/R and nephrotoxic agent-induced injuries, in a model of radiation-induced renal dysfunction, Epo administration concomitant with radiation was associated with dose-dependent deterioration of renal function (Andratschke *et al*, 2006). The pathophysiology of the deleterious effect of Epo in the setting of ionizing radiation-induced renal injury is unclear. The direct signal transduction pathways activated by Epo in the kidney, the specific cell types that constitute targets for Epo action and the mechanisms involved in renoprotective signalling activated by Epo require further characterization.

EpoR structure in non-haematopoietic cells

The structure of the cell surface receptor that mediates the biological effects of Epo and its derivatives in non-haematopoietic tissues remains to be characterized. The tissue-protective effects of Epo in the brain and heart appear to require a second distinct receptor component, the βcR subunit of the IL-3 receptor, also shared by IL-5 and granulocyte–macrophage colony-stimulating factor (Brines *et al*, 2004). In this study, a physical association between βcR subunit and EpoR was demonstrated by co-immunoprecipitation of the proteins in lysates of neuron-like cells. The requirement for βcR in the tissue-protective effect of Epo was shown using βcR knock-out mice in a spinal cord injury model where motor function recovery in response to Epo was absent in βcR knock-out mice compared with strain-matched wild-type control animals. When primary cardiac myocytes isolated from βcR knock-out mice were subjected to staurosporine-induced apoptosis, Epo failed to exert cardioprotection. Previous studies in haematopoietic cells have suggested a functional interaction between EpoR and βcR. Epo was reported to induce the tyrosine phosphorylation of βcR in UT-7 erythroleukaemia cells (Hanazono *et al*, 1995). In IL-3-dependent Ba/F3 cells transfected with EpoR and βcR, co-immunoprecipitation of the receptors was demonstrated upon Epo treatment. Moreover, increased expression of βcR potentiated Epo-dependent growth, whereas inhibition of βcR using anti-sense oligodeoxynucleotides blocked both IL-3 and Epo-dependent cell growth (Jubinsky *et al*, 1997). The physical and functional interactions between βcR and EpoR in haematopoietic cell culture models have been investigated in animal models as well. In mice lacking both the common βcR and the

IL-3-specific β-chain, erythroid burst-forming unit (BFU-e) and erythroid colony-forming unit (CFU-e) formation and Epo responsiveness were reported to be normal, suggesting lack of a functional interaction in erythroid cells (Scott *et al*, 2000). In contrast, in mice carrying null mutations in *Csf2* and *Il3* genes, CFU-e frequency was reduced, suggesting that βcR signalling may contribute to erythropoiesis (Jegalian *et al*, 2002). In other studies, constitutive βcR signalling was shown to activate EpoR via JAK2 activation in haematopoietic cell lines (Blake *et al*, 2002). Whether such cross-talk is a feature of Epo-induced signalling in non-haematopoietic cells (including cancer cells) will require further work to elucidate the biology of potential functional EpoR and βcR interactions. Fig 1 illustrates the potential crosstalk between $(EpoR)_2$ and βcR cell surface receptors and protective signalling pathways in non-haematopoietic cells.

The generation of Epo variants, including asialo-Epo and carbamylated Epo (Cepo), that retain the protective effects of Epo in non-haematopoietic tissues whilst exhibiting no biological effect in haematopoietic cells that express EpoR have indicated the presence of fundamental differences between the mechanisms of Epo-mediated cellular signalling activation in haematopoietic *versus* non-haematopoietic cells (Erbayraktar *et al*, 2003; Leist *et al*, 2004). In radioligand binding experiments, Cepo fails to bind EpoR expressed in UT7 erythroleukaemia cells, and in Chinese hamster ovary cells or BaF/3 cells overexpressing the classical EpoR homodimers $(EpoR)_2$. Although systemic Cepo injections fail to increase the haematocrit in mice, the neuroprotective effects of Epo in models of cerebral infarction, spinal cord trauma, experimental autoimmune encephalomyelitis and diabetic peripheral neuropathy were completely preserved (Leist *et al*, 2004). Subsequent studies demonstrated that Cepo retained not only the neuroprotective activities (Bianchi *et al*, 2004; Erbayraktar *et al*, 2006; Mennini *et al*, 2006; Villa *et al*, 2007; Wang *et al*, 2007) but also the cardioprotective effects of Epo (Fiordaliso *et al*, 2005; Moon *et al*, 2006). Cepo exhibits no haematopoietic effects in colony formation assays and does not compete with Epo (Coleman *et al*, 2006). Systemic Cepo administered to mice leads to the accumulation of $CD34^+$/Flk-1 EPCs in the bone marrow in a manner similar to Epo, but unlike Epo, Cepo is not mitogenic for cultured human umbilical vein endothelial cells. Systemic and renal hemodynamic measurements showed that, unlike Epo, the chronic administration of Cepo was not associated with elevation of blood pressure and renal cortical blood flow. Thus, the availability of tissue-protective Epo variants devoid of erythropoietic stimulatory effects may prove clinically useful by abrogating concerns over undesirable elevation of the red blood cell mass and possibly other systemic adverse effects, such as hypertension.

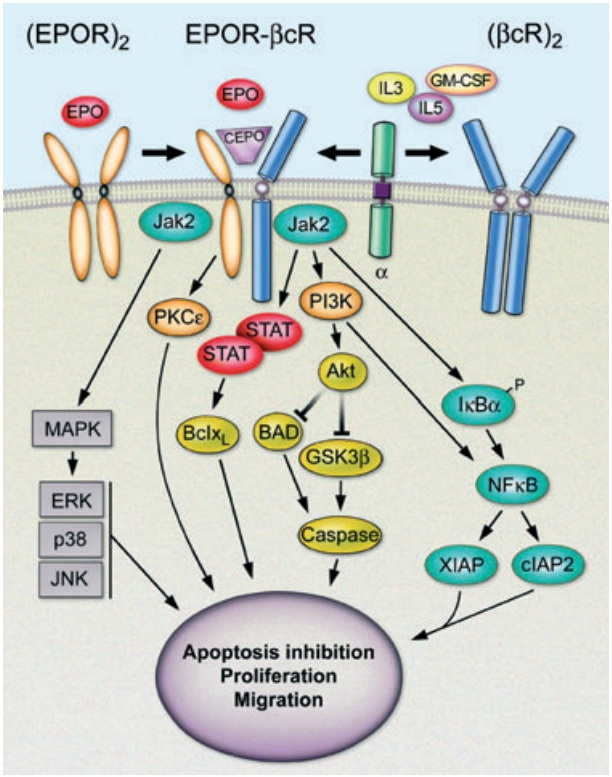

Fig 1. EpoR structure and signalling pathways in non-haematopoietic cells. A physical association between the βcR subunit and EpoR has been demonstrated by co-immunoprecipitation of the proteins in neuron-like cells. The tissue-protective effects of Epo appear to require the expression of βcR and a low-affinity, heterodimeric EpoR–βcR receptor (Brines *et al*, 2004). Epo-induced activation of PI3K-AKT pathway and inhibition of apoptosis is central to the diverse protective properties of Epo. In primary neurons, Epo induces the phosphorylation and degradation of IκB to release NF-κB in a JAK2-dependent manner to inhibit apoptosis by upregulating XIAP and c-IAP2 proteins (Digicaylioglu & Lipton, 2001). Epo-mediated translocation of PKCε isoform from the cytosol to the membrane fraction is involved in its cardioprotective effect (Shi *et al*, 2004; Hanlon *et al*, 2005). The JAK-STAT pathway does not appear to be uniformly activated by Epo in non-haematopoietic cells and its role in the protective effects of Epo requires further characterization. Epo-mediated activation of MAP kinase family members has been reported in cardiac cells (Parsa *et al*, 2004; Fu & Arcasoy, 2007) and cancer cells where Epo may promote cancer cell migration *in vitro* (Lester *et al*, 2005). The carbamylated Epo variant Cepo does not bind $(EpoR)_2$, exhibits no detectable erythropoietic activity yet retains the protective properties of Epo in non-haematopoietic tissues (Leist *et al*, 2004). Depicted in this hypothetical diagram, the role of an additional receptor subunit (α) and the heteromeric cell surface structure of the receptor remain to be characterized.

Potential risks of Epo therapy

As new applications for Epo therapy are being explored, the potential for adverse systemic and cardiovascular effects, such as hypertension, retinopathy, neurotoxicity and thrombotic events, as well as the impact of Epo therapy on survival outcomes will need to be considered carefully in the design of future clinical studies. Hypertension, which either develops or worsens in 20–30% of renal patients on recombinant Epo therapy, is usually controlled without serious consequences (Maschio, 1995; Bohlius *et al*, 2006). The early use of Epo in anaemia of prematurity has been associated with significant

Table I. The functions of endogenous Epo signalling.

Epo-modulated function	Models	Phenotype/effect	References
Erythropoiesis	*Epo*- or *Epor*-null mouse	Defective fetal liver erythropoiesis Severe anaemia and *in-utero* death	(Wu *et al*, 1995; Kieran *et al*, 1996; Lin *et al*, 1996)
Physiological angiogenesis			
Embryo development	*Epo*- or *Epor*-null mouse	Impaired angiogenesis and vascular networks	(Wu *et al*, 1999; Kertesz *et al*, 2004)
Cyclic uterine angiogenesis	Ovariectomized mouse	Inhibition of endometrial transition to proestrus stage by sEpoR antagonist	(Yasuda *et al*, 1998)
Wound healing	Fibrin-induced wound healing in rats	Delayed wound healing by local sEpoR or anti-Epo antibody administration	(Haroon *et al*, 2003)
Brain development	*Epo*- or *Epor*-null mouse	Increased neuron apoptosis	(Yu *et al*, 2001, 2002)
	Conditional EpoR knock-down mice	Embryonic neural defects	(Tsai *et al*, 2006)
	Selective EpoR-rescued mice	Decreased neurogenic progenitor cells	(Chen *et al*, 2007)
Neuroprotection	Gerbil forebrain ischaemia model	Epo blockade promotes neuron death	(Sakanaka *et al*, 1998)
	I/R injury in mouse retina	Intra-vitreal sEpoR increases damage	(Junk *et al*, 2002)
Cardiovascular protection	Erythroid-rescued $EpoR^{-/-}$ mouse:		(Tada *et al*, 2006)
	Myocardial I/R injury	Increased MI size, apoptosis in $EpoR^{-/-}$ mice	(Asaumi *et al*, 2007)
	Pressure overload-induced LV dysfunction	Accelerated LV dysfunction/reduced survival in $EpoR^{-/-}$ mice	
	Hypoxia-induced pulmonary hypertension	Accelerated vascular remodeling in $EpoR^{-/-}$ mice	(Satoh *et al*, 2006)
	Hindlimb ischaemia	Impaired angiogenesis and blood flow recovery in $EpoR^{-/-}$ mice	(Nakano *et al*, 2007)

I/R, ischaemia-reperfusion; LV, left ventricular; MI, myocardial infarction; EpoR, erythropoietin receptor.

increase in the risk of retinopathy (Ohlsson & Aher, 2006), probably by the stimulation of retinal neovascularization (Morita *et al*, 2003). In some preclinical studies, very high doses of Epo in conjunction with hypoxia has been associated with a paradoxical neurotoxic effect, suggesting the need for further work to determine dose-response conditions to optimize the neuroprotective effects of Epo (Weber *et al*, 2005; Buhrer *et al*, 2007; Kellert *et al*, 2007). Several clinical studies evaluating Epo therapy in chronic kidney disease patients have raised the possibility that higher target haemoglobin levels may be associated with increased cardiovascular events and all-cause mortality as well as a potential increased risk of arteriovenous access thrombosis (Besarab *et al*, 1998; Drueke *et al*, 2006; Singh *et al*, 2006; Phrommintikul *et al*, 2007). The baseline risk of venous thromboembolism in cancer patients is increased by Epo therapy (Khorana *et al*, 2005), with an overall increase in the relative risk by 67% in a large cohort (Bohlius *et al*, 2006). Thrombotic events associated with Epo therapy were also increased in critically ill patients in whom Epo significantly reduced mortality, particularly in trauma patients, compared with placebo (Corwin *et al*, 2007). The mechanisms involved in the increased thrombosis rate as a consequence of Epo therapy require further characterization, especially as increased haematocrit and viscosity may not necessarily be sufficient to promote thrombosis suggested by the absence of thromboembolic phenomena in transgenic mice with constitutive Epo overexpression and haematocrits in the 85% range (Shibata *et al*, 2003). In a canine model consisting of animals with arterio-venous shunts, Epo therapy was thrombogenic in the absence of a significant effect on the haematocrit, an effect that was associated with platelet activation that resolved after cessation of Epo (Wolf *et al*, 1997). Other studies have reported that Epo may modulate endothelial and platelet function, von Willebrand factor antigen levels and factor VIII activity and attenuate the effect of aspirin on prolongation of the bleeding time (Akizawa *et al*, 1991; Malyszko *et al*, 1995; Stohlawetz *et al*, 2000; Noguchi *et al*, 2001; Tang *et al*, 2007). Cepo, the carbamylated variant of Epo that does not stimulate erythropoiesis, was reported to result in fewer undesirable effects on vascular function and procoagulant activity suggesting that this cytoprotective ligand may exhibit a favourable adverse effect profile in the treatment acute ischaemic coronary syndromes and cerebrovascular accidents (Coleman *et al*, 2006).

In cancer patients, the findings of a series of recent published and unpublished randomized, placebo-controlled clinical trials involving patients with specific types of tumours, including head–neck cancer, metastatic breast cancer, lymphoproliferative malignancies and advanced non-small cell lung cancer, have found that Epo therapy exerts an adverse effect on survival when administered to target haemoglobin levels exceeding the currently recommended value of 120 g/l (Henke *et al*, 2003, http://www.dahanca.dk/get_media_file.php?mediaid=125, http://www.fda.gov/ohrms/dockets/ac/07/briefing/2007-4301b2-00-index.htm; Leyland-Jones, 2003; Leyland-Jones *et al*, 2005; Wright *et al*, 2007). Whether the reported unfavourable effects of Epo on survival outcomes are specific for certain tumour types is not known, but suggested by the apparent absence of a detrimental effect of Epo on overall survival in patients with small cell lung cancer (Grote *et al*, 2005, http://www.amgen.com/media/media_pr_detail.jsp?releaseID=987476). The mechanisms of the impaired survival reported in some of the trials and the direct role of Epo in tumour progression remain unclear. Well-designed preclinical studies and clinical trials are required to characterize Epo biology in cancer as the clinical practice guidelines for Epo therapy in chemotherapy-induced anaemia continue to evolve (Rizzo *et al*, 2007).

Conclusions and implications for future studies

An extensive body of preclinical work has resulted in the accumulation of scientific evidence indicating that Epo is a pleiotropic cytokine that confers broad tissue-protective properties as part of an innate response to stressors, promotes angiogenesis and modulates wound healing responses (Table I). Although Epo exerts selective erythropoietic activity within the haematopoietic system to stimulate the production of red blood cells, the diverse functions and potentially beneficial biological effects of Epo signalling in non-haematopoietic organs suggests that further investigations of new therapeutic applications for recombinant Epo are warranted. Further characterization of the non-haematopoietic effects of Epo may contribute to the optimization of its use for current indications in chronic kidney disease and cancer patients. Epo and its derivates are likely to find novel clinical applications that

Table II. Current and potential future uses of recombinant Epo and its derivatives in clinical practice.

Current indications for Epo
Anaemia associated with chronic kidney disease
Chemotherapy-induced anaemia in non-myeloid malignancies
Anaemia related to zidovudine therapy in human immunodeficiency virus-infected patients
Anaemic patients scheduled to undergo non-cardiac, non-vascular surgery
Other clinical uses in selected cases
Anaemia in chronic non-renal diseases
Anaemia in myelodysplastic syndromes
Anaemia of prematurity
Potential future applications as a tissue-protective agent
Ischaemic heart disease
Congestive heart failure
Cerebrovascular accident
Trauma and other critically ill patients
Neurodegenerative and psychiatric disorders
Acute renal failure
Wound healing
Diabetic neuropathy

Epo, erythropoietin.

are already being explored in trials involving critically ill patients and patients with acute ischaemic stroke, coronary syndromes and heart failure. Future clinical trials are expected to better define the role for Epo as a tissue-protective agent and the potential risks associated with Epo therapy (Table II). Additional future scientific challenges include, but are not limited to (i) the characterization of the structure of the cellular receptor that mediates the biological effects of Epo in non-haematopoietic cells; (ii) the mechanisms of tissue-specific expression of EpoR; (iii) the elucidation of the mechanisms of the prothrombotic effects of systemic Epo; (iv) the generation and availability of additional adult mouse models of tissue-specific EpoR knock-out; and (v) the characterization of Epo biology in cancer through the development of novel preclinical experimental models and well-designed clinical trials.

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